

## **DELIVERABLE**

### **DL.3.1.1. MAIN WELFARE ASPECTS OF STUNNING BROILER CHICKENS BY EXPOSURE TO CONTROLLED ATMOSPHERE**

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## 1. Introduction

Two main types of stunning methods are commercially used in broilers, electrical waterbath stunning (WBS) and controlled atmosphere stunning (CAS). The WBS is by far the most frequently applied method worldwide (European Commission, 2013; Berg and Raj, 2015). To know the popularity of both methods in the European Union (EU) in 2021, EURCAW-Poultry-SFA sent an internal survey to the Competent Authorities of the Member States asking for providing a list of slaughterhouses and which stunning method are using each. Twenty out of 27 Member States provided the data and approximately 84% of the slaughterhouses use WBS systems, 15% CAS systems and 1% mechanical methods. However, approximately 53% of broilers slaughtered are stunned with WBS and 47% with CAS. In this sense, while WBS is more used in small-scale compared to large-scale abattoirs, CAS is nearly always used in large-scale abattoirs, likely because CAS systems involve large investments but also increase the slaughter capacity (European Commission, 2013).

Although WBS is intended to result in immediate loss of consciousness, the stunning method is associated with several major welfare concerns. These concerns include the uncrating and shackling of conscious birds, which involves birds being inverted and hung by their legs in metal shackles causing severe pain and fear-provoking situations to the birds (Gentle and Tilston, 2000; Bedanova *et al.*, 2007; Shields and Raj, 2010; Berg and Raj, 2015; EFSA, 2019). Being hung upside down activates the nociceptors in the legs by the compression generated by the weight of birds on the legs in contact with the shackles (Gentle and Tilston, 2000). Most birds struggle and wing flap following shackling causing injuries and carcass downgrading. In addition, this welfare issue can be accentuated if birds have leg abnormalities (*e.g.*, lameness, injuries; Butterworth, 1999) and/or the operator is rough during the shackling. Moreover, in WBS systems, wings might be exposed to the electrified water prior to immersion of the heads at the entrance of the waterbath, causing pre-stun shocks and leading to severe pain to the bird. Furthermore, other hazards, such as delayed induction of unconsciousness or failure in onset of unconsciousness, may occur due to a lack of control of current applied to individual birds in a multiple bird waterbath stunner, leading to some birds receiving less current than others (Sparrey *et al.*, 1993). In addition, recovery of consciousness before death may occur even when electrical settings are conducting an effective stun due to inappropriate neck cutting practice or prolonged stun to stick interval. Furthermore, birds might completely avoid the WBS if they are too small for the head to reach the water, or when a bird lifts its head to avoid contacting the water, therefore with the risk of being neck cut while still conscious. Others may also enter the scald tank conscious if they missed the neck cutter or recovers from unconsciousness or when both stunning and neck cutting are inadequate (Shields and Raj, 2010). For these reasons, WBS requires monitoring and re-stunning of animals in birds showing outcomes of consciousness (HSA, 2016). However, in slaughterhouses that use rapid line speeds (> 8,000 birds per hour), the possibility of checking animal welfare on an individual basis and to applying backup stunning methods if needed, is limited (EFSA, 2004). A further complicating factor is that the visual appearance of effectively stunned birds can be indistinguishable from electro-immobilised but conscious birds subjected to an ineffective stunning.

CAS emerged as an alternative stunning method to eliminate the WBS welfare concerns (Berg and Raj, 2015). CAS consists in exposing the chickens to modified gas environments or by reducing the atmosphere pressure, which induce a gradual loss of consciousness. Depending on the gas mixture concentrations and the duration of exposure, CAS stunning can be either reversible or irreversible. However, in most of the slaughterhouses the duration of exposure is long enough to cause death in all the animals. This long exposure is used, since in CAS, birds are stunned at large numbers at the same time and thus would recover consciousness before death due to bleeding. The CAS is applied to birds

in their transport containers or on conveyers, and thus involves minimal or no handling of conscious birds, and shackling is only performed when birds are in an unconscious state or dead. Hence, all birds will be dead when shackled, neck cut and bled. However, other welfare concerns arise with the CAS method as the different gases and mixtures of gases in use must be operated very carefully to avoid undesirable incidents. The reason for this is that the induction of unconsciousness with CAS is not instantaneous but involves a transitional period during which negative welfare outcomes may occur (Verhoeven *et al.*, 2015). Furthermore, if the time of exposure is too short birds might not die and the duration of unconsciousness may not last until the end of bleeding.

