



How is the cardiac activity of broiler chickens affected by two phase carbon dioxide stunning during commercial slaughter?

My Svärd

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Swedish University of Agricultural Sciences, SLU
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How is the cardiac activity of broiler chickens affected by two phase carbon dioxide stunning during commercial slaughter?

Hur påverkas hjärtats aktivitet hos slaktkycklingar vid bedövning med koldioxid vid kommersiell slakt?

My Svärd

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Abstract

According to the Swedish Animal Welfare Act (SFS 2018:1192) all animals slaughtered in Sweden must be stunned prior to exsanguination. This means that the animal is rendered unconscious before it is killed. A slightly majority of the broiler chickens in Sweden are stunned through carbon dioxide gas stunning (Svensk Fågel 2024a). The main objective of this study was to examine if the broilers die during the stunning process, i.e. enter cardiac arrest. The study was performed at a commercial slaughterhouse during five days. In total 404 CO₂-stunned broilers were auscultated with an electronic stethoscope in order to detect any heartbeats. Two conscious birds were sent through the gas stunning machine with a pulse sensor attached, to determine when the broiler enter cardiac arrest during stunning. The study showed that none of the 404 auscultated birds had any detectable heartbeats. The result from the pulse sensor was difficult to interpret and it was not possible to determine at what time during stunning the broiler developed cardiac arrest. Since only a few of the 200 000 slaughtered broilers per day were examined in this study, and conscious birds showed up at the shackling point, it is difficult to draw general conclusions based on this study, although the results may indicate a high proportion of dead birds. Further research is required to with a greater accuracy determine the extent to which birds die in the CO₂-stunning system prior to exsanguination. The results from this study can be used to improve the understanding of how the CO₂-stunning affects the broilers' physiology. It also has practical implications for the slaughter industry in terms of regulations of stun-to-stick intervals, but also animal welfare.

Keywords: *Gallus gallus*, stunning, auscultation, humane slaughter, broiler, abattoir, heartbeat

Sammanfattning

Enligt den svenska djurskyddslagen (SFS 2018:1192) måste alla djur som slaktas i Sverige vara bedövade innan avblodning. Detta betyder att djuret har gjorts medvetslöst innan det avlivats. En knapp majoritet av alla slaktkycklingar i Sverige bedövas genom koldioxidbedövning (Svensk Fågel 2024a). Syftet med denna studie var att undersöka om slaktkycklingarna dör under bedövningsprocessen, d.v.s. om de får hjärtstopp. Studien utfördes på ett kommersiellt slakteri under fem dagar. Totalt auskultades 404 koldioxidbedövade slaktkycklingar med ett elektroniskt stetoskop för att upptäcka hjärtljud. Två medvetna, obedövade fåglar skickades genom gasbedövningen med en pulssensor på sig, för att avgöra när under bedövningsprocessen som hjärtstopp inträffar. Studien visade att ingen av de 404 undersökta fåglarna hade några detekterbara hjärtljud. Resultatet från pulssensorn var svårt att tolka och det var inte möjligt att fastställa vid vilken tidpunkt under bedövningen som slaktkycklingarna fick hjärtstopp. Eftersom endast några få av de 200 000 dagligen slaktade kycklingarna undersöktes i denna studie, samt att medvetna fåglar dök upp vid hängbordet, är det svårt att dra några generella slutsatser baserat på resultat från denna studie. Dock kan resultaten indikera på en hög andel döda fåglar. Det behövs vidare forskning inom ämnet för att med större säkerhet kunna fastställa hur många slaktkycklingar det är som dör i koldioxidbedövningen innan avblodning. Resultatet från denna studie kan användas för att förbättra förståelsen för hur koldioxidbedövning påverkar slaktkycklingarnas fysiologi. Det har också praktiska konsekvenser för slaktindustrin när det gäller regleringar av sticktid, men även för djurens välfärd.

Nyckelord: *Gallus gallus*, bedövning, auskultation, human slakt, broiler, slakteri, hjärtslag

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Glossary

Bleeding	The process when central arteries are cut to induce death of an animal through blood loss.
Bird/broiler	A 28-35 days old <i>Gallus gallus domesticus</i> bred for slaughter.
Cardiac arrest	When the heart suddenly stops pumping blood around the body.
Dead on arrival (DOA)	A broiler that has died during transport to the slaughterhouse.
Large bird	A broiler from final removal of the flock for slaughter. Is around 35 days old.
Slaughter	The killing of an animal designated for human consumption.
Small bird	A broiler from thinning, around 28 days old.
Stunning	Process of rendering an animal unconscious and insensible before bleeding.
Thinning	When a part of the birds in the flock are sent to the slaughterhouse before the end of the growing cycle.

1. Introduction

In 2024 approximately 110 million broiler chickens were slaughtered in Sweden (SJV 2025). According to the Swedish Animal Welfare Act (SFS 2018:1192) all animals slaughtered in Sweden must be stunned, i.e. rendered unconscious and unsensible, before exsanguination. This is crucial to minimise the risk of exposing animals to unnecessary suffering (EFSA 2004). Permitted stunning methods for poultry are, according to the Board of Agriculture's regulations and standard procedures for the slaughter and euthanasia of animals (SJVFS 2019:8, L22), captive bolts, free bullet rifle, blow to the head, electricity and group stunning with carbon dioxide (CO₂) gas. Electricity (water-bath stunners or dry head-only stunning) or CO₂-group stunning are usually used in commercial slaughterhouses.

Stunning can be either reversible or irreversible. A reversible stunning method leads to a temporary unconsciousness, while an irreversible stunning method leads to unconsciousness and death (Fuseini 2019). Gas stunning can constitute irreversible as well as reversible stunning, depending on the gas type, exposure time and gas concentration (EFSA 2013). However, at slaughter all stunned animals must be exsanguinated to ensure death. Death is confirmed when both brain and cardiac activity has ceased (EFSA 2004; SCAW 2017).

Studies have shown that broilers exposed to CO₂ at a high enough concentration during long enough time will be permanently dead. Gerritzen and co-workers (2013) exposed broiler chickens during four to six minutes to an increasing amount of CO₂, from 20% to 65%, which, according to the results, led to death in all animals. Raj and Gregory (1990) exposed broiler and hens to 55% CO₂ in air, which resulted in the death of all birds within two minutes. However, there is currently a gap in the literature concerning studies whether the CO₂ stunning method leads to cardiac arrest in commercial slaughter.

Against this background, the main objective of this study was to find out if the broilers die during gas stunning at a commercial slaughterhouse with given settings for CO₂ concentration and exposure time, i.e. if they are already dead (defined by cardiac arrest) before exsanguination. The results from this study can help food business operators determine a suitable stun-to-stick interval for CO₂ stunned broilers under certain conditions.

In this study, it was hypothesized that:

1. The exposure time in combination with the high CO₂ concentration in the stunning system will kill the majority of the birds.

2. The methods of measuring cardiac activity would result in no detected heart beats in a stunned bird, in the majority of the studied animals.

2. Literature review

2.1 The slaughter process of broilers

When the broiler chickens are approximately four to five weeks old, they are sent for slaughter. They are collected at the farm, placed in transport modules and transported to the slaughterhouse (Svensk Fågel 2024b). Before slaughter a veterinarian from the Swedish Food Agency must inspect the animals, with reference to food safety and animal welfare. For poultry, the inspection can be limited to a representative group of birds from each flock (Swedish Food Agency 2025). During slaughter the birds are stunned, i.e. rendered unconscious, before exsanguination. The next steps in the process are scalding, plucking and evisceration. The carcass is then inspected by veterinary assistants before chilling (Svensk Fågel 2024b).

2.1.1 Stunning methods

According to the Swedish Animal Welfare Act (SFS 2018:1192) all animals slaughtered in Sweden must be stunned before exsanguination. More than 50% of the broilers slaughtered in Sweden are stunned with gas (Svensk Fågel 2024a), but as a result of the structure of the industry, most medium-sized or large slaughterhouses still use electrical water-bath stunning, whereas the small slaughterhouses often use dry head-only electrical stunning (Länsstyrelserna 2023).

There are three main stunning methods used at slaughter of poultry: mechanical, electrical and gas/controlled atmospheres (CAS) (EFSA 2019). Mechanical stunning methods are captive bolt and percussive blow to the head. These methods are usually used as a back-up method if an animal has to be re-stunned or is injured and culled prior to the standard stunning intervention. Electrical stunning methods are water-bath, head-only and head-to body applications, but head-to-body is not used for slaughter in practice. In most parts of the world, water-bath stunning is the primary used stunning method for poultry. The birds are inverted and shackled in both legs on wet metal shackles, and the heads are put in the electrified water-bath. The current renders the animal unconscious by causing epileptiform activity in the bird's brain. A similar principle is used in head-only electrical stunning. The bird is shackled or manually restrained. Electrodes are placed on either side of the bird's head and the current cause generalised epilepsy in the brain and thereby unconsciousness is achieved. CAS is explained further below (*Gas stunning*).

