The Old School, Brewhouse Hill, Wheathampstead,
Hertfordshire AL4 8AN, UK
www.ufaw.org.uk

Animal Welfare 2013, 22: 5-12
ISSN 0962-7286
doi: 10.7120/09627286.22.1.005

Killing wild geese with carbon dioxide or a mixture of carbon dioxide and argon

MA Gerritzen*, HGM Reimert, A Lourens, MBM Bracke and MTW Verhoeven

Wageningen UR Livestock Research, Wageningen University and Research Centre, PO Box 65, 8200 AB Lelystad, The Netherlands
* Contact for correspondence and requests for reprints: marien.gerritzen@wur.nl

Abstract

The killing of animals is the subject of societal and political debate. Wild geese are caught and killed on a regular basis for fauna conservation and damage control. Killing geese with carbon dioxide (CO_2) is commonly practiced, but not listed in legislation on the protection of flora and fauna, and societal concerns have been raised against this method. In this study, an experiment was carried out killing 30 wild-caught geese using either CO_2 or a mixture of CO_2 and argon (Ar). Brain function (EEG) and heart function (ECG) were measured to determine loss of consciousness and onset of death. The stage of unconsciousness was reached on average within one minute in both treatments (56 s for CO_2 and 50 s for CO_2 and Ar). States of minimal brain activity and ineffective heart beat were reached more quickly using CO_2 compared to CO_2 and Ar (112 versus 178 s for minimal brain activity and 312 versus 394 s for ineffective heart beat for CO_2 and the mixture of CO_2 and Ar, respectively). The mixture of carbon dioxide and argon did not significantly reduce time to loss of consciousness or death. Further studies on behaviour and stress physiology are needed to determine conclusively whether CO_2 alone is a satisfactory agent to kill wild-caught geese as the lower CO_2 concentration in the CO_2-Ar treatment may act as a sedative and reduce the aversiveness of the animals during exposure to lethal gas concentrations.

Keywords: *animal welfare, argon, CO_2, geese, killing, stunning*

Introduction

Population numbers of wild animals sometimes need to be controlled. Welfare considerations are increasingly recognised in the area of 'pest control' (eg Littin 2010). Numbers of wild geese are increasing in The Netherlands, due to overabundance of food, reduced hunting and improved breeding areas (van der Jeugd *et al* 2006). Grazing of wild geese may contribute to species diversity in nature areas, but it may also cause overgrazing and financial damage for property owners. Between 2000 and 2004, on average, €185,000 were paid for damage caused by geese that visit during the summer, especially Greylag geese (*Anser anser*) (van der Jeugd *et al* 2006). In 2010, the total damage caused by these geese had increased to €1.8 m (€6.2 m for the total of all geese; Faunafonds 2010). The issue of how to deal with overabundant wild geese is hotly debated in The Netherlands. The Flora and Fauna Act allows for the capturing and killing of wild animals. A commonly used method in The Netherlands is to gather wild geese during moulting and to trap and kill the animals with carbon dioxide (CO_2). However, this method is not listed in the Dutch laws for the protection of flora and fauna, and societal concerns have been raised against this method. CO_2 is an acidic gas and causes aversive responses in poultry when administered in high concentrations due to irritation to mucous membranes (Raj *et al* 1992; McKeegan *et al* 2006). However, in low concentrations, CO_2 will act as a sedative, and as such, CO_2 can be used to support the killing of poultry by means of an inert gas like argon (Ar) or nitrogen (N_2) as is recommended in the UK (eg Raj 1998). Inert gases are odourless and do not cause irritation to mucous membranes (Raj & Gregory 1991). Effectivity of these gases is based on displacement in air available for breathing, by which a very low oxygen (O_2) supply to the blood is induced (hypoxaemia). It is advised that air that is inhaled consists of no more than 2% O_2.