## 2. List of CAS methods and legal requirements

In the EU, the welfare of poultry at the time of killing is under Regulation (EC) No 1099/2009 of 24 September 2009 on the protection of animals at the time of killing<sup>1</sup>. This Regulation describes seven CAS methods that can be used for poultry stunning at slaughter, for depopulation or for other situations than slaughter (*e.g.*, because unproductive, for disease control, etc.). Four out of these seven CAS methods are related to stunning poultry at slaughter for human consumption and are reviewed in this manuscript. According to the approach used to modify the atmospheric condition, these are:

1. Stunning with carbon dioxide in two phases
2. Stunning with inert gases
3. Stunning with carbon dioxide associated with inert gases
4. Low atmospheric pressure stunning (LAPS)

From the 20 out of 27 Member States that provided information of slaughterhouses and CAS methods used, none of them were with inert gases, nor carbon dioxide associated with inert gases nor LAPS (EURCAW-Poultry-SFA survey, 2021). Thus, it seems that carbon dioxide (CO<sub>2</sub>) in two phases is the most common CAS method by far applied in the EU. Legal requirements for each CAS stunning system are detailed as follows:

Description of the methods (RE 1099/2009 of 24 September 2009, Annex I, Chapter I):

- Carbon dioxide in two phases: *“successive exposure of conscious animals to a gas mixture containing up to 40% of carbon dioxide, followed, when animals have lost consciousness, by a higher concentration of carbon dioxide.”*
- Inert gases: *“direct or progressive exposure of conscious animals to an inert gas mixture such as argon or nitrogen leading to anoxia. Simple stunning for poultry if the duration of exposure to anoxia is of less than 3 minutes”*
- Carbon dioxide associated with inert gases: *“direct or progressive exposure of conscious animals to a gas mixture containing up to 40% of carbon dioxide associated with inert gases leading to anoxia. Simple stunning of poultry if the overall duration of exposure to at least 30 % of carbon dioxide is of less than 3 minutes.”*
- LAPS: *“Exposure of conscious animals to gradual decompression with reduction in available oxygen to less than 5 %. It can be used in broiler chickens up to 4 kg live weight.”*

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<sup>1</sup> This Regulation was amended by Regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March 2017 (Official Controls Regulation) and Commission Implementing Regulation (EU) 2018/723 of 16 May 2018 (Annexes I and II to Council Regulation (EC) No 1099/2009 as regards the approval of low atmospheric pressure stunning and corrected in Corrigendum to Regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March 2017 (Official Controls Regulation).

Requirements regarding carbon dioxide, use of inert gases or a combination of those gas mixtures (RE 1099/2009 of 24 September 2009, Annex I, Chapter II, point 8):

- *“Under no circumstances shall gases enter into the chamber or the location where animals are to be stunned and killed in a way that it could create burns or excitement by freezing or lack of humidity.”*

Requirements regarding layout, construction and equipment of slaughterhouses, regarding gas stunning equipment for poultry (RE 1099/2009 of 24 September 2009, Annex II, point 6):

- *“Gas stunners, including conveyor belts, shall be designed and built to: (a) optimise the application of stunning by gas; (b) prevent injury or contusions to the animals; (c) minimise struggle and vocalisation when animals are restrained.”*
- *“Gas stunners shall be equipped to measure continuously, display and record the gas concentration and the time of exposure, and to give a clearly visible and audible warning if the concentration of gas falls below the required level. The device shall be placed so as to be clearly visible to the personnel. These records shall be kept for at least one year. Gas stunners shall be designed in a manner that, even at the maximum permitted throughput, the animals are able to lie down without being stacked on each other.”*

Requirements regarding LAPS (RE 1099/2009 of 24 September 2009, Annex I, Chapter II, point 10):

- *During the first phase, the decompression rate shall not be greater than equivalent to a reduction in pressure from standard sea level atmospheric pressure 760 to 250 Torr for a period of not less than 50 seconds.*
- *During a second phase, a minimum standard sea level atmospheric pressure of 160 Torr shall be reached within the following 210 seconds.*
- *The pressure time curve shall be adjusted to ensure that all birds are irreversibly stunned within the cycle time.*
- *The chamber shall be leak tested and pressure gauges calibrated before each operational session and not less than daily.*
- *Records of absolute vacuum pressure, time of exposure, temperature and humidity shall be kept for at least one year.*

Requirements regarding layout, construction and equipment of LAPS (RE 1099/2009 of 24 September 2009, Annex II, point 7):

- *LAPS equipment shall be designed and built to ensure a vacuum of the chamber enabling slow gradual decompression with reduction in available oxygen and holding at minimal pressure.*
- *The system shall be equipped to measure continuously, display and record the absolute vacuum pressure, the time of exposure, the temperature, the humidity and to give a clearly visible and audible warning if the pressure deviates from the required levels. The device shall be clearly visible to the personnel.*

When these CAS methods are applied, normal functioning of the neurons in the brain is prevented, inducing a state of reversible or irreversible unconsciousness depending on the methods and

parameters used. However, with the exception of carbon dioxide stunning in two phases, the other CAS methods are not frequently used in the poultry industry neither globally nor in EU-27 yet.

