

## Physiological and behavioral responses of poultry exposed to gas-filled high expansion foam

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**ABSTRACT** Disease control measures require poultry to be killed on farms to minimize the risk of disease being transmitted to other poultry and, in some cases, to protect public health. We assessed the welfare implications for poultry of the use of high-expansion gas-filled foam as a potentially humane, emergency killing method. In laboratory trials, broiler chickens, adult laying hens, ducks, and turkeys were exposed to air-, N<sub>2</sub>-, or CO<sub>2</sub>-filled high expansion foam (expansion ratio 300:1) under standardized conditions. Birds were equipped with sensors to measure cardiac and brain activity, and measurements of oxygen concentration in the foam were carried out. Initial behavioral responses to foam were not pronounced but included headshakes and brief bouts of wing flapping. Both N<sub>2</sub>- and CO<sub>2</sub>-filled foam rapidly induced ataxia/loss of posture and vigorous wing flapping in all species, characteristic of anoxic death. Immersion in air-filled, high expansion foam had little effect on physiology or behavior. Physi-

ological responses to both N<sub>2</sub>- and CO<sub>2</sub>-filled foam were characterized by a pronounced bradyarrhythmia and a series of consistent changes in the appearance of the electroencephalogram. These were used to determine an unequivocal time to loss of consciousness in relation to submersion. Mean time to loss of consciousness was 30 s in hens and 18 s in broilers exposed to N<sub>2</sub>-filled foam, and 16 s in broilers, 1 s in ducks, and 15 s in turkeys exposed to CO<sub>2</sub>-filled foam. Euthanasia achieved with anoxic foam was particularly rapid, which is explained by the very low oxygen concentrations (below 1%) inside the foam. Physiological observations and postmortem examination showed that the mode of action of high expansion, gas-filled foam is anoxia, not occlusion of the airway. These trials provide proof-of-principle that submersion in gas-filled, high expansion foam provides a rapid and highly effective method of euthanasia, which may have potential to provide humane emergency killing or routine depopulation.

**Key words:** electroencephalogram, foam, poultry, euthanasia, welfare

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## INTRODUCTION

Disease control measures require poultry to be killed on farms to minimize the risk of disease being transmitted to other poultry, and in some cases, to protect public health. Several techniques (mechanical, electrical, pharmacological, gaseous, and fire-fighting foam) have been employed for this purpose, each of which presents practical challenges, raises welfare concerns, or both. Recently, the use of CO<sub>2</sub> as a lethal gas (delivered in a liquid that then vaporizes) in poultry houses has been advocated because of its practicality, particularly

because it eliminates the need to handle the birds, a vital advantage if worker health is at risk as in the case of highly pathogenic avian influenza strains. Furthermore, killing animals in their production housing reduces animal welfare risks associated with live animal handling. However, unlike some other methods of killing, it is not instantaneous. Recent research (Gerritzen, 2006; Sparks et al., 2010; McKeegan et al., 2011) shows that although whole house gassing is effective, birds remain conscious for considerable periods during the process and may experience prolonged respiratory distress. Moreover, not all poultry houses are suited for whole house gassing because it is required that the buildings are sealable to a certain extent (Gerritzen et al., 2006b).

An alternative method is the use of medium expansion (expansion ratio 25:1 to 140:1) fire-fighting foam

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filled with air, and although not currently approved for use in the European Union, this approach has been tested and conditionally approved in the United States for use in specific circumstances (Dawson et al., 2006; USDA-APHIS, 2006; Benson et al., 2007). These are depopulation of floor-reared poultry (i.e., broiler chickens and turkeys) in accordance with USDA-APHIS performance standards; animals infected with a potentially zoonotic disease; animals experiencing an outbreak of a rapidly spreading infectious disease that cannot be contained by conventional means of depopulation; or animals housed in structurally unsound buildings that would be hazardous for human entry, such as those that may result from a natural disaster. Application of foam has several potential advantages including reducing the number of people in contact with the birds, and reduced use of gas, which may be in short supply in the event of a disease outbreak. Furthermore, high expansion foam (expansion ratio more than 250:1) can be applied for whole flock treatment in open buildings. The foam used in the United States is medium-expansion, medium-density foam with small diameter bubbles. It operates as a killing agent by rapidly occluding the airways of the birds, causing death by hypoxia (Benson et al., 2007). Because of welfare concerns surrounding this approach, alternatives such as gas-filled high expansion foam are now being investigated. Anoxic gases have not thus far been used for whole-house gassing because of the practical impossibility of sealing the house to the extent required to adequately eliminate oxygen. The use of high expansion gas-filled foam containing an anoxic gas presents a potentially feasible alternative delivery method of anoxic killing (McKeegan et al., 2007; Gerritzen and Sparrey, 2008) because as the foam envelops the bird, oxygen availability will be effectively eliminated. Raj et al. (2008) presented preliminary results on the use of high expansion foam filled with N<sub>2</sub> and concluded that this approach may have welfare advantages over use of CO<sub>2</sub> gas or medium-expansion foam. Alphin et al. (2010) reported the use of CO<sub>2</sub> filled foam as a euthanasia method, but the expansion ratio of the CO<sub>2</sub>-infused foam they applied was only 21:1; therefore, it is unlikely that the gas held in the foam matrix was available to be respired by the bird. It may in fact have acted as an irritant and the mode of action would still have been occlusion of the airway. Therefore, the effectiveness and welfare consequences of the application of gas-filled high-expansion foam remain to be rigorously examined, and issues such as initial aversion to foam, potential inhalation of foam, and time to loss of consciousness after immersion must be considered. The aim of this study was to investigate whether, in principle, high expansion foam filled with N<sub>2</sub> or CO<sub>2</sub> could be a humane medium for killing of poultry. Broilers, ducks, turkeys, and hens were exposed to anoxic, hypercapnic, or control (air filled) foam under standardized conditions while their behavioral and physiological responses were monitored. The work required the development of a small-scale system