Reversible and irreversible stunning

Stunning before slaughter can be reversible or irreversible, and both approaches are accepted as long as the animals remains unconscious until it dies from exsanguination. If the stunning method is reversible the animal only has a temporary loss of consciousness and will recover. The stun-to-stick interval is then extra important, in order to ensure that the animal does not regain consciousness (EFSA 2019). After successful irreversible stunning, the animal will not be able to regain consciousness. Which method is categorized as irreversible or reversible stunning depends on the species and the method. For poultry the following methods are, slightly simplified, categorized as reversible stunning methods:

- head-only electrical stunning,
- water-bath stunning (if the electrical frequency is above 50 Hz),
- carbon dioxide combined with inert gases (if exposure time of >30% CO₂ is < 3 min) and
- inert gases (if exposure time is < 3 min) (Gregory & Grandin 2007; EFSA 2019; EC 1099/2009).

The following methods are categorized as irreversible stunning:

- captive bolt stunning (according to a study from Raj & O'Callaghan (2001))
- water-bath stunning (if the electrical frequency is 50 Hz or below)
- low atmospheric pressure stunning (LAPS)
- carbon dioxide, carbon dioxide combined with inert gases and inert gases (depending on concentration and exposure time) (EC 1099/2009)

It should be noted that also when theoretically irreversible methods are applied, failure in terms of correct application of the method (incorrect localisation, concentration, duration, or other settings) may lead to birds regaining consciousness. Both reversible and irreversible stunning should be followed by a procedure ensuring rapid death, such as the cutting of both carotids or the application of electrocution (EC 1099/2009).

Gas stunning

There are several advantages related to using gas as a stunning method. The birds can remain in the transport modules, as they can be stunned in the modules. This reduces the risk of injury when emptying the modules and improves workers' conditions (Berg & Raj 2015). There is no need for human interaction with the live (or at least conscious) birds, as it is when using electrical stunning methods where the birds have to be shackled while conscious (EFSA 2004). This lowers the pre-slaughter stress for the birds (Gregory & Grandin 2007). Furthermore, gas

stunning tolerates a bigger variation in bird size than water-bath stunning (Berg & Raj 2015).

There are different kinds of gas mixtures that can be used for stunning. According to the EU Council Regulation (EC) No 1099/2009 the following gas combinations can be used at slaughter of broilers: (i) carbon dioxide in two phases, (ii) carbon dioxide combined with inert gases such as argon or nitrogen, (iii) inert gases alone or (iv) low atmospheric pressure stunning (LAPS). The most common gas combination in EU is carbon dioxide in two phases (FCEC 2012). In Sweden the regulations are stricter, and carbon dioxide is currently the only gas used for stunning broilers (SJVFS 2019:8, L22).

When using the two-phase carbon dioxide stunning method, the conscious birds are gradually exposed to a gas mixture containing up to 40% CO₂ during the first phase (EC 1099/2009; Gerritzen *et al.* 2013). The high levels of CO₂ (and decreasing amount of O₂) makes the birds unconscious. In the second phase, the CO₂ concentration increase up to 80% and the birds fall into a deeper and more long-lasting state of unconsciousness. The high levels of CO₂ inhaled causes respiratory acidosis (Sjaastad *et al.* 2016). pCO₂ in the blood is increased and the pH falls. Chemoreceptors detect changes in the pCO₂ and sends signals to the respiratory centre in the *medulla oblongata* and the *pons*. As a response to the high levels of CO₂, the bird starts breathing faster and more deeply. In a bird exposed to the open air, this response would restore the acid base balance in the blood. In a bird located in a constant CO₂ rich environment this adjustment is not possible.

CO₂ is an aversive gas for many animals, including humans, according to several studies. Mice and rats show aversion above 25% CO₂ (Leach *et al.* 2002), humans find 50% CO₂ "highly unpleasant" (Danneman *et al.* 1997) and turkeys avoid feeding chambers containing 72% CO₂ (Raj 1996). Studies on broilers and laying hens have shown reactions from the birds at different levels of CO₂ in air. Therefore, it's not allowed to expose conscious birds to more than 40% CO₂ during the initial phase of the stunning. When animals have lost consciousness, the gas concentration can be increased (EC 1099/2009).

Indicators of a satisfactory stunning

The EFSA Panel on Animal Health and Welfare (AHAW) has published a *Scientific Opinion on monitoring procedures at slaughterhouses for poultry* (2013), where they have listed definitions of the indicators of consciousness (i.e. an unwanted outcome) in poultry after gas stunning. A selection of these indicators are listed in Appendix 1.

2.1.2 Legalisations and regulations during slaughter

General regulations about animal protection during slaughter

According to article 3(1) in EC 1099/2009, animals “shall be spared any avoidable pain, distress or suffering during their killing and related operations”. Article 4 in the same regulation states that animals should be stunned before killing and that unconsciousness and insensibility must be maintained until the death of the animal. The exception is if the animal is a part of religious slaughter at a slaughterhouse (EC 1099/2009). In Sweden all animals must be stunned before exsanguination (SFS 2018:1192), and there is hence no exception made for religious slaughter. The business operator of the slaughterhouse must perform regular checks to ensure that the stunned animal does not show any signs of consciousness or sensibility in the period between stunning and death. If the animal shows signs of not being correctly stunned, the staff must take appropriate measures according to their *standard operating procedures* (EC 1099/2009).

The definition of a standard operating procedure (SOP) is “a set of written instructions aimed at achieving uniformity of the performance of a specific function or standard” (EC 1099/2009 art. 2(i)). The SOPs are mandatory and should ensure that the animals are protected from avoidable pain and suffer during killing and related operations. For each process and each stunning method used at the slaughterhouse, there should be SOPs defined. These can cover for example, minimum exposure time in gas, and details on gas concentration, gas temperature and stun-to-stick interval. The SOPs can differ between different food business operators (FBOs) and equipment types, but they must be based on available scientific evidence (EC 1099/2009).

Animal welfare officer

Every poultry slaughterhouse slaughtering more than 150,000 birds annually must have an assigned animal welfare officer, employed by the FBO (EC 1099/2009). The animal welfare officer works close to the business operators and assist them in ensuring compliance with regulation EC 1099/2009 and is also the contact person for the competent animal welfare authorities. He or she must have a certificate of competence issued for the operations he or she is responsible for, and the responsibilities have to be defined in the SOPs. Any action that the animal welfare officer has taken to improve animal welfare at the slaughterhouse, shall be documented and available upon request.

Slaughter for private consumption

Regarding slaughter of poultry for private domestic consumption, the regulations in EC 1099/2009 does not apply (EC 1099/2009, art 1(3b)). Instead, it is up to

each country to cover it by its national legalisation (European Commission n.d.). In Sweden, slaughter for private consumption is covered by SJVFS 2019:8, L22. According to this regulation, the person handling and/or stunning the poultry must have knowledge in animal protection and the stunning method he or she uses and know the relevant legalisation. SJVFS 2019:8 states that permitted stunning methods for broilers in Sweden are captive bolt gun, free bullet rifle, blow to the head, electricity and carbon dioxide. If any of the first two mentioned stunning methods are used, exsanguination must begin within 60 seconds after stunning. Please note that this time limit formally only applies to slaughter for private consumption. At slaughterhouses the maximum stun-stick interval is determined by the SOPs and must be accepted by the competent authorities.

The SJVFS 2019:8, L22 also states that poultry below 1 kg body weight, also can be euthanised by decapitation (must be rendered unconscious before this method is applied), cervical dislocation (must be made unconscious first, if they are heavier than 250g), electricity or by a blow to the head. In practice this does not relate to the regular slaughter situation. Although, a blow to the head can be used as a back-up method at commercial slaughter.

2.2 Definition of clinical death

Death is biologically univocal and irreversible, claims Huang and Bernat (2019). This means that it happens to all biological organisms, no matter if it is a mouse or a human, and once someone is dead, it will not return to life. But what is the scientific definition of death? Bernat (1998) defines death as “the permanent cessation of critical functions of the organism as a whole”, where spontaneous breathing and autonomic control of circulation, consciousness and a functioning homeostasis are the three categories of *critical functions*. An organism cannot be pronounced dead before it permanently loses the functions in all three categories.