### 3. Carbon dioxide in two phases

Chickens are first exposed to low concentration of carbon dioxide (CO<sub>2</sub>; up to 40%) until unconsciousness occurs. Thereafter, the CO<sub>2</sub> concentration is increased in the second phase inducing a deeper state of unconsciousness and then death (EFSA, 2019).

#### 3.2. Available equipment

Commercial equipment for stunning with CO<sub>2</sub> differs in design, either being tunnels, pits or closed cabinets. In tunnels and pits, birds enter the system in their transport crates, or they are uncrated by tilting the container (EFSA, 2019) and using a conveyor. Birds enter continuously at one end of the system, and while they are conveyed to the opposite end, they are exposed to different gas concentration phases (the pit and the tunnel are pre-filled with the different concentrations of CO<sub>2</sub>). In contrast, with closed cabinet equipment, birds enter the system in their transport crates as one batch at a time. Once the birds have been loaded within the system the gas is then added in two (or more) phases into the cabinet and removed upon completion of the stunning cycle. The birds remain static within the system, offering a greater level of control and uniformity over the gas killing process than both tunnels and pits.

The most commonly used equipment in EU involves tunnels, followed by closed cabinets and pits (Figure 1).

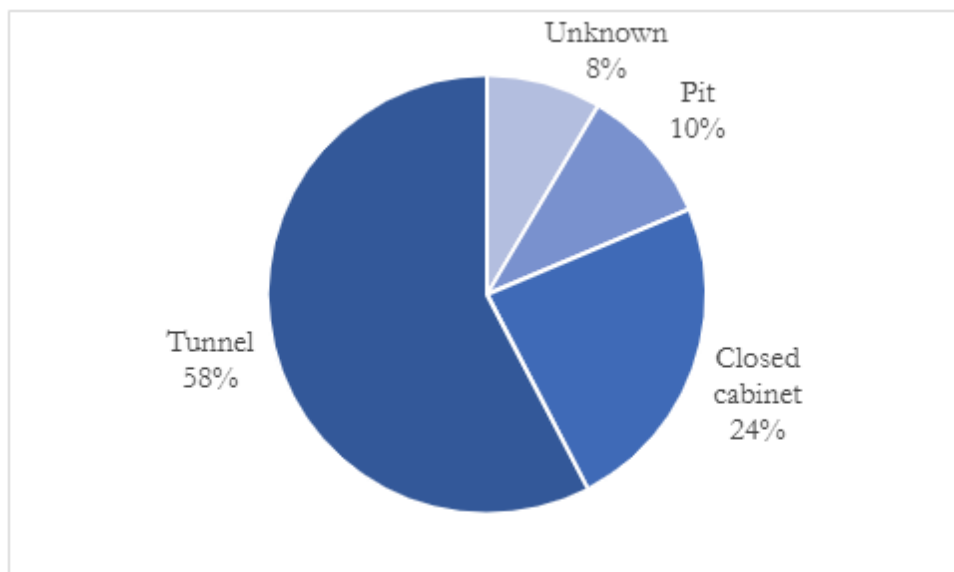


Figure 1. Percentage of the different CO<sub>2</sub> stunning equipment that are currently in use in EU. Data extracted from 20 out of 27 European Member States that replied a EURCAW-Poultry-SFA survey in 2021.

The CO<sub>2</sub> used for stunning is not produced at the processing plant but obtained from the industry (Raj *et al.*, 2006). Regardless of the equipment design, either if the gas is pre-filled in the system or introduced once the animals are there, the gas is added in two (or more) phases. Nevertheless, these two phases can be divided into more stages in order to gradually increase the CO<sub>2</sub> concentration with each stage, but always ensuring the loss of consciousness before increasing the concentration of CO<sub>2</sub>

above 40%. Once the process is completed, the CO<sub>2</sub> is fully evacuated (in closet cabinets) or the animals reach a part of the tunnel or pit without CO<sub>2</sub>. The birds are unloaded, and the process continues with the unconscious or dead birds being shackled and killed by neck cutting (Gerritzen *et al.*, 2013).

Tunnel equipment has windows positioned along sides allowing to assess animal welfare based on bird behaviour during induction to unconsciousness. On the other hand, observation of animals in pits are through cameras whereas in closed cabinets, animals can be monitored throughout a window positioned at the rear of the system. Furthermore, all of them have gas sensors to monitor gas gradient throughout stunning phases.