to deliver gas foam with similar specifications to that which would be used in the operational disease control situation with regard to expansion ratios, surfactant type, temperature of delivery, speed of delivery, method of gas delivery, bubble diameter, and bubble composition. The experiments took place in the United Kingdom (broilers and hens, N<sub>2</sub> foam) and the Netherlands (broilers, ducks, and turkeys, CO<sub>2</sub> foam), in each case using the same testing apparatus, recording equipment, and foam generator.

## MATERIALS AND METHODS

### Test Apparatus

The foam trials were carried out in a large clear plastic box (1 m × 1 m × 1 m). One wall of the box was removable to allow full access for bird placement and bird retrieval and foam removal after each trial. The floor of the box was covered by plastic mesh (aperture ~10 mm) to prevent birds from slipping on the smooth surface. To record each trial and allow detailed behavioral observations, a video camera (JVC, London, UK) was positioned with a complete view of one side of the box, and a web cam (Logitech, Vaud, Switzerland) in a waterproof box was positioned under the box to view the base. The apparatus was fitted with instrumentation to measure oxygen concentration in the foam; these were zirconium dioxide dynamic oxygen sensors (Teda MF420-O-Zr, J Dittrich Elektronik GmbH, Baden-Baden, Germany) with a signal processing and recording system developed by Solutions for Research Ltd. (Bedford, UK). Each sensor was mounted in a short stainless steel tube behind a sintered bronze plug to prevent the ingress of moisture; in addition, the probe was heated so that any moisture coming into contact with the sensors evaporated. Three oxygen sensors were positioned at heights of 10, 30, and 90 cm in one corner of the box and were protected from bird movement by a wide metal mesh grid (aperture 20 mm).

### Foam Generation

Many high-expansion foam generators work by spraying foam solution onto a mesh through which air is driven by a fan, but for the current experiment a system to generate high expansion from compressed gas was developed. Simplification of construction, reduction of the complexity of effective cleansing, and disinfection after deployment and price were also considerations in the design. The laboratory method of foam generation was originally developed in Holland by LST International BV for use with CO<sub>2</sub> gas. It consisted of a pressure vessel containing a premixed solution of water and 3% foam concentrate (HTF 1000) per the manufacturers' recommendation (Ajax BV, Netherlands). The HTF 1000 was selected because of the expectation that the approach would ultimately be required to generate a structurally strong foam; this foam concentrate was de-

signed especially for high and very high expansion uses to build foam up to 12 m high.

The liquid tank was connected to the foam generator by a hose with a maximum working pressure of 20 bar. The foam generator itself consisted of a spray nozzle and a pair of wire mesh screens mounted inside a stainless steel cylinder of diameter 150 mm and length 250 mm. The gas was supplied directly to the generator from a compressed N<sub>2</sub> cylinder through a separate hose to the generator. The gas was released inside the closed body of the generator through an annulus drilled with 2.5-mm holes on a 2-cm pitch. The holes were directed to the back (sealed end) of the generator to reduce the effect of the gas jets distorting the spray cone. For the foam generator, a premix tank was pressurized by a separate compressed gas cylinder (Figure 1); the pressure vessel was used at small scale as an alternative to a pump. Changing the pressure altered the spray pattern of the nozzle, the aim being to generate a cone of spray that delivered a uniform flow of premix across the first mesh. The characteristics of the cone were also changed by the flow of gas through the generator, which distorts the flow pattern. It was therefore necessary to balance the pressure and flow of the premix with the flow of the gas through the generator.

With a designed expansion rate of 300:1 and a nominal premix flow rate of 2.5 l per min, the theoretical foam output of the system was 0.75 m<sup>3</sup> per min. The gas was supplied from compressed N<sub>2</sub> or CO<sub>2</sub> cylinders through high-flow regulators. The air-filled foam was generated by opening the rear of the generator cylinder. The jet effect of the spray nozzle caused a pressure drop across the cylinder and therefore an air flow through

the cylinder sufficient to develop the high expansion foam. This method of creating air-filled foam resulted in a slightly lower expansion rate of 250:1.