The legal definition of death in humans may differ between countries, but according to the Swedish Act on criteria for determining human death (SFS 1987:269), a person is dead when they permanently have lost all their brain functions. The 2023 determination of death guideline in Canada has the same definition: death is the permanent cessation of brain function (Shemie *et al.* 2023). The guideline also clarifies what characterizes permanent loss of brain function. That is complete absence of consciousness and absence of brainstem reflexes, as well as inability to breath independently.

A definition of death in animals is presented in EFSA’s report *Welfare aspects of animal stunning and killing methods* (2004). They define death as a physiological state of an animal, where breathing and circulation is absent because of an

irreversible inactivity in the respiratory and circulatory centres in the *medulla oblongata*. The lack of nutrients and oxygen to the brain leads to an irreversible unconsciousness. In a statement from the Swedish Centre of Animal Welfare (SCAW) the author points out that the most straight forward definition of death in an animal during slaughter is irreversible cardiac arrest (SCAW 2017). The author adds that it is not an ideal definition since studies has showed that the heart can continue to beat for a while after the point when EEG-signals has ceased, i.e. when the animal is brain dead. Therefore, a practically applicable definition of death at slaughter, according to SCAW, is when a stunned animal has bled out, when permanent cardiac arrest is present and the animal has no blood circulation, no breathing and no brain functions.

In this study, the definition of death used will be based on EFSA's (2004), SCAW's (2017) and Bernat's (1998) definitions. An animal is dead when spontaneous breathing, circulation and consciousness is absent, in this study identified by permanent cardiac arrest.

2.2.1 Determination of death

Being able to determine the death of an animal during slaughter is very important for animal welfare. Before carcass processing, the bird should be confirmed dead. This should be made visually on all animals after exsanguination (SJVFS 2019:8, L22). If a bird would enter the scalding tank alive, it could experience pain and fear (EFSA 2019), which is not acceptable.

Terlouw and co-workers (2016) mention three points on how to verify absence of life in a slaughter environment. These three points are: absence of breathing, no brainstem reflexes and end of bleeding. If all points are confirmed the animal can be considered dead. Examples of brainstem reflexes are pupillary light reflex, corneal reflex and gagging (EFSA 2004). It is important to remember that spinal reflexes still can be present, even if the animal is brain dead. The mechanism is not fully understood, neither in animals (EFSA 2004) nor in humans (Nair-Collins 2022). According to Nair-Collins (2022), some scientists claim that there might be residual brainstem functions that allow these reflexes and movements, while others suggest that the movements solely originate from the spinal cord. This question is bigger in human medicine than in the slaughter context. Doctors need to have a way to determine if a person on life support is truly brain dead and when they can declare death. In a slaughter context, we want the animal to be dead before carcass processing commences. However, if the animal is not breathing, has no brainstem reflexes and has bled out, it can be considered dead even if minor muscular activity can be seen. There is no definition of death in Council

Regulation No. 1099/2009, which would be needed if we want a common definition of when death can be determined during slaughter.

As mentioned earlier, end of bleeding is one of the requirements when declaring absence of life in slaughter environment (Terlouw *et al.* 2016). Exsanguination is what formally kills the animal during slaughter, since stunning only makes the animal unconscious and rarely leads to immediate cardiac arrest, not even when irreversible methods are used (except for low frequency water-bath stunning). In broilers both carotid arteries are cut through neck sticking or the bird is decapitated (Gregory & Grandin 2007). Within 29 seconds after neck cutting, the bird has lost around 63% of all the blood that can leave the body through bleeding (Barnett *et al.* 2007). This is approximately 40% of a broiler's total blood volume. When a human loses 30-40% of their blood volume they develop hypotension, tachypnoea and tachycardia (Gutierrez *et al.* 2004). If the blood loss is not treated, loss of coronary perfusion pressure will lead to reduction of myocardial contractility, which then leads to decreased blood flow to the brain, coma and death.

An electroencephalogram (EEG) can be used to examine the brain activity. Gregory and Wotton (1986) studied the effect of slaughter on spontaneous and evoked activity in chickens' brains. When using decapitation as exsanguination method, the spontaneous activity in the brain has decreased by 95% within 32 seconds and the visual evoked activity in the brain has decreased by 95% within 136 seconds. The same study showed that the spontaneous activity in the brain had decreased by 95% within 60 seconds and the visual evoked activity has decreased by 95% within 163 seconds, if both carotid arteries were cut. If loss of evoked activity would be the definition of brain death, this means that an exsanguinated broiler dies two minutes after decapitation and approximately three minutes after bilateral cutting of the carotid arteries.

2.2.2 Irreversible stunning and death

Reversible stunning is when the animal only has a temporary loss of consciousness, while irreversible stunning is the opposite: the animal – if correctly stunned – remains unconscious and will not regain consciousness even if exsanguination is – theoretically – not carried out. Becerril-Herrera and co-workers (2009) explains that irreversible stunning methods cause both unconsciousness and death. The animals do not necessarily die immediately, but they are unable to regain consciousness and will eventually die (Fuseini 2019). When Vimini and co-workers (1983) studied heart activity in cattle stunned with captive bolt (irreversible stunning) and thereafter were not exsanguinated, the heart stopped eight to ten minutes after stunning. It should be noted that exsanguination is nevertheless a strict requirement, regardless of whether the stunning method is considered reversible or not, as one cannot guarantee complete irreversibility for all

individuals. Therefore, the animals in that study were decapitated 15 minutes after stunning, i.e. five to seven minutes after cardiac arrest. Correctly performed penetrative captive bolt stunning leads to severe and permanent damage to the brain but the heart can continue to beat since there is oxygen in the blood (Grandin 2015). Eventually, the heart runs out of oxygen since the brain damage has stopped breathing, and no new oxygen is supplied. The heart then stops and the animal is dead.

For reversible methods, the animals may regain consciousness, unless exsanguinated and possibly be completely unharmed. One example of a reversible method is low-frequency (50 Hz) head-only electrical stunning and high frequency (> 300 Hz) head-to-body electrical stunning. According to a study from Beausoleil *et al.* (2024), an animal that has been stunned using head-only (50 Hz) or head-to-body (two seconds head-only, followed by 2,000 Hz head-to-body) will wake up and go back to normal with no side effects. If, on the other hand, low-frequency (50 Hz) head-to-body electrical stunning is used it may cause cardiac fibrillation and death (Gregory & Wotton 1984), but some animals may recover rhythmic breathing and corneal reflex (Llonch *et al.* 2015). Therefore, this is a theoretically irreversible stunning method but must nevertheless be followed by exsanguination in the case of slaughter (EC 1099/2009).

2.3 Blood volume and heart rate

There is a correlation between the body mass of an animal and its heart rate. Travasso and co-workers (2025) have put up a model where the body weight (kg) of a mammal can be used to calculate the diameter (cm) of the aorta and the heart rate (Hz). The model is based on documentation from 95 mammal species with body size ranging from 1 kg to 3,800 kg. Since birds have a larger heart and a lower heart rate than mammals with the same body mass, the results from Travasso and co-workers are nontransferable. Fortunately, a couple of studies in birds has been made. Grubb (1983) carried out a similar study in birds, where relationships between body weight, heart mass, cardiac output and heart rate was found. Berger and co-workers (1970) studied the heart rate in flying birds and Calder (1968) examined the respiratory rate and heart rate in resting birds. All three studies found a connection between increasing body weight and decreasing heart rate. However, the equations for calculation of heart rate based on body weight differ. According to literature, the heart rate of an 25-30 day old broiler chicken ranges between 200 to 320 BPM in laboratory environment (Hossain *et al.* 2023). Younger chicks have a resting heart rate between 350-450 BPM (MSD Veterinary Manual 2025). The heart rate varies between individuals of the same species and is influenced by physique, age and by activity in the parasympathetic

and sympathetic system (Sjaastad *et al.* 2016). The sympathetic system activates when an individual for example is scared. The heart rate increases temporarily and goes then back to normal. A resting animal has a lower heart rate than normal because of increased stimulation from the parasympathetic system.

The blood volume in an animal is affected by the nutritional status, potential lactation or pregnancy, physical fitness and the size of the animal (Sjaastad *et al.* 2016). A fat animal has less blood than a lean animal of the same body mass. Depending on the source of information, a chicken has between 60-100 ml/kg blood in their body (Morton *et al.* 1993; Sjaastad *et al.* 2016; University of Kentucky n.d.).