Few studies have been conducted on the effects of gas stunning in geese, and the primary focus of these studies was on product quality. One study investigated effects of controlled atmosphere stunning (CAS) and conventional electrical stunning on meat and liver quality in geese (Turcsán *et al* 2001). During the first phase, geese were placed for one minute in a gas mixture that consisted of 30% O_2, 40% CO_2 and 30% N_2. In a second phase, geese were placed for two minutes in a gas mixture consisting of 5% O_2, 80% CO_2 and 15% N_2. A reduction in wing flapping was observed, which may indicate better welfare during stunning (McKeegan *et al* 2007a). However, no behavioural observations were conducted in the study by Turcsán *et al* (2001) and responses of geese to increased CO_2 concentrations were not recorded.

Fernandez *et al* (2010) compared electrical stunning in a water-bath, head-only electrical stunning, mechanical stunning (penetrating captive bolt) and controlled-atmosphere stunning (CAS: phase 1, CO_2 (40%) O_2 (30%) N_2 (30%), for two minutes followed by phase 2, CO_2 > 85% in air, for two minutes) in both ducks and geese. During the first five minutes after slaughter, the stunning techniques that did not kill the animals were associated with a high incidence of head movements (mechanical and electrical head-only stunning), convulsions and convulsive wing flapping (mechanical stunning). CAS was associated with the lowest rate of fractures of the humeral bone.

Ducks previously responded to high CO_2 concentrations in a similar way as turkeys and broilers (Gerritzen *et al* 2006). It is therefore expected that results from work on poultry may, in general, apply to geese as well. However, exceptions may occur and pose a risk for animal welfare. Therefore, killing methods need to be studied for the specific species in question.

The objective of this study was to examine the welfare implications of killing of wild-caught geese with either CO_2 or a mixture of CO_2 and Ar. Brain function (EEG) and heart function (ECG) were measured to determine the onset of unconsciousness and death.

Materials and methods

Study animals and housing

Two groups of wild geese (with partly domestic genotypes, various ages) were caught at two different locations in The Netherlands. Group A (n = 18) was caught in mid-November 2009 within a period of approximately 30 min using gates, as they were relatively tame. Group B (n = 12) was taken from another area on another day in early January 2010. For the latter group, time of capture was not recorded. Transporting the animals to the location of stunning took approximately an hour for each group, after which time they were provided with *ad libitum* food and water, and kept in a fenced piece of land with trees for two days.

Experimental protocol

Each group was subjected to the experimental treatments two days after capture. On an experimental day, geese were removed individually from the herd and equipped with measuring equipment (see later). Three birds were tested at any one time (run) on each experimental day. On the first experimental day there were three runs for Group A followed by three runs for Group B starting at 1000h with intervals of 40 to 80 min between runs. On the second experimental day there were two runs for Group A followed by two runs by Group B starting at 1000h with intervals of 40–70 min between runs. During each run, three geese were randomly subjected to Treatment 1, an increasing gas mixture of only CO_2 (80%) and three geese were subjected to Treatment 2, a hypercapnic anoxic mixture of CO_2 (30%) and Ar (70%) with less than 1% O_2.

Treatments were imposed by placing three geese together in an iron cage in a specially developed plastic container measuring 740 × 580 × 1,100 mm (length × width × height). The container was equipped with a fixed CO_2 meter and a portable gasmeter (Oxybaby®, Witt-Gastechnik, Witten, Germany) measuring O_2 and CO_2 concentrations every 10 s. Both gasmeters were attached to the inside of the container at animal level. Since the gas mixture inside the container was sampled at each recording, relatively fast changes in gas concentrations could be measured. For Treatment 1 (the CO_2 treatment), after the container was closed, CO_2 was added until a concentration of 80% CO_2 was reached. Since it was not possible to add both CO_2 and Ar simultaneously, in Treatment 2, CO_2 was injected first until the concentration reached 30%. Subsequently, the injection of Ar started until a level of less than 1% residual O_2 was reached in the gas mixture. The total procedure for both treatments lasted approximately 4 min after which time the container was opened and the dead animals were removed. Data loggers were removed 5 min after removal from the container.