### Subjects and Husbandry

In the United Kingdom, 20 broilers (Ross 308) were obtained at 1 d old from a commercial supplier and reared in a single group under commercially relevant conditions. The rearing pen was furnished with deep wood shavings litter and equipped with heat lamps. The birds had ad libitum access to food and water. Twenty adult hens (ISA Brown) were obtained at 42 wk of age from a commercial supplier and housed in individual cages. The cages had individual ad-lib feeders and drinkers, and each hen had visual and auditory contact with neighbors.

In the Netherlands, 20 broiler chickens (Ross 308) were obtained at 3 wk of age from a commercial supplier and reared individually for 2 wk in wire mesh pens. All broilers had visual and auditory contact with their neighbors. The rearing pen was furnished with a deep litter of wood shavings. The birds had ad libitum access to food and water. Ten white Peking ducks and 10 broad-breasted turkeys were obtained at 6 wk of age from a commercial supplier and reared individually for 2 wk in wire mesh pens. All birds had visual and auditory contact with their neighbors. The rearing pen was furnished with a deep litter of wood shavings. The birds had ad libitum access to food and water.

All experiments in the United Kingdom were carried out at Royal Veterinary College under Home Office Authority, which followed ethical approval. Experiments

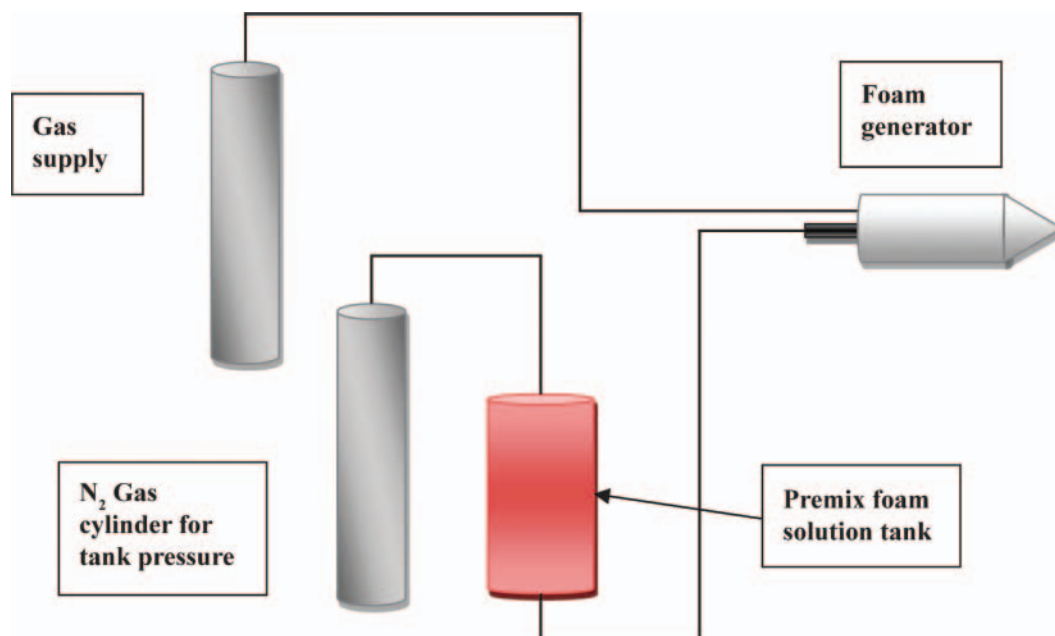


Figure 1. Diagram of laboratory-scale foam generator system. Color version available in the online PDF.

that took place in the Netherlands were authorized specifically by the Animal Ethical Committee of the Wageningen UR Animal Sciences Group.

### **Implantation of Electroencephalogram Electrodes**

At 28 d of age, broilers underwent surgery to implant electroencephalogram (EEG) electrodes. Hens underwent surgery after 10 d of acclimatization. Ducks and turkeys underwent surgery after 7 d of acclimatization. In all cases, EEG electrode implantation methods were identical. After an analgesic premedication (Buprenorphine, 0.2 mg/kg), general anesthesia was induced and maintained with Sevoflurane (United Kingdom) or Halothane (the Netherlands). The EEG was recorded by two 0.35-mm diameter Teflon insulated silver electrodes connected to a socket (DIN, RS Components, Corby, UK). The electrodes were placed on the dura through holes drilled in the skull, one on each of the dorsal surfaces of the right and left telencephalon at their approximate rostro-caudal and medio-lateral mid-points. An indifferent electrode placed between the skull and the overlying tissue under the comb was also connected to the socket. The EEG implant was secured to the skull with dental cement, and the surrounding skin was closed with sutures. After initial recovery from the anesthetic, all birds were housed in individual cages or pens with ad libitum access to food and water and visual and auditory contact with neighbors. All subjects were allowed to recover for a minimum of 5 d before undergoing any further experimental procedure.