2.3.1 Methods for measuring heart rate

Stethoscope

It is said that Dr. René Théophile Hyacinthe Laënnec is the one who invented the concept of a stethoscope in 1816 (Roguin 2006). He rolled a sheet of paper and put one end on the patient's chest and his ear over the other end. Nowadays there are digital stethoscopes that can amplify sounds, are noise cancelling and can be paired with a smartphone (Littmann 2025). By using an application on the phone, it's possible to see a real time visualisation of the heart beats, record the heart sounds and analyse heart murmurs.

Heart auscultation on a bird is a quite insensitive tool because of the rapid heart rate (Fitzgerald & Beaufrère 2016). It's possible to get a subjective assessment of the heart rate, rhythm and sound quality using a stethoscope. The chest piece is placed on the cranial keel and can then be moved around the keel, thoracic inlet, and dorsum to examine the heart.

Electrocardiogram (ECG)

The electrocardiograph was constructed by Willem Einthoven in 1903 (Nobel Prize Outreach 2025). In 1924 he was awarded the Nobel Prize in Physiology or Medicine for his invention. An electrocardiograph uses electrodes on the surface of the body to record voltage differences (Sjaastad *et al.* 2016). Muscle contractions in the heart are initiated by electrical activity. Repolarisation and depolarisation in the muscle fibres cause voltage differences between the electrodes, producing diagrams of the electrocardiograms. A standard ECG consists of a P wave, a QRS complex and T wave. The P wave represents the depolarization of the atria, the QRS complex represents the depolarization of the ventricles, and the T wave represents the repolarization of the ventricles. The shape and size of the

waves, as well as the time interval between them, provides information about the function of the heart.

2.4 Death in poultry in relation to exposure of CO₂

Gerritzen and co-workers (2013) carried out a study on broiler chickens, exposing them to CO₂ at different concentrations. During one experiment of the study, Gerritzen and co-workers evaluated the induction of unconsciousness in the broilers by exposing them to two different treatments. In treatment one, which lasted for six minutes, the broilers were gradually exposed to CO₂ in five stages: at 20, 30, 35, 40, and 64%. In treatment two, which lasted for four minutes, the broilers were gradually exposed to CO₂ in four stages: 20, 35, 45 and 65%. ECG and EEG were used to measure the brain- and heart activity. The study showed that all broilers were stunned and killed, regardless of if the four- or six-minute treatment was used.

A small study on 18 broiler chickens was conducted by Goncalves and co-workers (2022). The broilers were divided into three groups: dead on arrival (DOA; n = 6), stunned (n = 10) and sensible (n = 2) broilers. Stunned and DOA chickens had been transported through a five-chamber gas stunning system prior being classified as DOA or stunned. The CO₂ concentration in each chamber was 13.6, 21.9, 34.7, 62.5, and 72.2% respectively and the exposure time was approximately one minute per chamber. No heart beats in the stunned chickens could be auscultated with neither classic nor electronic stethoscopes. According to the authors, the electronic stethoscope picked up abdominal noises, making it hard to determine possible heart beats. A pulse oximeter with a clip sensor was used on 6 stunned, 5 DOA and 2 sensible broiler chickens. The clip was placed on a clean toe. No heart beats were detected in any DOA, but in all sensible and stunned chickens. The beats per minute (bpm) in sensible chickens (n=2) ranged between 20 and 121 and in stunned chickens (n=6) it ranged between 24 and 159 bpm. The authors pointed out that the reading ability of the pulse oximeter can be influenced by vibrations or movements of the broilers, but concluded that pulse oximeters were meeting the requirements for a reliable method in detecting signs of life in broilers.

The UK researcher Raj has done much research on animal welfare at slaughter. He states that both chickens and turkeys can be killed if they are exposed to 50% CO₂ in air during minimum two minutes (Raj 2024). In a study from 1990, Raj and Gregory investigated the effect of carbon dioxide stunning on brain function in chickens. Broilers and hens were subjected to 35, 45, 55 or 65 % carbon dioxide in air. 18 broilers and 10 hens survived a five-minute exposure at 35%.

Two broilers and zero hens survived a five-minute exposure at 45%, but at 55% and 65% CO₂ all broilers and hens died within two minutes. The authors suggested that a concentration of at least 55% CO₂ would be suitable for killing hens and broilers in commercial situations (Raj & Gregory 1990).

3. Materials and methods

3.1 Ethical considerations

For this study, the need of an ethical approval for animals in research was investigated. The conclusion was that there was no need for such an ethical approval since the study was non-invasive. The study did not affect the welfare of the broilers during slaughter. The birds were stunned according to the slaughterhouse's standard routine, exsanguinated in line with the SOP, and were unconscious during handling. The only conscious birds that were handled were the two broilers that had the pulse sensor on, since it had to be attached before stunning. For this part of the study, an owner permission was given, since the birds at that point of time belonged to the slaughterhouse.

3.2 Study design and the animals

The practical part of the study took place during five days in October and November 2025. One commercial broiler chicken slaughterhouse in Sweden, equipped with a gas stunning system, was selected. A total of 404 broilers were examined, and the individuals were chosen through convenience sampling. In practice this meant that a new bird was chosen every four to five minutes. The birds came out from the gas stunning in their transport modules containing around 30 broilers each and a random unconscious bird was selected. The initial plan was to examine the same number of small birds as large birds, but since only 20% of the slaughterhouse's volume consists of small birds this was not possible. In addition, slaughter of small birds was only carried out late in the day, and the stun-to-stick interval was longer, which lead to fewer examined birds per hour in comparison with large birds since each small bird was examined for a longer time.

3.2.1 Description of the slaughterhouse

The slaughterhouse in this study slaughters approximately 50 million broiler chickens annually (*Gallus gallus* Ross 308 hybrid). Of these, 80% are large birds, from final removal of the flock for slaughter and 20% are smaller birds, from thinning. The birds are transported on conveyor belts into the gas stunning system, still inside their transport modules. The slaughterhouse uses a two-phase (or multiphase, but with two main phases) CO₂ system where the broilers are initially gradually exposed to a maximum of 40% CO₂. Slowly the bird modules are moved deeper down within a pit where the gas concentration is higher. In the bottom of the pit the CO₂ concentration is approximately 80%. After the birds have been exposed to the gas, the transport modules are elevated, and the

transport modules leave the gas stunning system on a conveyor belt. The birds are then individually inverted (upside down) and hung by their legs in moving shackles, transporting them to an automatic rotating knife where they are decapitated. If a non-stunned bird leaves the gas stunning, staff will manually re-stun the bird and exsanguinate it before returning it to the slaughter line.

The exposure time for the birds in the gas may vary by a few seconds from day to day but is around 6 minutes 57 seconds for the larger birds and 8 minutes 40 seconds for the smaller ones (Table 1). The stun-to-stick interval for all broilers varies from 99 to 154 seconds according to statistics from the slaughterhouse. During the study, the stun-to-stick interval for the last broiler in each transport module was measured five times. To know which bird was the last in each transport module, animal friendly spray paint was used and the staff at the shackling point were instructed to take that bird last. The mean value for large birds was 151 seconds (2 min 31 seconds) and the mean value for the small birds was 203 seconds (3 min 23 seconds). These values were meant to be used in the study.

Table 1: Descriptive information about the recorded values each observation day. Information about the exposure time is from the slaughterhouse. If not anything else is written, the information refers to large birds. n = number of measurements per day.

	Gas concentration (%)			Gas temperature (°C)			Exposure time
	Mean	SD	Min-max	Mean	SD	Min-max	
Observation day 1 (n= 7)	69.4	0.78	68.0-70.6	23.4	0.96	21.8-24.5	7 min 43 s
Observation day 2 - large birds (n= 4)	69.5	1.48	67.9-71.0	23.7	0.58	23.1-24.6	6 min 39 s
Observation day 2 - small birds (n= 2)	66.6	0.45	66.1-67.0	23.2	0.05	23.1-23.2	8 min 40 s
Observation day 3 (n= 6)	71.0	2.14	68.3-73.4	23.5	0.73	22.8-25.0	6 min 29 s
Observation day 4 (n= 8)	71.2	2.00	69.2-74.5	23.3	0.69	22.2-24.5	6 min 58 s
Observation day 5 (n= 6)	70.5	1.34	69.2-73.2	23.3	0.58	22.5-24.1	6 min 55 s

3.3 Literature search

Literature has been searched through a library and databases such as Scopus and Web of Science, using search terms such as carbon dioxide, gas stunning,

slaughter, broiler, chicken, cardiac activity and death. References from articles found through searching have been used to find additional relevant literature.