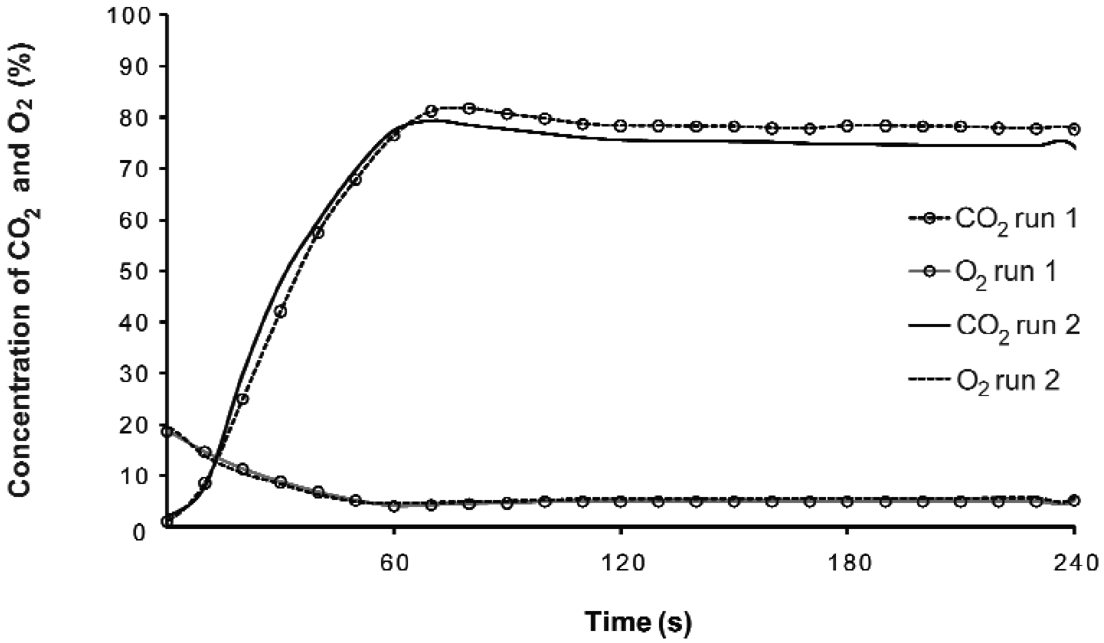
ECG and EEG measurements

To measure heart (ECG) and brain (EEG) activity, wireless data loggers (Royal Veterinary College, Hatfield, UK) were placed in a stretch jacket on the backs of the animals. The electrocardiogram (ECG) was measured using self-adhesive ECG electrodes (Blue Sensor, Ambu, Schiphol Airport, The Netherlands), with press-stud electrical connections, which were adhered to cleaned skin overlying the *pectoralis* muscle either side of the sternum. A harmless, cyanoacrylate, tissue adhesive (Vetbond, 3M, St Paul, USA) was applied to the ECG electrodes prior to placement onto the skin to improve adhesion. The electroencephalogram (EEG) electrodes (10-mm long and 1.5 mm in diameter; 55% silver, 21% copper, 24% zinc) were placed subdurally by pressing the electrode needle through the skin and the skull: one electrode 0.3 cm to the right and one 0.3 cm to the left of the sagittal suture, and both electrodes 0.5 cm caudally to the imaginary transverse line at the caudal margin of the eyes. The earth electrode for both the EEG and ECG was placed subcutaneously laterally on the right leg. Animals were not anaesthetised prior to this procedure, since anaesthetic agents can interfere with the ECG and EEG measurements (Modica *et al* 1990). To establish a baseline, ECG and EEG measurements were made for 1 min prior to the start of injecting the gas after the birds were placed inside the container. Data were saved onto the dataloggers for later analysis. Heart rate was calculated from the ECG data at 10-s intervals from the baseline up to 5 min after removal from the container. The ECG diagram was also analysed for irregular and ineffective heart beats (as indicated by, for example, fluttering and ST deviations) by a trained observer.