### **Telemetry/Logging Units**

In previous work, a telemetric data logging system which enables electrocardiogram (ECG) and EEG waveforms to be simultaneously collected from freely moving birds was described (Lowe et al., 2007; Coenen et al., 2009). Logging units of the same design were used in the current study, and the challenge of moisture from the foam was dealt with by minor modifications to waterproof the loggers as far as was practical. Briefly, the telemetric logging units were battery powered; each was small enough to be worn by a bird in a Lycra backpack, thus requiring no trailing leads. Two physiological waveform input channels were provided and were used to record ECG (from external noninvasive exercise electrodes, see below) and EEG (from implanted electrodes). The loggers also had a radio communication facility, allowing bidirectional radio communication with a base station connected to a standard laptop computer. This allowed bursts of waveform to be requested and verified from the waveform channels during setup, demonstrating that the sensors were correctly placed and working, and permitted the level of signal amplification to be adjusted before the commencement of the trial. Logging was triggered and stopped over the radio link

and logged data were recorded onto industry-standard, micro-SD memory cards.

As in previous work, birds already fitted with permanent EEG electrodes were also fitted with ECG electrodes immediately before each trial (see above). These were commercially available, disposable, self-adhesive ECG electrodes (Blue Sensor, Ambu, St. Ives, UK), with press-stud electrical connections, which were adhered to cleaned skin overlying the pectoralis muscle on either side of the sternum. A harmless, cyanoacrylate, tissue adhesive (Vetbond, 3M, St. Paul, MN) was applied to the ECG electrodes before placement on the skin to improve bonding. Each bird was also fitted with a reusable Lycra harness which was secured using Velcro fastenings behind the bird's head and incorporated a pocket positioned on the bird's back to contain the telemetry/logging system.

### **Experimental Procedure**

Identical experimental procedures were used with all species. Individual birds were assigned randomly to N<sub>2</sub>-, CO<sub>2</sub>-, or air-filled foam treatments. Immediately before each trial, each bird was fitted with ECG electrodes and a Lycra harness containing a telemetry unit. The telemetry function was used to verify the existence of high-quality physiological signals on each channel, and adjustments were made as necessary. Signal logging was triggered and a short baseline period (2 min) was allowed during which the bird was placed in an open cardboard carrier in a room adjacent to the test area. After baseline recording, the bird was carried to the test area and placed in the center of the test apparatus. A clapper board with bird number and treatment was held in front of both cameras for identification purposes. The removable wall of the clear plastic box was replaced and a further baseline (2 min) was recorded. Foam was then introduced from the top of the box; care was taken not to aim the foam directly at the test subject. Timings of foam start, foam-touching bird, complete bird submersion and foam off (filling process stopped when foam depth was approximately 1 m) were noted and later confirmed with web cam recordings. Synchronization of timings of telemetry recordings and the web cam recordings ensured behavioral changes could be related to physiological responses. In N<sub>2</sub> and CO<sub>2</sub> foam trials, all measurements continued for 3 min after birds exhibited cessation of movement. In control (air foam) trials, all measures continued for 60 s after submersion, after which the bird was rapidly retrieved and immediately euthanized (barbiturate overdose, administered intravenously).

### **Behavioral Observations**

Visual obscuration by the foam limited the extent of detailed behavioral measurements. Nevertheless several observations were carried out from the video record-

ings of each trial. As foam was introduced, counts of gasping, headshakes, foam avoidance, and escape attempts were noted. After submersion, time to ataxia, time to loss of posture, wing flapping (flapping onset, number of bouts, total flapping duration), and cessation of movement were recorded (from the underside of the clear plastic box).

### Postmortem Examination

After removal from the apparatus, all birds were subject to a postmortem examination of the mouth, esophagus, and upper airway, specifically the trachea from the glottis to the syrinx. Presence of foam and any other abnormalities were noted and photographed.

### Analysis

The logged data files were uploaded into a data acquisition and analysis program (Spike 2 Version 4.2, Cambridge Electronic Design, Cambridge, UK). A combination of automated and manual analysis techniques were used to produce dedicated event channels representing heartbeats per min (2-s bins) from the raw traces during baseline and after foam application. Where clear waveforms were present, heart rate was measured every 5 s. Visual inspection of the EEG traces allowed estimation of the timing of onset of different types of EEG activity: baseline, transitional, suppressed, and isoelectric. Statistical analysis in the form of a GLM with bird type (broiler, hen, duck, and turkey) and gas (CO<sub>2</sub>, N<sub>2</sub>) as factors was performed for times to transitional, suppressed and isoelectric EEG and time to ataxia, flapping onset, and cessation of movement. This was followed by post-hoc paired *t*-tests to highlight differences between bird types and gases.

## RESULTS

During initial trials with laying hens, it became apparent while reviewing physiological traces that some type of noise interference was affecting EEG recordings. This was eventually traced to moisture interacting with the temperature and respiration sensors, and these were

disconnected so that in later trials only ECG and EEG were recorded. To compensate for the loss of EEG data, a total of 12 hens were exposed to anoxic (N<sub>2</sub>-filled) foam, whereas 8 were exposed to control (air-filled) foam. Ten broilers were exposed to anoxic (N<sub>2</sub>-filled) foam and 10 were exposed to control (air-filled) foam. Eight broilers, 9 ducks, and 10 turkeys were exposed to CO<sub>2</sub>-filled foam.