3.4 Data collection

This study took place during regular slaughter activities. A room close to the gas stunning system was prepared, where the auscultation of the broilers was made. Every 30-60 minutes the gas concentration at the conveyor belt in the gas stunning system, as well as the temperature, was recorded (Table 1). With the same time interval, the number of re-stunned birds was recorded (Table 3), irrespective of whether these particular birds were included in the study or not. The definition of a re-stunned bird was a broiler that had left the gas stunning system with signs of consciousness (muscle tonus, swallowing, wing flapping, etc.) and had to be manually stunned and exsanguinated by staff before shackling. However, not all re-stunned broiler chickens during a slaughter day (approximately 16-17 hours) were registered, only the re-stunned birds during the time when the data collection took place (approximately eight hours).

3.4.1 Pilot study

A small pilot study was conducted at the Swedish Livestock Research Centre at Swedish University of Agricultural Sciences. Two ten-week-old laying hens, not included in any other study, were auscultated to find the best spot to place the stethoscope to hear the heartbeats. The hens were also used to check if the pulse sensor would suit the broilers and how to attach the equipment (Figure 1). The pulse sensors were also turned on to see if the recordings would give any readable result.



Figure 1: The pulse sensor attached to a laying hen during the pilot study. The sensor is attached over the brachial vein under the yellow bandage.

3.4.2 Auscultation

A Littmann CORE Digital Stethoscope was used to auscultate the broiler chickens. The stethoscope was turned on during examination to use the sound amplification and the frequency visualisation in the Eko app. The noise cancelling feature was not used the first three days (232 birds) but was on the last two days (172 birds).

A bird was fetched through a hatch between the gas stunning system and the point of shackling. A timer was turned on when the bird was collected. The consciousness of the bird was evaluated by the staff while bringing the bird to the prepared room. In the room, the bird was placed in dorsal recumbency on a metal table. Auscultation of the heart started and was made on the right side of the chest, close to the right wing. The auscultation began approximately 15-20 seconds after the bird was fetched from the hatch and continued until 2 minutes 51 seconds (large birds) or 3 minutes 23 seconds (small birds) had passed (Table 2). It was meant to be 2 minutes 31 seconds for the large birds, which was the mean value of the stun-to-stick interval for the last bird in each transport module. Due to a typing error, 2 minutes 51 seconds was the time actually applied. After 2 minutes 51 seconds or 3 minutes 23 seconds, the bird was exsanguinated by staff by cutting off both carotid arteries or by decapitation. During sticking no auscultation was made for safety reasons. Auscultation of the heart was resumed after sticking and continued for one more minute. During the whole auscultation period, potential signs of consciousness were studied. The time when the bird was brought to the room, size of the bird (large/small), signs of consciousness, stun-to-stick interval, muscle vibrations, possible heart beats and potential time for cardiac arrest was recorded in a protocol (Appendix 2). The occurrence of muscle vibrations in the birds was not necessary data for this study but was written down since the intense muscle vibrations theoretically could have concealed possible heart beats. The definition of intense muscle vibration was vibrations from two or more muscles, lasting for more than five seconds and must be heard clearly through the stethoscope. Two birds with intense muscle vibrations were opened one minute after exsanguination, to confirm cardiac arrest.

3.5 Additional data collection with pulse sensor

An optical pulse sensor was used to try to determine when the heart stopped beating during gas stunning. The device was constructed by students from Chalmers University of Technology to be used on horses, using photoplethysmography (PPG) to measure the heart rate. The device consists of a pulse sensor (pulsesensor.com) using LED light and a sensor to measure the blood flow in a vessel. The sensor was connected to a development board with a memory

card and a battery (Figure 2 & 3). The data was saved as a CSV-file on a micro-SD-card.

A development board is a printed circuit board (PCB) with a pre-assembled microcontroller (Hemeixin Electronics 2024). The board is usually used by engineers during prototyping and development phases. A development board comes with memory, input/output ports, power supply, etcetera. It can be connected to a computer to transfer codes to the microcontroller, programming the development board.

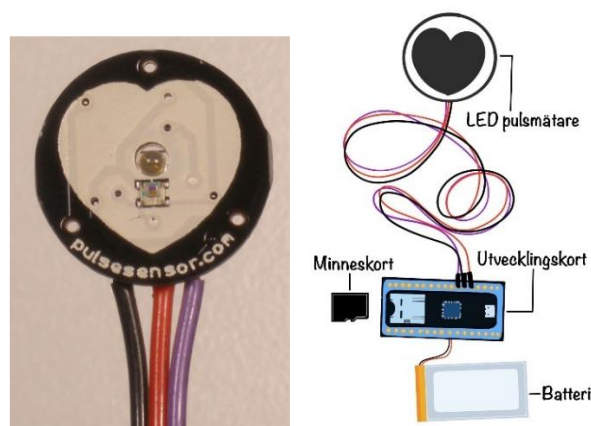


Figure 2 & 3: The pulse sensor and a sketch of the sensor, sketched by the students. The sketch shows the LED pulse sensor, the memory card, the development board and the battery. Used with permission from the owners (Sofia Bosson, Hedvig Arosenius, Sofia Dahl, Patrik Emanuelsson, Hanna Hallberg & Emma Karlsson)

The pulse sensor was tested on two small birds. One live and presumably healthy bird was picked up from its crate at the DOA checkpoint before stunning. The sensor was turned on and placed over the brachial vein and attached using skin friendly tape. Simultaneously a timer was turned on. An elastic self-adhesive bandage (EickWrap) was wrapped around the sensor and the wing (Figure 1). The development board, together with the battery and the memory card, was fastened on the bird's back by using a tube bandage. The tube bandage was wrapped around the bird's body, keeping the wings close to its body but allowing the head and legs move freely. The bird was put back in a crate at the DOA checkpoint and transported on a conveyor belt to the gas stunner. At the hatch between the gas stunning system and point of shackling, the bird was fetched, and the recording was turned off. The bird was shackled and returned to the slaughter line for exsanguination and processing. The same procedure was repeated on the second bird and the files from the memory card was uploaded to a website¹ that creates graphs from CSV-files (Figures 4A and 4B).

¹ <https://patrik.github.io/TimeSeries-Graph-CSV/>

4. Results

4.1 Auscultation

In this study, no heartbeats were detected with stethoscope in any of the 404 examined broiler. There was no difference between large birds (n = 375) and small birds (n = 29). There was neither any difference between using the noise cancelling function on the stethoscope (n = 172) nor not using the function (n = 232).

Table 2: Stun-to-stick interval for the broilers in the study. All values are for the large birds, unless anything else is stated.

	Mean value (s)	SD (s)	Min (s)	Max (s)
Day 1 (n= 67)	171.1	0.63	168.0	172.0
Day 2 - Large (n= 49)	170.9	0.27	170.0	171.0
Day 2 - Small (n= 29)	203.0	0.53	201.0	204.0
Day 3 (n= 87)	170.9	0.27	170.0	171.0
Day 4 (n= 102)	171.0	0.22	170.0	172.0
Day 5 (n= 70)	171.0	0.20	170.0	171.0

4.2 Result from the additional test with the pulse sensor

The optical pulse sensor was tested the second day on two small birds. The recordings produced graphs with too big uncertainty to interpret, leading to the decision of not continuing using this method. It is difficult to determine from these graphs when the heart activity disappears. In addition, it is not possible to see how long the broiler has been in the gas before cardiac arrest. The sensor records everything from when it is turned on, which includes the time it takes to attach the sensor on the bird and the transport time to the gas stunner. The graphs are presented below (Figures 4A and 4B).