The moment of loss of consciousness during stunning was determined based on changes in amplitude and frequency of the EEG recordings using LabChart Pro 7 software (AD Instruments Ltd, Oxford, UK). Baseline EEG activity consisted of low amplitude, high frequency activity reflecting the birds' state of alertness (McKeegan 2007a). Consistent changes in the EEG were assigned by a trained observer to characteristic phases namely: 'suppressed' comprising a substantially suppressed EEG containing some slow-wave activity, indicating loss of consciousness; 'minimal activity' comprising a strongly

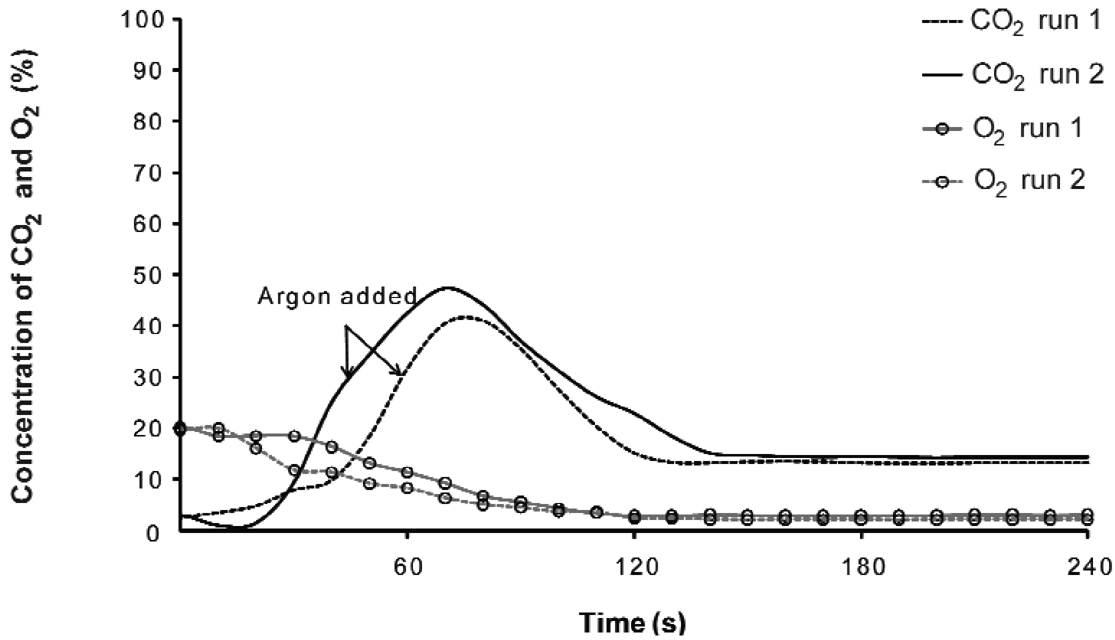
https://doi.org/10.7120/09627286.22.1.005 Published online by Cambridge University Press

Figure 1



CO_2 and O_2 concentrations during two runs of the CO_2 treatment.