### Oxygen Concentration Measurements

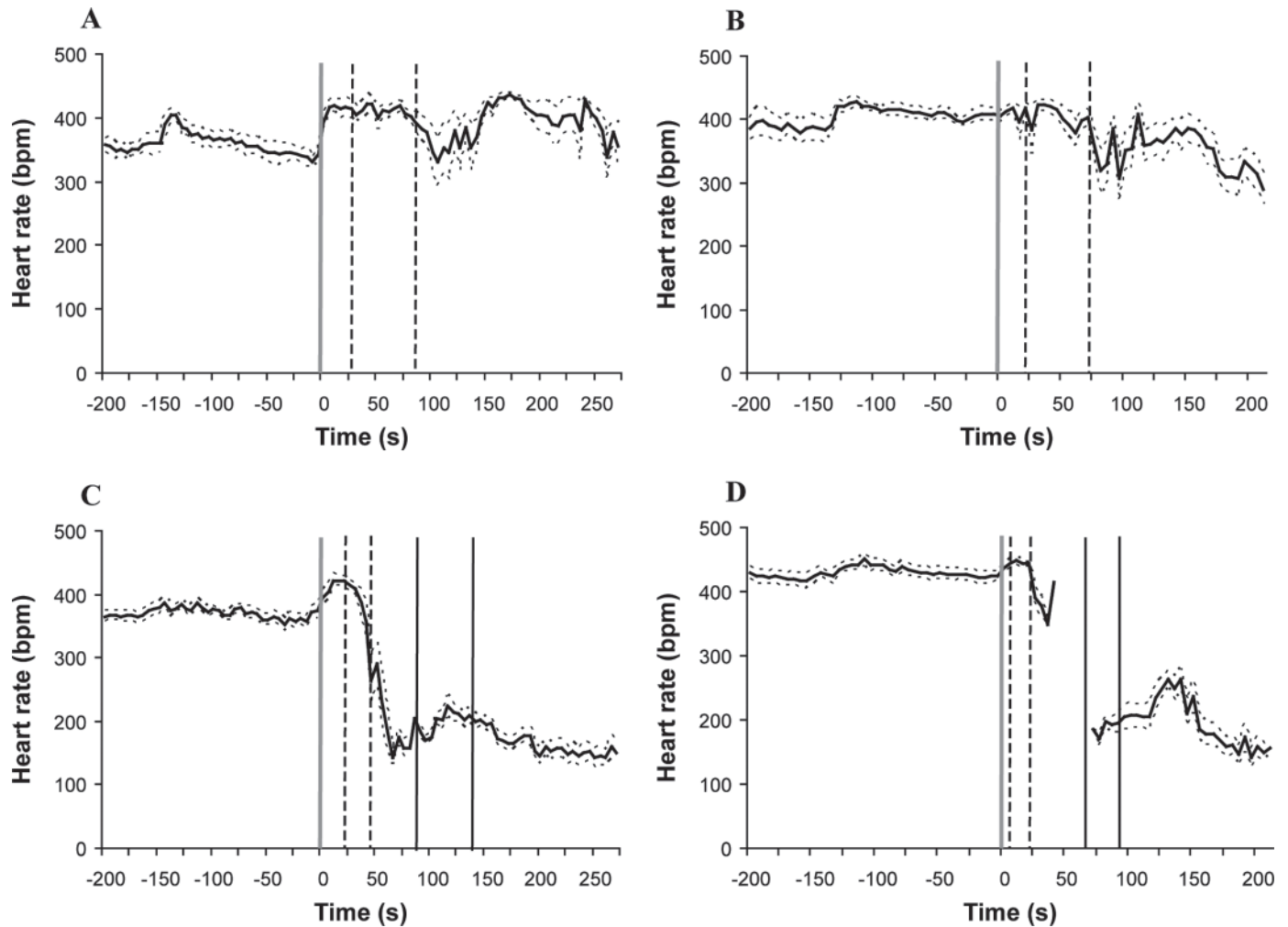
Mean measurements of oxygen concentration in N<sub>2</sub>-filled foam are shown in Table 1 (trials with hens) and were very similar for trials with the other gases and species. Mean values were taken from 1 min after each sensor was submerged in foam and calculated over the following 5 min. It was apparent that very low oxygen concentrations were achieved in the foam (regularly below 1% and the majority below 2%) at 10 and 30 cm heights. The uppermost sensor (90 cm) was frequently not immersed in foam, which accounts for the higher oxygen values recorded. Oxygen concentrations in the air-filled foam (data not shown) were very similar to ambient, falling in some cases to a reading of 15%, most likely due to occlusion of the sensor or the presence of slightly denser foam with a higher water content.