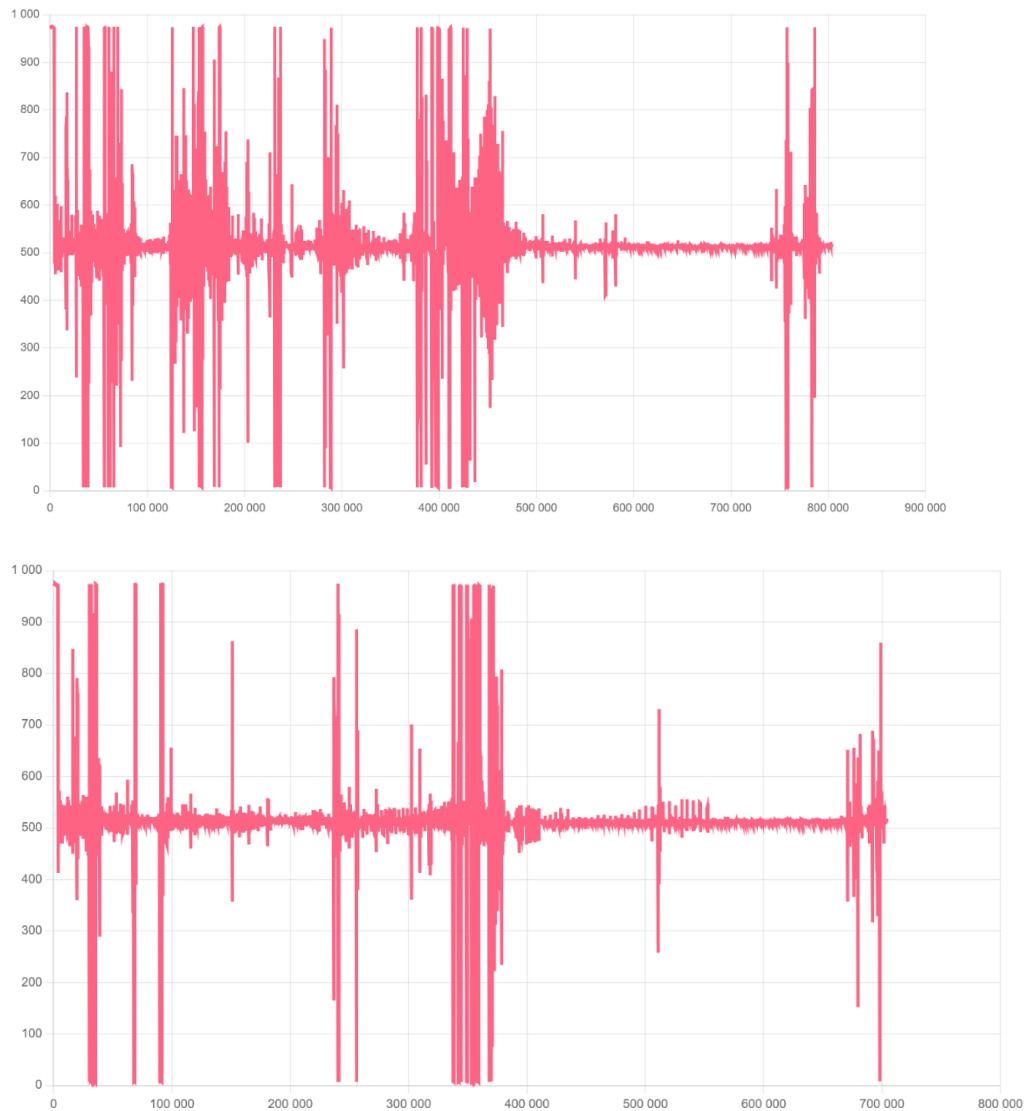


Figure 4A and 4B: The raw PPG-graphs from the recording of bird no 1 and 2. x-axis is time (milliseconds) and y-axis show the PPG signal amplitude (light intensity).

4.3 Secondary findings

4.3.1 Re-stunned birds

The number of re-stunned birds due to inadequate stun quality per observation day varied between 8 to 38 birds (Table 3). The total number of re-stunned broilers per slaughter day was not recorded. The re-stunned birds were not evenly distributed throughout the observation day; the number of re-stuns varied between 0 to 14 birds per hour. These registrations are based on slaughterhouse data. It is not known what signs of consciousness the bird showed. Therefore, the stun quality cannot be assessed within the framework of this study.

Table 3: Descriptive information of re-stunned and auscultated chickens during observation day 1-3, when the stethoscope without noise-cancelling was used, and during observation day 4-5, when the noise-cancelling function was used.

Slaughter_o = number of slaughtered birds during the observation hours.

Slaughter_F = all slaughtered birds during the whole day.

	Observation days				
	Day 1	Day 2	Day 3	Day 4	Day 5
Number of auscultated broilers	67	78	87	102	70
Percentage auscultated broilers of slaughter _o (%)	0.073	0.065	0.080	0.087	0.068
Percentage auscultated broilers of slaughter _F (%)	0.033	0.039	0.043	0.051	0.034
Number of re-stunned broilers	38	8	19	30	9
Percentage re-stunned broilers of slaughter _o (%)	0.042	0.007	0.018	0.026	0.009
Percentage re-stunned broilers of slaughter _F (%)	0.019	0.004	0.010	0.015	0.004

4.3.2 Muscle vibrations

In 60 of the 404 observed birds intense muscle vibrations were displayed. These only occurred in the large birds.

5. Discussion

The aim of this study was to find out if the broilers enter cardiac arrest during gas stunning at a commercial slaughterhouse. It was hypothesized that the majority of the birds would be dead post stunning because of the exposure time and the high CO₂ concentration. It was also hypothesized that the methods of measuring cardiac activity would result in no heart beats in the majority of the studied broilers, after exiting the stunner. The pulse sensor was only used twice, and the results was difficult to interpret. Therefore, only the result from the auscultation will be discussed unless anything else is mentioned.

5.1 The occurrence of dead and alive birds after stunning

All observed birds in this study were dead, i.e. had no heartbeat, when they left the gas stunning. On the contrary, a number of birds leaving the gas stunner had to be re-stunned every day. Studies have shown that hens and broiler chickens do not survive longer than two minutes in environments with > 55% CO₂ (Raj & Gregory 1990; Raj 2024). In this study, the broilers were exposed to a gradually increasing CO₂ concentration with a maximum of 74.5 % CO₂ during stunning (Table 1). The mean exposure time for the large birds in this study was 6 minutes 57 seconds. This is far more than the two-minute exposure that according to earlier mentioned studies, is enough to kill broilers in an environment with >50% CO₂. In this study, I did not examine for how long time the broilers were in a <40% CO₂ environment and in a >40% CO₂ environment respectively. Therefore, it is not known if the birds were in a > 55% CO₂ environment for longer than two minutes, but it is believed to be so. However, the study that Gerritzen and co-workers (2013) conducted, where broiler chickens were gradually exposed to CO₂ in four to five stages (20-65% CO₂) during four to six minutes, is similar to this study. All broilers in their study died, which was also the outcome for the auscultated birds this study.

As mentioned, every day a number of stunned birds at the slaughter line had to be re-stunned (Table 3). These animals were not examined within this study, but a few were observed and they were standing or laying upright, could blink with their eyes, and showed other signs of inadequate stunning. Nevertheless, they had been exposed to the gas in the gas stunner for as long time as the other broilers, which died during the stunning process. Studies have shown that some chickens die faster than other during exposure of CO₂. In the study conducted by Raj and Gregory (1990), two out of twenty broilers survived being in 45% CO₂ during

five minutes. The authors did not discuss possible reasons for this, but it indicates that there are differences between individuals, although it is unknown if it was related to differences in sensitivity, actual exposure e.g. related to ‘pockets of air’ in the plumage, or other factors. Also Zeller and co-workers (1988) saw individual differences when they exposed broiler chickens and parent stock to CO₂ in different concentrations and exposure times. Especially in roosters, they saw a large individual difference whether the bird died or not during exposure. The authors conclude that age and individual affect how the bird reacts to exposure of CO₂.

A theory from animal welfare employees at the slaughterhouse where this study was conducted, is that air pockets were created between the birds, and therefore some birds would not become unconscious. The birds are packed tightly in the transport module and somehow fresh air may get trapped between the birds and might create an air pocket where the CO₂ concentration is lower than in the surroundings within the gas stunning system. A bird in the air pocket may become unconscious, but will possibly not die, and in contact with fresh air at the shackling point, the bird regains consciousness. Please note that this theory has not been investigated or mentioned in other literature.

5.1.1 Practical considerations for the industry

This study examined less than one percent of the approximately 200,000 broilers that were stunned every day at the slaughterhouse in question (Table 3). It is not possible to claim that all (i.e. 100 %) of the broilers that leave the gas stunning system are dead, especially since there were a few birds each day that displayed signs of consciousness and therefore had to be re-stunned. But assuming that a majority of the stunned birds are dead, how would that impact the industry?

Stun-to-stick interval

Even if a study would show that the majority of the broilers leaving the gas stunning system are dead, there will be birds that are only unconscious or even conscious. These birds are at risk for regaining consciousness or sensitivity to pain after a certain amount of time. It is therefore still important for the FBOs to set up suitable stun-to-stick interval, to prevent birds from regaining consciousness before bleeding. Assuming that all birds are dead, would have negative effects on the animal welfare.

Bleeding efficiency

Exsanguination is what per definition kills the animal during slaughter, since stunning is only to render the animal unconscious (EFSA 2019). In this study, the bleeding efficiency was not investigated. Since the result show that at least some

of the stunned broilers are dead when leaving the gas stunner system, this raises the question of if the exsanguination efficiency, e.g. amount of blood loss, will be affected. After a quick search, only one study regarding exsanguination of dead poultry was found, but since there was no information about for how long the chicken had been dead before exsanguination, no conclusions can be drawn. It is generally acknowledged that the draining of blood after sticking can mainly be attributed to gravity and is not dependent on a beating heart, and hence bleeding can be efficient also if the bird is no longer alive after stunning. However, the blood may start to coagulate at some point in time after cessation of circulation, so it would still be interesting to look closer into possible negative effects of death prior to exsanguination.