Figure 2



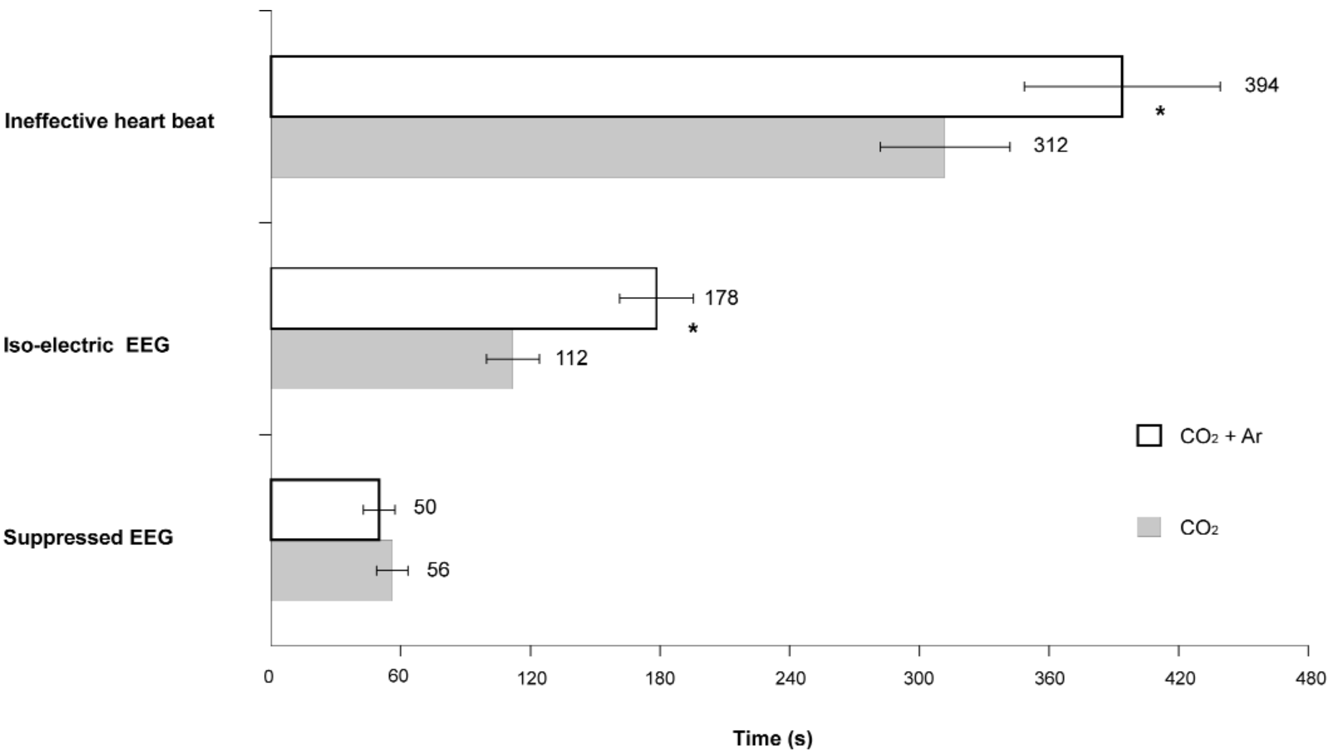
CO_2 and O_2 concentrations during two runs of the treatment with Ar and CO_2.

suppressed EEG which is close to but not a fully achieved isoelectric state; and 'isoelectric' or a flat EEG recording comprising residual low-level noise indicating lack of EEG activity (McKeegan 2007a). Time of death was based on brain and heart activity, ie iso-electric EEG signal together with absence of an effective heart beat.

Statistical analysis

Data saved in the data loggers were entered in LabChart Pro 7 software (AD Instruments Ltd, Oxford, UK) and analysed with the Residual Maximum Likelihood (REML) procedure in GenSat (Lawes Agricultural Trust 2007). The onsets of suppressed, minimal and iso-

Figure 3



Mean (± SEM) time from exposure to gas to reaching unconsciousness (suppressed EEG), minimal brain activity (iso-electric EEG) and ineffective heart beat in the two treatments (CO_2 versus CO_2 and argon). * Differ significantly at $P < 0.05$.

electric EEG were determined in each individual as well as the onset of a irregular and ineffective heart beat. Differences in time between the treatments were analysed using pair-wise comparisons (ppair).

Ethical aspects of the experiment were judged and approved by the Animal Ethical Committee of the Animal Sciences Group, The Netherlands.

Results

Gas concentrations during stunning

Measured concentrations of CO_2 and O_2 during two runs are depicted in Figure 1 (only CO_2) and Figure 2 (CO_2 and Ar). In trials using only CO_2, the concentration of CO_2 rapidly and consistently increased to almost 80% within 60 s. Oxygen concentrations decreased simultaneously from 18 to approximately 4.5% and remained at this level until ventilation with normal air started.

In the CO_2-Ar treatment, oxygen levels dropped to below 1% within approximately 120 s. After the CO_2 supply was shut down at 30%, Ar was added. CO_2 levels hereafter peaked at 47% at approximately 70 s after the onset of gas injection. The final mixture contained approximately 19% CO_2, 79% Ar and less than 2% O_2.