### ECG Responses

Figure 2 shows mean ( $\pm$ SE) changes in heart rate in response to exposure to either air- or N<sub>2</sub>-filled foam. Initial exposure to air-filled foam was associated with a rise in heart rate in hens (likely associated with a fear response due to novel environmental stimuli (e.g., noise of foam generation), but this response was less apparent in broilers. During submersion in air-filled foam, both hens and broilers exhibited a trend for a slight temporary reduction in heart rate, but after 60 s rates were similar to baseline. Anoxic (N<sub>2</sub>-filled) foam was associated with an initial heart rate increase (again likely a fear response that was more pronounced in hens), followed by rapid and pronounced bradyarrhythmia, which is an expected response to anoxia. An almost identical pattern of response was seen during exposure to CO<sub>2</sub>-

**Table 1.** Mean oxygen concentrations in N<sub>2</sub>-filled foam at 10, 30, and 90 cm during trials with hens

Bird number	10 cm, % oxygen (mean)	30 cm, % oxygen (mean)	90 cm, % oxygen (mean)
2	0.82	1.13	19.80
3	0.33	1.08	13.40
4	1.82	0.81	19.93
5	0.76	0.84	19.88
6	3.00	0.87	19.82
9	0.64	4.98	19.50
11	0.96	0.87	19.64
14	0.25	1.56	15.38
15	0.93	1.05	19.57
17	1.64	0.96	19.45
18	0.58	0.91	17.86
19	2.67	2.28	19.93



**Figure 2.** Graphs showing changes in mean heart rate ( $\pm$ SE) in hens exposed to air-filled foam (A), broilers exposed to air-filled foam (B), hens exposed to  $N_2$ -filled foam (C), and broilers exposed to  $N_2$ -filled foam (D). Line markers indicate timings of foam start (solid gray line), earliest and latest submersion (dashed lines), and earliest and latest time to cessation of movement (solid black line, C and D only).

filled foam (data not shown). Generally, the trend of a substantial fall in heart rate was followed by varying degrees of recovery (tachycardia), stabilization, or both, before a final decline. Throughout recording, the ECG waveform was sometimes obscured due to electromyogram activity arising from the pectoral muscles or movement artifacts.

### EEG Responses

As explained above, some of the EEG files from hens exposed to  $N_2$ -filled foam were unusable, particularly toward the end of the traces (as the equipment became more affected by moisture ingress). In all species, baseline EEG activity consisted of low-amplitude, high-frequency activity reflecting the birds' alert state. In some birds exposed to air-filled (control) foam, the EEG pattern did not deviate from the baseline state, with no noticeable EEG changes (Figure 3A,B), whereas in others there was evidence of slow wave activity during submersion (Figure 3C).

During exposure to  $N_2$ - or  $CO_2$ -filled foam, a series of consistent changes in the appearance of the EEG were apparent. Visual inspection of the traces was used to assign portions of the EEG to 1 of 4 phases with particular characteristics where baseline was as before foam introduction (Figure 3D); transitional was high-amplitude, low-frequency activity or high frequency but reduced amplitude signal; suppressed was a greatly suppressed EEG but containing some slow wave activity; and isoelectric was residual low-level noise indicating lack of EEG activity. Transitional EEG tended to be characterized by slow wave (high amplitude, low frequency) activity, and this response was seen in all birds exposed to anoxic foam (Figure 3E). Table 2 shows the timings of phase changes in all species exposed to  $N_2$ - and  $CO_2$ -filled foam. Time to transitional EEG was affected by gas ( $P = 0.003$ ), whereas time to suppressed EEG was influenced by species ( $P < 0.001$ ).

Suppressed EEG is a reliable indicator of loss of consciousness. On this basis, the mean measured time to unequivocal loss of consciousness (in relation to time

of submersion) with N<sub>2</sub>-filled foam was 30 s in hens and 18 s in broilers. In CO<sub>2</sub>-filled foam, time to loss of consciousness (in relation to time of submersion) was 16 s in broilers, 1 s in ducks, and 15 s in turkeys. The *t*-test comparisons revealed that in response to N<sub>2</sub>-filled foam, broiler chickens exhibited suppressed and isoelectric EEG sooner than hens ( $P < 0.001$ ,  $P = 0.004$ , respectively). In CO<sub>2</sub>-filled foam, time to suppressed EEG was more rapid than with N<sub>2</sub> ( $P = 0.024$ ). As can be clearly seen in Table 2, in all species exposed to CO<sub>2</sub> foam, transitional EEG was apparent before submersion. With N<sub>2</sub>-filled foam, transitional EEG was seen only after submersion. In ducks exposed to CO<sub>2</sub> foam, there was a trend toward earlier appearance of suppressed EEG ( $P = 0.075$ ). Notably, no differences in time to onset of isoelectric EEG between species or gas used to fill the foam were observed.

### Behavioral Responses

Birds exposed to air-filled foam exhibited headshakes, escape attempts, and wing flapping. Headshaking was seen in both hens and broilers in response to initial foam delivery [mean number  $2.1 \pm 1.3$  (SE) in hens and  $2.5 \pm 0.8$  in broilers]. Escape attempts (vertical jumps at box wall) were seen in one hen (2 attempts) in response to control foam delivery. After submersion, some birds exhibited brief and sometimes repeated wing flapping/struggling responses (mean number of bouts  $1.4 \pm 0.4$  in hens and  $3.5 \pm 0.7$  in broilers).