### 3.3. Positive welfare aspects of the method/equipment

Stunning broilers using CO<sub>2</sub> in two phases render the bird unconscious when using a low level of CO<sub>2</sub> (< 40%) followed by a second phase where birds are killed painlessly with exposure to a lethal CO<sub>2</sub> concentration.

### 3.4. Negative welfare aspects of the method/equipment

Concentrations of CO<sub>2</sub> at above 30% are detected by intrapulmonary chemoreceptors that are acutely sensitive to CO<sub>2</sub> and cause unpleasant sensation to broilers when inhaled (Webster and Fletcher, 2004; Raj *et al.*, 2006). However, most broiler chickens seem to tolerate concentrations up to 40% CO<sub>2</sub> (EFSA, 2004). Broilers that inhale 40% CO<sub>2</sub> show withdrawal reactions from the gas but at above 40% cause painful stimulation of the nasal mucosa and aversive reactions (McKeegan *et al.* 2006; Gerritzen *et al.*, 2007). In this sense, if the time of exposure during the first phase (<40%) is not enough to induce unconsciousness, the birds will be conscious and experience aversion when CO<sub>2</sub> concentrations are increased in the second phase. If time of exposure is too short, and birds do not die and/or CO<sub>2</sub> concentrations are too low to induce unconsciousness (EFSA, 2019), birds might be conscious during shackling and bleeding. In addition, effectiveness of stunning can be improved avoiding bunching or overcrowding of the birds exposed to the gas mixture.

The CAS system has usually windows, fixed or mobile video cameras, allowing for assessment of the behaviour of the birds when exposed to CO<sub>2</sub>, which is of particular importance during the first phase where birds are still conscious, and the induction of unconsciousness should be as smooth as possible. However, the behaviour assessment through windows or cameras does not allow a clear view of all animals so the personnel in charge of assessing cannot ensure that all animals are unconscious when approaching the second phase of the stunning procedure.

Other considerations are related to the system design. A negative welfare aspect occurs in systems where uncrated birds enter the system using a conveyor belt (instead of in their transport crates). Conscious birds are tipped out of the transport crates onto the conveyor belt and during this process, birds fall on top of each other, causing stress and injuries, especially for the last birds that still remain in the crate and are removed often by mechanical shaking of the containers (OIE, 2011; EFSA, 2019).

Moreover, negative welfare aspects might occur linked to the physical properties of CO<sub>2</sub>. This gas becomes liquid with freezing temperatures when compressed into a container and readily vaporise when it is released into atmospheric air. Thus, the low temperature of the gas is equilibrated to environmental temperature during expansion by absorbing heat from the environment. In this sense, if the administration is rapid, it may freeze the regulators and associated plumbing, and if this happens during stunning, it may interrupt the supply of gas. Additionally, the cold gas could be potentially

painful during breathing and induce hypothermia in conscious birds (Raj *et al.*, 2006). To avoid this, CO<sub>2</sub> should be heated to ambient temperature before entering in contact with birds.

## 4. Inert gases

Chickens are induced to unconsciousness with inert gases maintained within the system at a level that reduces the available oxygen to below 2% by volume. Inert gases displace oxygen from the inhaled atmospheric air and this ensures that the birds are stunned by anoxia (*i.e.*, lack of oxygen). Simple stunning occurs when the process is prolonged enough (EFSA, 2019).

### 4.2. Available equipment

The method consists in direct exposure of conscious animals to an inert gas. The most frequently used gas is nitrogen whereas others like argon, xenon and krypton are avoided in commercial slaughterhouses due to their prohibitive price (Raj and Tserveni-Gousi, 2000; Landymore, personal communication).

Birds are stunned in their transport crates in tunnels. Crates enter continuously at one end of the system, and while they are conveyed to the opposite end, birds are exposed to inert gases. However, in commercial slaughterhouses stunning with inert gases was found to be too expensive and, currently, only systems using carbon dioxide associated with inert gases are used (see section 5). Available equipment allows animal welfare assessment based on the observation of bird behaviour during induction to unconsciousness (*i.e.*, windows, fixed or mobile video cameras). Furthermore, it has gas sensors to monitor oxygen gradient.

### 4.3. Positive welfare aspects of the method/equipment

Inert gases are colourless, odourless and tasteless. Thus, inhalation of inert gases does not cause aversive reactions after initial exposure, as they are imperceptible to birds (Raj, 1996; Webster and Fletcher, 2004). In addition, the reduction of available oxygen is not perceived either (Velarde and Raj, 2016). When broilers enter in a chamber filled with inert gases their behaviour does not differ from when they were breathing regular air (Webster and Fletcher, 2004), they do not withdraw (McKeegan *et al.*, 2006) and they barely show behavioural signs of distress (Wooley and Gentle, 1988; Gent *et al.*, 2000).