Heart rate and EEG measurements

In the 80% CO_2 treatment, geese showed ineffective heart beat and minimal brain activity significantly quicker than in the CO_2-Ar treatment ($P < 0.05$). The time taken to reach unconsciousness (ie suppressed EEG) did not differ between the two treatments (Figure 3).

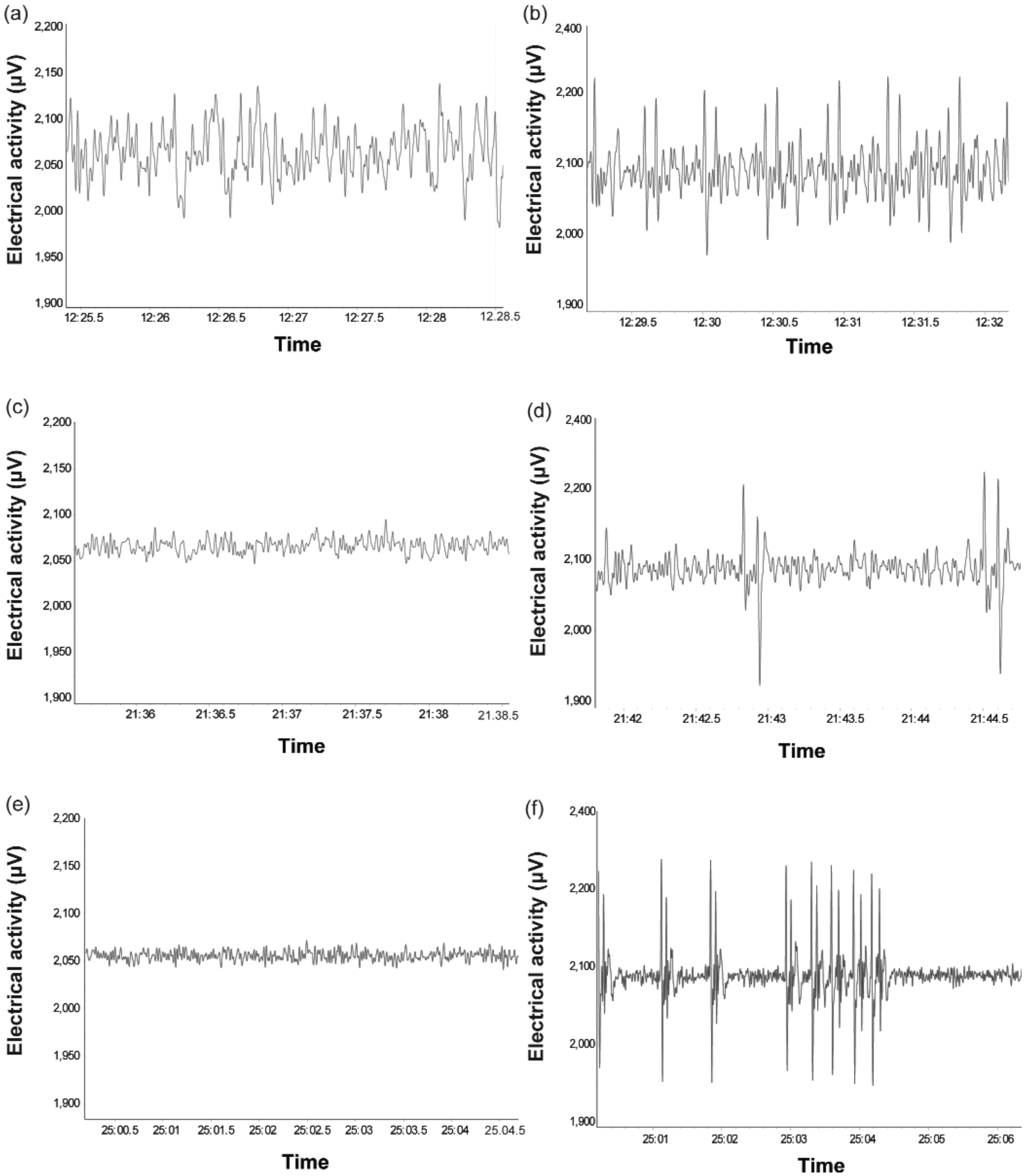
Representative EEG and ECG traces of different situations, such as iso-electrical line and ineffective heart beat from randomly selected geese from the CO_2 treatment, are shown in Figure 4.