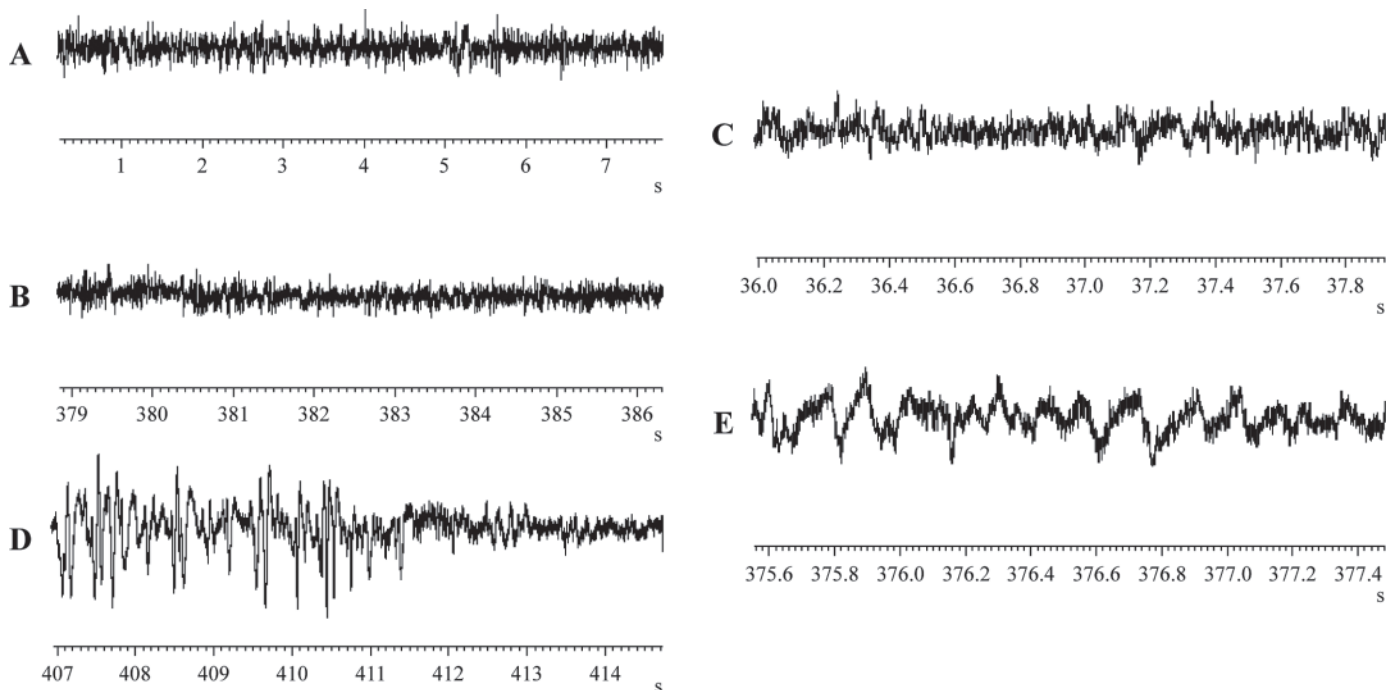
Table 3 summarizes the behavioral responses exhibited by hens and broilers in response to N<sub>2</sub>-filled foam.

**Table 2.** Mean  $\pm$  SE timings (s) of electroencephalogram phase changes in hens, broilers, ducks, and turkeys exposed to N<sub>2</sub>- or CO<sub>2</sub>-filled foam<sup>1</sup>

Item	Transitional	Suppressed	Isoelectric
Hens, N <sub>2</sub>	10 $\pm$ 1	30 $\pm$ 2	65 $\pm$ 3
Broilers, N <sub>2</sub>	8 $\pm$ 1	18 $\pm$ 1	47 $\pm$ 2
Broilers, CO <sub>2</sub>	-2 $\pm$ 1	16 $\pm$ 1	66 $\pm$ 9
Ducks, CO <sub>2</sub>	-11 $\pm$ 1	1 $\pm$ 1	58 $\pm$ 8
Turkeys, CO <sub>2</sub>	-2 $\pm$ 1	15 $\pm$ 1	60 $\pm$ 3

<sup>1</sup>All timings are in relation to submersion in foam.

As in control birds, headshakes were observed (frequency not different from control foam in hens or broilers, confirmed by *t*-test), as were escape attempts (hens only, 2 individuals). Anoxic foam induced ataxia/loss of posture and vigorous wing flapping characteristic of anoxic death. There was an effect of species on time to cessation of movement ( $P = 0.005$ ) and effects of gas on time to ataxia ( $P = 0.025$ ) and flapping onset ( $P = 0.001$ ). Time to ataxia was seen between on average  $16 \pm 1.1$  s after submersion in hens but earlier ( $9 \pm 1.3$  s) in broilers ( $P = 0.001$ , *t*-test). Mean onset of vigorous wing flapping was  $18 \pm 1.1$  s in hens and  $15 \pm 1.3$  s in broilers and was not different between species. Number of flapping bouts also did not differ between hens and broilers exposed to N<sub>2</sub> foam (both mean  $4 \pm 0.3$ ). Flapping duration was also not different (mean  $14 \pm 1.1$  s and  $14 \pm 1.9$  s for hens and broilers, respectively) Time to cessation of movement was shorter in broilers than hens (hens  $65 \pm 3.5$  s, broilers  $51 \pm 2.3$  s;  $P = 0.004$ , *t*-test).