### 4.4. Negative welfare aspects of the method/equipment

After loss of consciousness, birds can perform severe convulsion (Webster and Fletcher, 2004; Gent *et al.*, 2020), which may produce wing fractures as well as injuries and distress to other birds that have not yet lost consciousness (McKeegan *et al.*, 2007; Berg and Raj, 2015). In some cases, it is not entirely clear whether the observed convulsion are reflexive reactions occurring after the bird loses consciousness or whether the birds are still conscious (McKeegan *et al.*, 2007; Coenen *et al.*, 2009; Shields and Raj, 2010). Moreover, the duration of these behaviours is longer than when stunning with CO<sub>2</sub> in two phases but with a lower severity (Gent *et al.*, 2020).



The exposure time to lose consciousness with inert gases is longer than with CO<sub>2</sub>. If birds are not exposed enough time to this modified atmosphere, birds can recover consciousness rapidly if breathing atmospheric air at the exit of the stunning system (Velarde and Raj, 2016). For this reason, depending on the residual oxygen concentration and the duration of exposure, stunning can be either reversible or irreversible (see Regulation 1099/2009).

## 5. Carbon dioxide associated with inert gases

Birds are induced to unconsciousness by direct or progressive exposure to a gas mixture containing carbon dioxide (<40%) associated with inert gases. CO<sub>2</sub> concentration is sufficient to cause unconsciousness to chickens, but the addition of inert gases displace oxygen from the inhaled atmospheric air (Poole and Fletcher, 1995; Hoen and Lankhaar, 1999; EFSA, 2004; Webster and Fletcher, 2004) and contributes to ensuring that the birds are not only stunned but killed by hypercapnic anoxia at the end of the process if it is prolonged enough (EFSA, 2019).

### 5.2. Available equipment

Birds are stunned in their transport containers in tunnels. Crates enter continuously at one end of the system, and while they are conveyed to the opposite end, birds are exposed to the modified atmosphere. Although this CAS method is not yet used in the EU, some slaughterhouses in the UK do. However, they use nitrogen in combination with CO<sub>2</sub> due to the higher cost when using argon or other inert gasses. Available equipment allows animal welfare assessment based on the observation of bird behaviour during induction to unconsciousness through windows positioned along sides. Furthermore, it has gas sensors to monitor the different gas gradients.

### 5.3. Positive welfare aspects of the method/equipment

Stunning with CO<sub>2</sub> associated to inert gases seems to be the best stunning method from a welfare point of view since broilers tolerate the inhalation of concentrations below 40% CO<sub>2</sub> (EFSA, 2004), inert gases are imperceptible to birds (Raj, 1996; Webster and Fletcher, 2004) and the reduction of available oxygen is not perceived either (Velarde and Raj, 2016).

On the other hand, the occurrence of vigorous convulsions expressed as wing flapping in unconscious birds are less pronounced than when using only inert gases (McKeegan *et al.*, 2007; Coenen *et al.*, 2009) and thus, the likelihood of unconscious animal while convulsing to harm neighbouring birds that are still conscious is lowered.

### 5.4. Negative welfare aspects of the method/equipment

Welfare aspects related to the equipment are associated to the physical properties of CO<sub>2</sub> (as in carbon dioxide stunning in two phases, see subsection 3.3) and inert gases (see subsection 4.4) and the rate of induction, depth and duration of unconsciousness, which depend on both CO<sub>2</sub> and inert gas concentration and exposure time. Therefore, both should be controlled during the exposure to minimise negative effects on the welfare of the birds (EFSA, 2019).

On the other hand, if there is too low concentration of gas or the exposure time in the applied atmosphere is too short, induction of unconsciousness in all birds may not be accomplished or unconscious birds may recover consciousness before or during bleeding (EFSA, 2019).

Equipment has windows and fixed or mobile video cameras to assess bird's behaviour when exposed to gases. However, it does not allow a clear view of all animals.

## 6. Low atmospheric pressure stunning (LAPS)

Broilers are induced to a non-recovery state of consciousness through progressive hypobaric anoxia (*i.e.*, lack of oxygen due to lowered atmospheric pressure; Mackie and McKeegan, 2016; Martin *et al.*, 2016a, b, c).