Discussion

The main aim of this experiment was to investigate the welfare effects of two gas mixtures during the killing of wild geese in terms of heart and brain activity (ECG and EEG). Gas mixtures consisted of 80% CO_2 or a combination of 30% CO_2 and 70% Ar. Geese killed with only CO_2 reached a minimal brain activity and ineffective heart beat significantly faster than geese treated with a combination of CO_2 and Ar. However, the time to reach unconsciousness as indicated by a suppressed EEG did not differ significantly between the treatments. Gases used for stunning slaughter animals can be divided into gases that displace O_2 from the breathing air, such as Ar and N_2, and gases that directly affect the central nervous system, such as CO_2 (Van den Bogaard

https://doi.org/10.7120/09627286.22.1.005 Published online by Cambridge University Press

Figure 4



Examples of changes in brain activity (EEG, left) and heart beat (ECG, right) in geese during CO_2 treatment.

et al 1985). CO_2 is an anaesthetic gas that produces rapid unconsciousness when inhaled at high concentrations (Forslid *et al* 1986). High CO_2 concentrations in the blood (pCO_2) decrease the respiratory rate, followed by slower and deeper breathing (gasping). This reaction of the body is directed at reducing the CO_2 concentrations in the blood. However, because the CO_2 concentration in the breathing air is greatly increased, the pCO_2 value will continue to rise and the pH of the blood will decrease. Inhaling CO_2 concentrations above 15% induces a decrease in blood pH (average of 7.4 to 7.0), immediately followed by a reduction in the pH of the cerebrospinal fluid, which causes unconsciousness at pH < 6.8 (Martoft *et al* 2003; Gerritzen *et al* 2006). Chickens are already responding to a small increase in CO_2 concentration by breathing faster (Jukes 1971). Even at slightly elevated CO_2 concentrations (< 1%), the animals respond and this occurs even before any changes in the blood can be measured by the chemoreceptors in the lungs (Hempleman & Posner 2004). Broilers responded aversively to concentrations of CO_2 gas above 40%, showing rejection and avoidance behaviour (Raj *et al* 1998). These aversive reactions did not occur when chickens were suddenly exposed to the inert gas Ar (McKeegan *et al* 2007a,b). With a sudden exposure to CO_2 concentrations above 40%, mucous membranes are irritated and breathing is painful (Raj *et al* 1998). When the CO_2 concentrations gradually increase, chickens already begin to lose consciousness at 20% CO_2 (Gerritzen *et al* 2006). In such cases, the animals do not perceive the elevated CO_2 concentrations as painful. Therefore, it seems better to kill animals using gradually increasing CO_2 concentrations. At CO_2 concentrations of around 5%, more blood is pumped around without causing any significant increase in heart rate (Wideman *et al* 1999). An increase in heart beat was also seen in this study in both treatments. Independent of O_2 concentration, a CO_2 concentration of greater than 40% will quickly result in delayed and irregular heart beat (bradycardia). Within 10–20 s, the heart rate is reduced by 30–50% (McKeegan *et al* 2007a,b). An oxygen-free environment also reduces the heart rate, but only by 10% (Coenen *et al* 2000).

McKeegan *et al* (2007a) showed four different phases of the EEG in chickens anaesthetised with a two-phase CO_2 stunner (CAS). The first stage shows a normal EEG pattern in the chicken that is yet to be exposed to CAS, with a characteristically low amplitude and high frequency. The second phase (initial phase) shows a higher amplitude with a reduced frequency. The third phase is characterised by a depressed and irregular EEG signal. Phase 4 shows a very strongly suppressed EEG and an almost isoelectric line. The start of the second phase is not dependent on the gas mixtures used, however its duration is. Phase 3 occurs later under anoxic conditions than under high CO_2 concentrations (McKeegan *et al* 2007a). Phase 4 starts later when chickens are stunned in a two-phase CO_2 system, with 40% CO_2 and 30% O_2 for 1 min and 80% CO_2 for 2 min.