Meat quality

If the exsanguination efficiency would be negatively affected because birds die during the stunning process, there is a risk that the meat quality also can be affected. Blood is a very good medium for microbial growth. A CO₂ killed, not exsanguinated broiler chicken, has a higher count of bacteria in the meat than an exsanguinated bird (Alvarado *et al.* 2007). It also has a greater increase of bacteria growth in 4 °C between day 0 and 5, than what was seen in meat from exsanguinated broilers with different stunning methods. Gas stunned birds at slaughterhouses are always exsanguinated, but the study from Alvaro and co-workers may help us understand how the meat quality could potentially be affected if not enough blood is lost during bleeding. This is only speculations since we do not know if the exsanguination efficiency is affected.

5.2 Methodological considerations

In this study, only one person listened to all birds. This has many advantages, but it can be a disadvantage because the number of consecutive working hours is high, which may involve risk of operator fatigue. In the beginning of the day, the person is more alert than after many hours of listening. Even with regular breaks, it is difficult for one person to be alert all day and analyse possible heart sounds in the stethoscope. There is an increased risk a faint heartbeat will be unnoticed because the person is tired.

An electronic stethoscope will not only amplify the sound of the heart beats, but also all internal noises such as moving intestines. If there would be a faint heartbeat, there is a risk it would not be heard. Even when the noise cancelling function was turned on, some interference from internal bowel sounds were inevitable. The stethoscope was also sensible to fingers moving or if someone touched the table where the bird laid.

Intense muscle vibrations were heard in 60 of the 404 observed broilers. The vibrations could last from 10 seconds to longer than a minute. During this time, it was not possible to hear anything else through the stethoscope. If the heart would beat during the vibrations, it would not be possible to detect. In these cases, another technique of heart monitoring might be suitable, e.g. ECG.

Not only sounds from inside the broiler did disturb the auscultation. Even though the study took place in a room with a closed door, noise from the slaughter activities was noticeable through the stethoscope. Additionally, the person that fetched and left the birds had to open and close the door twice per auscultated chicken. During the few seconds when the door was opened, loud noise from the slaughter hall filled the room.

5.3 Suggestions for future research

This study examined less than 1% of all broiler chickens slaughtered per day (Table 3). To achieve a higher level of representativity and statistical significance, a higher proportion of randomly selected birds should be examined. This would require more people in the research group, so it would be possible to listen to many broilers simultaneously or carry out the study over a longer period of time. More staff from the slaughterhouse would be required to fetch and leave the examined birds. It would also be necessary to examine the conscious birds that leave the gas stunning system, to find out if there is any correlation to age, sex, weight, gas concentration, localisation in the transport module or other factors. Are there any surrounding factors or is it down to the individual level whether the broiler becomes unconscious or not during gas stunning?

In this study, all examined birds were dead post stunning. The pulse sensor was used to try to find out when during stunning the bird enter cardiac arrest. Unfortunately, it was difficult to interpret the results and to know for how long time the broiler had been in the gas before it died, since the recordings from the sensor showed the time from when the sensor was turned on and not from when the bird entered the gas. A stopwatch was turned on simultaneously as the pulse sensor when the pulse sensor was used on the second bird, but the time when the bird entered the chamber was not recorded. If the decision to continue with the pulse sensor method would have been made, there would have been done more detailed planning around the method. It is encouraged to in the future examine more thoroughly when cardiac arrest occurs during gas stunning.

As mentioned earlier, there were difficulties to detect heart sound through electronic stethoscope since it amplifies all sounds from the bird. Goncalves and colleagues (2022) encountered the same problem when they used an electronic

stethoscope, evaluating methods in detecting signs of life after CO₂ gas stunning in broilers. They also tried a classic stethoscope and experienced no improvement. In their study, they concluded that using a pulse oximeter was a reliable and practical method in detecting signs of life in stunned broilers. However, these results were based upon data from six stunned, two conscious and five DOA broilers and it is unclear if the results are repeatable. The scientists found no difference in heartbeat between the conscious and the stunned broilers, which would have been expected since the stunned broilers in that study were in the gas for almost five minutes. More research regarding methods to detect heartbeat in birds during slaughter should be carried out, focusing on methods that are reliable even in noisy environments.

As mentioned earlier, it is also important to study the effects on bleeding and meat quality, in relation to possible differences between an unconscious stunned bird or a dead stunned bird. Are there any microbiological disadvantages if the bird dies during stunning or does it affect the meat quality such as the water holding capacity (WHC), protein content or meat colour? The number of published studies in this area is limited, especially for poultry. Hence, modern studies focusing on this aspect would be useful.

6. Conclusions

None of the broilers examined in this study displayed any heartbeats after leaving the gas stunner. Even though internal sounds and muscle vibrations might have concealed a faint heartbeat, it is most likely that the bird had developed cardiac arrest at that point of time. The method where the pulse sensor was used did not give a satisfactory result for interpretation, and the method should be refined if to be used in the future.

Despite the result that all broilers that were examined after leaving the gas stunning were considered dead, it is not possible to state that all gas stunned broilers are dead. Only a low proportion of the approximately 200,000 broilers slaughtered per day were examined and, in addition, a few conscious birds were identified by the staff at the shackling point every day. It is therefore important for animal welfare that the staff consider the animals alive until after exsanguination, and that a reasonable stun-to-stick interval is applied, to minimise the risk of birds regaining consciousness prior to or during bleeding.

Further research is required to determine the proportion of birds that dies during gas stunning, and if this has any effect on exsanguination efficiency or meat quality.

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Populärvetenskaplig sammanfattning

År 2024 slaktades drygt 110 miljoner slaktkycklingar i Sverige. Enligt svensk djurskyddslag (SFS 2018:1192) måste alla djur bedövas, d.v.s. göras medvetslösa, innan slakt för att skydda dem mot onödigt lidande och obehag. De vanligaste bedövningsmetoderna vid slakt av slaktkyckling är gasbedövning eller elektricitet. De som blir bedövade med elektricitet kan bli det i ett så kallat vattenbad, där medvetna kycklingar hängs upp och ner och deras huvud förs ner i ett strömförande vattenbad. Den andra typen av elektrisk bedövning är ”torr bedövning” över fågelns huvud, där kycklingen hålls fast manuellt. Mer än hälften av slaktade kycklingar i Sverige bedövas med gas. I EU tillåts olika typer av gasblandningar, även om koldioxid är helt dominerade, men i Sverige är endast koldioxidbedövning godkänt. Koldioxid är en gas som enligt studier är aversiv hos många djur inklusive människor, vilket innebär att inandning upplevs smärtsam och obehaglig. När under bedövningsutförandet som obehaget kommer beror på gaskoncentrationen, dvs hur mycket koldioxid det finns i atmosfären. Man har kommit fram till att kycklingar tolererar upp till 40 % koldioxid utan att visa tecken på obehag. Bedövning av slaktkyckling på slakteri sker därför i två faser där första fasen innehåller max 40 % koldioxid. Det är i denna fas som kycklingarna blir medvetslösa. I nästa fas ökas gaskoncentrationen upp till 80 % koldioxid för att få en bestående medvetslöshet. Beroende på inställningar och tillverkare varierar det hur länge kycklingen vistas i gasen, men i Sverige är det över fem minuter. Efter bedövning, när fåglarna är djupt medvetslösa, är det dags för avblodning. De hängs upp i benen och en automatiskt roterande kniv skär av de stora halskärlen eller hela huvudet. När djuret har blött ur räknas det som dött och kan gå vidare till styckning och förädling.

Det saknas vetenskaplig litteratur som systematiskt undersökt huruvida kycklingarna är medvetslösa eller döda efter bedövning med koldioxid vid storskalig kommersiell slakt. Det har tidigare gjorts mindre studier där man utsatt slaktkycklingar för koldioxid i olika koncentrationer och exponeringstider. En studie undersökte överlevnaden hos höns och slaktkycklingar som exponerats för olika koncentrationer av koldioxid. Studien visade att alla höns och slaktkycklingar dog inom två minuter när de exponerades för 55 % koldioxid eller mer. I andra studier, där koldioxiden gradvis har gått från 20 % till 65 % under fyra till sex minuter, har också alla fåglarna dött.