### 6.2. Available equipment

Broilers enter the system in their transport crates into a stunning chamber where air is gradually removed by using a vacuum pump to reduce oxygen tension in the atmosphere. It is intended to achieve a gradual decompression with reduction in available oxygen to less than 5%. Currently, this method is only approved for the stunning of broilers of less than 4 kg of body weight according to a prescribed pressure curve carefully described in the implementing regulation. During the first phase, the decompression rate must not be greater than equivalent to a reduction in pressure from 101 kPa to 33 kPa for a period of not less than 50 s. During a second phase, a minimum of 21 kPa is reached within the following 210 s. Further, the pressure time curve must be adjusted to ensure that all birds are irreversibly stunned within the cycle time. It is recommended that LAPS should be conducted in darkness, as the presence of light reduced the time to reach unconsciousness (Martin *et al.*, 2016b).

### 6.3. Positive welfare aspects of the method/equipment

According to the scientific opinion of EFSA (2017), the LAPS stunning method is at least equivalent, in terms of animal welfare outcomes, to other currently available stunning methods. Compared to WBS, LAPS-stunned birds had lower blood corticosterone concentration, which indicates a lower stress level (Vizzier-Thaxton *et al.*, 2010). In addition, broilers enter the system in their transport crates. Thus, broilers are never tipped or dumped on conveyors and therefore, the hazards of being injured and bunched on conveyor do not occur. Moreover, LAPS produces a non-recovery state of consciousness and thus, it does not compromise the welfare of the bird during the following slaughtering procedures.

### 6.4. Negative welfare aspects of the method/equipment

EFSA (2017) revised the neurological events of LAPS based on EEG quantitative analysis (illustrated in Figure 3). EFSA pointed out that at 40 s after start of the LAPS process, broilers seem to progressively fall into a state of drowsiness possibly induced by low availability of oxygen and, to a lesser extent, the darkness in the chamber. However, at 50 s of the LAPS process there is an awaking of the birds and possible explanation revolved around the occurrence of dryness of the mucosae due to rapidly decreasing humidity in the chamber, the expansion of gases in the organ cavities and life-threatening oxygen levels. It is not until 72 s of the LAPS process that birds are in a state of unconsciousness. EEG in combination with behaviour assessment highlighted that there is a span of time of 20 s (between 51

and 72 s of LAPS process) that birds are experiencing behaviours of aversion caused by the rapid loss of atmospheric pressure, and changes in partial pressure in oxygen, humidity and gas tension condition.

However, this sequence of events in time are the calculated average among all individuals tested. It should be noticed that actually, a wide range exist. In fact, aversion have been observed from the span of 1 to 157 s, unconsciousness from 41 to 147 s and death from 86 to 260 s of the LAPS cycle. In this sense, this heterogeneity among individuals represents a matter of welfare concern.

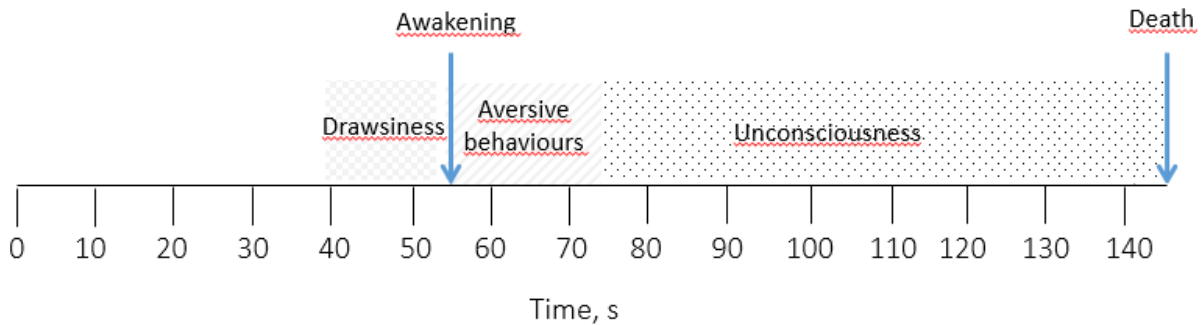


Figure 3. Graphical representation of the main welfare events in one LAPS cycle combining different sources of information according to EFSA (2017).

In addition, there are some hazards identified in LAPS (EFSA, 2019). If decompression is too fast, birds may experience pain and distress due to expansion of gas trapped in body cavities. Defecation in all birds were observed during LAPS that never occurred systematically in other CAS methods suggesting expansion of gas trapped in gut and probably also in other body cavities (EFSA, 2017). In addition, Gent *et al.* (2020) pointed out that LAPS exposed birds bled from the head (haemorrhagic changes seen in the calvarium coming from haemorrhage in the underlying trabeculae) and showed prolapses of cloaca likely related to the rate of decompression/recompression. In 2020, Martin *et al.* assessed bird's organ integrity post-mortem after LAPS and they stated that organs were presumably not affected, but this does not fully guarantee that the welfare of the bird is not compromised, since pain may happen even if the absence of visible lesions post-mortem. Despite of this, the LAPS method is not considered to be worst on a welfare ground to other currently available stunning methods.