In an experimental design where CO_2 was gradually increased and O_2 reduced, Gerritzen *et al* (2004) noted that the moment at which chickens failed to mainain a standing or sitting position (falling) coincided with the appearance of theta (4–8 Hz) and delta waves (0–4 Hz) in the EEG. Therefore, a large proportion of low frequencies on the EEG is indicative of the onset of unconsciousness. The appearance of theta and delta waves in the EEG took place earlier when the animals were exposed to increasing CO_2 concentrations resulting from a 100% CO_2 source, compared to when the animals were exposed to increasing concentration resulting from a source with 50% CO_2 and 50% nitrogen (Gerritzen *et al* 2004). This indicates that an increase in CO_2 more strongly suppressed brain activity than a reduction of O_2.

When making decisions concerning the method of killing from an animal welfare point of view, it is important to look at the whole process instead of only the method of killing itself.

For the application of most stunning methods, it is necessary to restrain the animals. Animals may be free-moving when stunned in a gas chamber. Killing wild geese by means of a high concentration of CO_2 or a mixture of Ar and CO_2 can be an acceptable method under certain circumstances. In fast-rising gas concentrations, both in CO_2 and CO_2-Ar, geese lost consciousness within 1 min, which was the maximum duration of potential discomfort caused by exposure to the gas. Discomfort can be associated with difficulty in breathing, anxiety, muscular contractions, escape attempts and possible irritation of the mucous membranes (Ewbank 1983; Raj & Gregory 1994; Raj & Gregory 1995; Ludders *et al* 1999). The degree of discomfort will depend on the speed with which the CO_2 concentrations are increased. A slower increase of the CO_2 concentration causes a milder induction of unconsciousness than a faster increase. Duration of the discomfort, however, will be prolonged by a slow increase in CO_2 concentration (Gerritzen *et al* 2007). In this study, convulsions were registered when heard from the outside, since no cameras were placed inside the container. Convulsions occurred in both treatments, but were more severe and lasted longer in the CO_2-Ar treatment. When inert gases such as Ar are used, severe muscle convulsions or uncontrolled muscles spasms may occur. It is commonly assumed, however, that convulsions take place after animals have gained unconsciousness. However, muscle movements have also been observed in poultry prior to reaching unconsciousness (McKeegan *et al* 2007a,b). The occurrence of uncontrolled muscle spasms during consciousness will have a strongly negative impact on the welfare of the animals. Since animals do not lose consciousness at all the same time, prevention of muscle convulsions is recommended.

It is likely that animals experience a certain degree of stress during capture and transport which is only partially avoidable. In herding and capturing the animals in this study, using the standard applied method of Duke Fauna management, possible stress and fear have been taken into account (Duke Fauna management, undated). Calmly herding and luring using food created the opportunity for geese to observe the route they were supposed to take. Luring geese with food is not an option, when geese are not used to being fed (as may be the case in some urban areas). Stress and discomfort caused by transport may be reduced further by using sedation and killing of animals in transport trailers suited to perform gas stunning. It is important to provide a controlled and safe gas administration, by which both animal welfare and the safety of the procedures for humans can be guaranteed.

Animal welfare implications and conclusion

For multiple reasons, but mainly due to the anaesthetic function of CO_2 and the non-irritating advantages of Ar, a gas mixture of 30% CO_2 and 70% Ar was used (Raj 2006; McKeegan *et al* 2007a). From this study it cannot be concluded that the addition of Ar improved the welfare of the geese killed, compared to rapidly increasing CO_2 concentrations. Lowering the CO_2 concentration in the CO_2-Ar treatment may reduce aversiveness during exposure and in this way improve animal welfare.

Acknowledgements

This research was funded by the Ministry of Agriculture, Nature and Food Quality, project number BO-07-011-063.

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