Syftet med den här studien var att undersöka huruvida slaktkycklingarna endast blir medvetslösa eller om de även dör under koldioxidbedövningen på ett kommersiellt slakteri. Resultatet kan bara appliceras på den aktuella typen av bedövningsutrustning, koncentration och exponeringstid och skildrar inte

koldioxidbedövning av kyckling på andra slakterier med annan utrustning och inställningar. Hypotesen var att de flesta av de undersökta fåglarna skulle vara döda. Resultatet kan hjälpa slakteribranschen när de ser över sina riktlinjer angående sticktid, d.v.s. tiden det tar från att kycklingen bedövas tills att den avblodas, men bidrar även till förståelse för hur gasbedövningen påverkar kycklingarnas fysiologi.

Försöket utfördes under fem dagar på ett kycklingslakteri med koldioxidbedövning. Studien utfördes under normala slaktförhållanden och påverkade inte djurskyddet. För att undersöka de bedövade kycklingarna under förhållanden så lika som vid ordinarie slakt, mättes först en genomsnittlig sticktid för den sista kycklingen i varje transportlåda. Under försöket avblodades de undersökta fåglarna manuellt och det var därför nödvändigt att få fram en sticktid som stämmer med vad som praktiskt tillämpas på denna anläggning. Den uppmätta sticktiden var 2 minuter och 31 sekunder, men ett skrivfel vid omvandling från sekunder till minuter resulterade i att det istället blev 2 minuter och 51 sekunder, vilket var tiden som användes under försöket. Sticktiden för de mindre kycklingarna var 3 minuter och 23 sekunder. Under försöksdagarna antecknades gaskoncentrationen i bottenvåningen av gasmaskinen samt temperaturen i gasen en gång i timmen. Detta för att senare ha som underlag vid diskussion av resultaten. Med samma tidsintervall antecknades också antalet ombedövade kycklingar i den samtidigt pågående ordinarie slakten. Bedövningskvaliteten bedöms av personal på alla kycklingar som lämnar gasbedövningen innan de hängs upp i fotbyglar för att transporteras till avblodningen. Kriteriet för en ombedövad kyckling var en kyckling som på något sätt visade tecken på medvetande efter att den kommit ut från bedövningen. Exempel på dessa tecken kunde vara stelhet i kroppen, andning eller rörelser. Om en kyckling visade tecken på att vara dåligt bedövad togs den åt sidan och ombedövades manuellt samt blev avblodad direkt.

Den utrustning som användes i studien var ett elektroniskt stetoskop från Littmann samt en pulsmätare som konstruerats av studenter vid Chalmers tekniska högskola. Ett elektroniskt stetoskop liknar ett vanligt stetoskop, men har funktioner så som att det förstärker hjärtljuden, kan göra inspelningar och spara på en app samt har noise cancelling (bullerreducerande) funktion. Under försöket användes noise cancelling-funktionen under två dagar, medan tre dagar var utan.

Undersökningen gick till på följande sätt: En kyckling plockades efter bedövning och togs åt sidan in i ett tystare rum, där ett elektroniskt stetoskop användes för att lyssna efter hjärtljud. Kycklingen lades på rygg och stetoskopet placerades på sidan av slaktkycklingens bröst. Efter 2 minuter och 51 sekunder avblodades kycklingen manuellt genom att antingen skära av hela huvudet eller skära av de

större halskärnen. Efter avblodningen placerades stetoskopet återigen på kycklingen och förekomsten av eventuella hjärtljud undersöktes under ytterligare en minut. Eventuella tecken på medvetande, hjärtljud och övrigt som observerats antecknades i ett protokoll. Därefter plockades en ny kyckling från bedövningen att undersöka.

Pulssensorn användes på två mindre kycklingar innan bedövning. Mätaren fästes över ett blodkärl på insidan av ena vingen och tillhörande utrustning sattes fast på fågelns rygg med elastiskt bandage. Kycklingen sattes sedan ner i en transportlåda och åkte genom gasbedövningen. Den fångades upp när den kom ut och mätningen stängdes av.

I studien undersöktes 404 slaktkycklingar varav 375 stycken var stora och 29 var små. Varken hjärtslag eller medvetande noterades hos någon av de undersökta kycklingarna. Som ett bifynd sågs kraftiga muskeltvibrationer hos 60 av de 404 undersökta kycklingarna. Pulsmätningen gav inget tydligt resultat och upprepades därför bara två gånger. Andelen ombedövningar i den ordinarie slakten varierade från åtta till 38 kycklingar per observationspass, d.v.s. de räknades inte under en hel slaktdag utan bara under den tiden på dagen som studien skedde på plats.

Resultatet av denna studie pekar mot att flertalet kycklingar dör under gasbedövning av denna typ. Då endast 0,04 % av slaktade djur undersöktes varje dag bör dessa resultat tolkas med stor försiktighet. Det fanns även svårigheter med att lyssna på kycklingarna då stetoskopet tog upp ljud från exempelvis kycklingens tarmar, muskeltvibrationer och omgivningen. Risken finns att ett svagt hjärtljud missades hos någon av de undersökta kycklingarna. Studien visade också att ett antal kycklingar dagligen kom ut ur gasen otillräckligt bedövade, trots den höga koncentrationen koldioxid och den långa tiden fåglarna vistats i gasen. Orsaken till varför detta sker är oklar, men kan ha att göra med skillnader på individnivå. Det kan möjligen också orsakas av någon slags luftficka som skapas mellan kycklingarna i lådan och innehåller ren luft, d.v.s. atmosfär med mycket låg koldioxidhalt, som kycklingen kan andas i. Hur stor andel av kycklingarna som verkligen dör av gasbedövningen och orsaker till varför vissa dör och andra inte, behöver undersökas i ytterligare studier. Det uppmuntras att även undersöka om avblodningskvaliteten eller köttkvaliteten påverkas av att kycklingarna är döda innan avblodning.

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Appendix 1

Indicators of consciousness after gas stunning.

Freely interpreted from EFSA Panel on Animal Health and Welfare (AHAW) (2013).

Indicator	Explanation	Satisfactory stunning
Breathing	Rhythmic breathing showing inspiration and expiration. Can be seen as regular movements of the lower abdomen.	No signs of breathing
Eye movements	Rotation of the eyeball or nystagmus. Require a close examination of the eyes to recognise.	Fixed eyes, wide open
Reflexes	Reaction when provoking corneal, palpebral or pupillary reflexes.	No reflexes
Spontaneous blinking	When a bird, on its own, opens or closes its eyes without any stimulation	No blinking
Spontaneous swallowing	When a bird is swallowing.	No swallowing
Vocalisation	Loud screaming from the bird.	No vocalisation
Wing flapping	Rhythmic movements of both wings. Should not be confused with rapid trembling of the whole body.	No movements

EFSA (2013). Scientific Opinion on monitoring procedures at slaughterhouses for poultry. EFSA Journal, 11 (12), 3521. <https://doi.org/10.2903/j.efsa.2013.3521>

Appendix 2

Protocol

Time: The time when the crate leaves the chamber.

Type: Small vs big birds.

Consciousness? Does the bird show consciousness? Indicators listed below. Is observed from when the bird is picked up from its crate to 1 minute after exsanguination. (Yes/No)

Heart beats: Does the focal animal have any heart beats when it leaves the chamber? (Yes/No)

Cardiac arrest: Time from start when the bird leaves the chamber to cardiac arrest. Is only measured if the bird has heart beats when it comes out from the chamber. (s/-)

Indicators of consciousness

Following indicators of consciousness is used in this protocol to determine if the focal animal is conscious. Indicators and definitions adapted from EFSA (2013) and Contreras Jodar et al (2022).

Breathing: Rhythmic breathing showing inspiration and expiration. Can be seen as regular movements of the lower abdomen/cloaca or the beak. The movements must occur more than once to count. An effective stunning should result in apnoea.

Muscle tone: An ineffectively stunned bird can show tension in its neck or in another body part. It may be recumbent or making attempts to stand up. An effectively stunned bird shows a relaxed body with no muscle tone or tension.

Spontaneous blinking: When a bird, on its own, opens or closes its eyes without any stimulation. Is a sign of consciousness and ineffective stunning.

Spontaneous swallowing: When a bird is swallowing.

Vocalisation: Single or repeated vocalisation is a sign of consciousness.

Wing flapping: Rhythmic flapping with both wings. Should not be confused with trembling of the entire body.

Contreras-Jodar, A., Varvaró-Porter, A., Michel, V. & Velarde, A. (2022). Inter-Observer Repeatability of Indicators of Consciousness after Waterbath Stunning in Broiler Chickens. *Animals*, 12 (14), 1800. <https://doi.org/10.3390/ani12141800>
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