In addition, the time to onset of unconsciousness and death is variable and depends on the genotype, weight and age of the bird. Thus, heavier birds (*e.g.*, broiler breeders) might need longer periods of decompression for respiration to cease (EFSA, 2017). Signs of aversive behaviours before onset of unconsciousness are observed (EFSA, 2017). Aversive behaviours can be minimized through speed of pressure reduction, which is achievable through precision in the controls of the equipment (Purswell *et al.*, 2007).

## 7. Conclusions

- WBS is still the most commonly commercially used stunning method for poultry in the EU. However, CAS systems are gaining ground, especially at large-scale slaughterhouses.
- The authorised CAS methods in the EU are carbon dioxide in two phases, inert gases, carbon dioxide associated with inert gases and low atmosphere pressure. However, among these CAS methods only carbon dioxide in two phases seems to be currently in use in EU and is estimated to stun almost half of the broilers slaughtered.

- CAS systems provides some welfare benefits over WBS, such as reduced or no manual handling of conscious birds, avoiding the need to invert and shackle live birds by their legs. CAS also prevents uneven stunning of birds differing in size and unintended pre-stunning electrical shocks further present a risk of impaired welfare that may happen in a WBS. In most cases, birds are dead when exit from CAS and there is no risk from recovery.
- Stunning with carbon dioxide in two phases consists in exposing birds to a controlled, gradually increasing concentration of carbon dioxide that results in a smooth transition to unconsciousness and is strongly encouraged over WBS.
- Unlike carbon dioxide, inert gases and carbon dioxide associated to inert gases have been reported to be from low to non-aversive to poultry and can therefore offer a less aversive induction to unconsciousness.
- Stunning with carbon dioxide associated to inert gases seems to be the best stunning method from a welfare point of view since inert gases are not aversive to birds and carbon dioxide has narcotic effect when inhaled at concentrations used.
- LAPS is the latest developed stunning method, and it produces a non-recovery state of birds and thus, it does not compromise the welfare of the bird during the following slaughtering procedures. However, the time to onset of unconsciousness and death is variable and defecation in all birds were observed during LAPS that never occurred systematically in other CAS methods suggesting expansion of gas trapped in gut and maybe also in other body cavities. Nevertheless, the LAPS method can be considered not worst on a welfare ground to other currently available stunning methods.

## 8. References

- Bedanova I, Voslarova E, Chloupek P, Pistekova V, Suchy P, Blahova J, Dobsikova R and Vecerek V. 2007. Stress in broilers resulting from shackling. *Poult. Sci.*, 86:1065–1069.
- Berg C and Raj M. 2015. A review of different stunning methods for poultry — Animal welfare aspects (stunning methods for poultry). *Animals*. 5(4):1207–1219.
- Butterworth A. 1999. Infectious components of broiler lameness: a review. *World's Poult. Sci. J.* 55(4):327–352.
- Coenen AM, Lankhaar J, Lowe JC and McKeegan DE. 2009. Remote monitoring of electroencephalogram, electrocardiogram, and behavior during controlled atmosphere stunning in broilers: implications for welfare. *Poult Sci.*, 88(1):10–9.
- European Union. 2009. Council Regulation (EC) No 1099/2009 of 24 September 2009 on the protection of animals at the time of killing. Off. J. Eur. Union, L303, pp. 1–30.
- European Commission. 2013. Report from the Commission to the European parliament and the Council on the various stunning methods for poultry. Retrieved from the European Commission website: [https://ec.europa.eu/food/system/files/2016-10/aw\\_practice\\_slaughter\\_com\\_2013\\_915\\_report\\_en.pdf](https://ec.europa.eu/food/system/files/2016-10/aw_practice_slaughter_com_2013_915_report_en.pdf)
- EFSA (European Food Safety Authority). 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. *EFSA Journal*, 45:1–29.
- EFSA (European Food Safety Authority). 2013. Scientific Opinion on monitoring procedures at slaughterhouses for poultry. *EFSA Journal*, 11(12):3521.

- EFSA (European Food Safety Authority). 2017. Scientific Opinion on the low atmospheric pressure system for stunning broiler chickens. *EFSA Journal*,15(12):5056.
- EFSA (European Food Safety Authority). 2019. Poultry welfare at slaughter: hazards identified, measures proposed. *EFSA Journal*, 17(11):5849.
- Gent TC, Gebhardt-Henrich S, Schild SA, Rahman AA and Toscano MJ. 2020. Evaluation of poultry stunning with low atmospheric pressure, carbon dioxide or nitrogen using a single aversion testing paradigm. *Animals (Basel)*. 10(8):1308.
- Gentle MJ and Tilston VL. 2000. Nociceptors in the legs of poultry: implications for potential pain in preslaughter shackling. *Anim. Welf.*, 9:227–236.
- Gerritzen MA, Lambooi B, Reimert H, Stegeman A and Spruijt B. 2007. A note on behaviour of poultry exposed to increasing carbon dioxide concentrations. *Appl. Anim. Behav. Sci.*, 108:179–185.
- Gerritzen MA, Reimert HG, Hindle VA, Verhoeven MT, Veerkamp WB. 2013. Multistage carbon dioxide gas stunning of broilers. *Poult Sci.*, 92(1):41–50.
- Hoehn T and Lankhaar J. 1999. Controlled atmosphere stunning of poultry. *Poult Sci.*, 78:287–289.
- HSA (Humane Slaughterhouse Association). 2015. Electrical waterbath stunning of poultry. Wheathampstead, UK. 104 pp. Available from: <https://www.hsa.org.uk/downloads/hsagn7electricalwaterbathpoultry1.pdf>
- Mackie N and McKeegan D. 2016. Behavioural responses of broiler chickens during low atmospheric pressure stunning. *Appl Anim Behav Sci.*, 174:90–98.
- Martin JE, Christiansen K, Vizzier-Thaxton Y, McKeegan DEF. 2016a. Effects of analgesic intervention on behavioural responses to low atmospheric pressure stunning. *Appl. Anim. Behav. Sci.*, 180:157-165
- Martin JE, Christensen K, Vizzier-Thaxton Y, McKeegan DEF. 2016b. Effects of light on responses to low atmospheric pressure stunning in broilers. *Br Poult Sci.*, 57(5):585-600.
- Martin JE, Christensen K, Vizzier-Thaxton Y, Mitchell MA, McKeegan DEF. 2016c. Behavioural, brain and cardiac responses to hypobaric hypoxia in broiler chickens. *Physiol Behav.*, 163:25-36.
- Martin JE, McKeegan DEF, Magee DL, Armour N, Pritchard DG. 2020. Pathological consequences of low atmospheric pressure stunning in broiler chickens. *Animal*, 14(1):129-137.
- McKeegan DEF, McIntyre J, Demmers TGM, Wathes CM and Jones RB. 2006. Behavioural responses of broiler chickens during acute exposure to gaseous stimulation. *Appl Anim Behav Sci.*, 99:271–286.
- McKeegan DE, Abeyesinghe SM, McLeman MA, Lowe JC, Demmers TG, White RP, Kranen RW, van Bommel H, Lankhaar JA and Wathes CM. 2007. Controlled atmosphere stunning of broiler chickens. II. Effects on behaviour, physiology and meat quality in a commercial processing plant. *Br Poult Sci.*, 48(4):430–42.
- OIE (World Organisation for Animal Health). 2011. Terrestrial animal health code. Available from: <https://www.oie.int/doc/ged/D10905.PDF>
- Poole GH and Fletcher DL. 1995. A comparison of argon, carbon dioxide, and nitrogen in a broiler killing system. *Poult Sci.*, 74(7):1218–23.

- Raj M. 1996. Aversive reactions of turkeys to argon, carbon dioxide and a mixture of carbon dioxide and argon. *Vet Rec.*, 138:592–593.
- Raj M and Tserveni-Gousi A. 2000. Stunning methods for poultry. *Worlds Poult. Sci. J.*, 56(4):291–304.
- Raj AB, Sandilands V and Sparks NH. 2006. Review of gaseous methods of killing poultry on-farm for disease control purposes. *Vet Rec.*, 159(8):229–35.
- Shields SJ and Raj M. 2010. A critical review of electrical water-bath stun systems for poultry slaughter and recent developments in alternative technologies. *J. Appl. Anim. Welf. Sci.*, 13(4):281–299.
- Velarde A and Raj M. 2016. *Animal welfare at slaughter*. 1st ed. Sheffield: 5m Publishing.
- Verhoeven MTW, Gerritzen MA, Hellebrekers LJ and Kemp, B. 2015. Indicators used in livestock to assess unconsciousness after stunning: a review. *Animal*, 9(2):320–330.
- Vizzier-Thaxton Y, Christensen K, Schilling M, Buhr RJ and Thaxton J. 2010. A new humane method of stunning broilers using low atmospheric pressure. *J. Appl. Poult. Res.*, 19, 341-348.
- Webster AB and Fletcher DI. 2004. Assessment of the aversion of hens to different gas atmospheres using an approach-avoidance test. *Appl. Anim. Behav. Sci.*, 88:275–287.
- Wooley SC and Gentle MJ. 1988. Physiological and behavioural responses of the domestic hen to hypoxia. *Res. Vet. Sci.*, 45:377–382.