**Figure 3.** Electroencephalogram (EEG) trace excerpts from 2 birds in response to submersion in air- or N<sub>2</sub>-filled foam. A) Hen EEG during baseline; B) hen EEG after 50 s of submersion in air-filled foam; C) broiler EEG during baseline; D) broiler EEG showing slow wave activity after 30 s of submersion in air-filled foam; and E) broiler EEG showing a transitional pattern in response to N<sub>2</sub>-filled foam.

**Table 3.** Behavioral responses exhibited by hens and broilers in response to N<sub>2</sub>-filled foam<sup>1</sup>

Item	Headshakes	Ataxia/loss of posture (s)	Flapping onset (s)	Flapping bouts	Flapping duration (s)	Time to cessation of movement (s)
Hens	2 ± 1	16 ± 1	18 ± 1	4 ± 0	14 ± 1	65 ± 3
Broilers	2 ± 1	9 ± 1	15 ± 2	4 ± 0	14 ± 2	51 ± 2

<sup>1</sup>Data are shown as mean ± SE. All timings indicated are in relation to submersion in foam.

Responses to CO<sub>2</sub>-filled foam were broadly similar to N<sub>2</sub>-filled foam in terms of the order of events, though as noted above the effects of exposure to the gas started before submersion. For this reason, the timings of behavior responses shown in Table 4 are in relation to foam onset, not submersion. Unlike with N<sub>2</sub> foam, gasping was seen in response to CO<sub>2</sub>-filled foam. Times to ataxia, flapping onset, and time to cessation of movement were not different between broilers, ducks, and turkeys exposed to CO<sub>2</sub>-filled foam (Table 4).

### Postmortem Observations

In response to air-filled foam exposure, 4 out of 8 birds swallowed foam so that it was visible in the mouth and esophagus. A few birds regurgitated food during exposure to air-filled foam. Many birds exposed to control foam also had small amounts of foam in the tracheal opening, but the trachea was never occluded. Birds exposed to N<sub>2</sub>-filled foam also had foam in the mouth and esophagus, and some regurgitated food. Birds exposed to N<sub>2</sub>-filled foam regularly had foam present deeper in the trachea than controls (10/12 hens and 9/10 broilers). In one hen, foam was present as far down as the syrinx, but foam was more usually observed 3 to 10 cm from the tracheal opening. The foam was visible as a few tiny bubbles clinging to the tracheal wall, and in no instance was the trachea even partially occluded by foam. No other abnormalities were observed in the mouth, esophagus, or upper airway. The postmortem results of birds exposed to CO<sub>2</sub>-filled foam were very similar to those of birds exposed to N<sub>2</sub>-filled foam, with a few tiny bubbles occasionally present but no evidence of occlusion of the airway.

## DISCUSSION

Behavioral responses to initial foam delivery varied between birds but were not particularly pronounced.

The responses observed seemed to be more related to the noise from the foam generator rather than the foam per se, and birds tended to stay still while the foam enveloped them. Transient increases in heart rate corroborate that fear was likely to be experienced during initial foam introduction. Responses to submersion in air-filled foam provide a control to determine responses to the foam per se (rather than any gas it contained), and there was some evidence that being submerged was associated with some distress. Most birds exposed to control foam swallowed or inhaled small amounts of foam (or both), some regurgitated food, and all appeared distressed by the procedure on removal. However, it is worth pointing out that control birds were submerged conscious for 60 s, which is more prolonged than the duration experienced by birds exposed to the N<sub>2</sub> or CO<sub>2</sub> foam (on average conscious for between 18 and 30 s and 1 to 16 s after submersion, respectively). During submersion in air-filled foam, some birds exhibited slow-wave EEG patterns characteristic of sleep or reduced vigilance, and these are probably associated with protective eye closure in response to the foam. Eye closure and the generation of slow wave EEG are closely associated in birds (D. E. F. McKeegan, unpublished observations). A slight reduction in heart rate was also apparent during submersion in air-filled foam, and this may be related to the dive reflex in response to submersion during which a bradycardia is evoked to conserve oxygen (Borg et al., 2004).

Consistencies in responses such as headshaking suggest that the hens did not perceive any difference between the air-filled and N<sub>2</sub>-filled foam before submersion. However, birds exposed to CO<sub>2</sub>-filled foam displayed gasping and increased headshaking before losing consciousness than those exposed to N<sub>2</sub>-filled foam. This is likely to be due to a boundary layer of gas surrounding the foam, caused by CO<sub>2</sub> that was fed to the generator but not trapped in bubbles, or escaping from bursting bubbles as they hit the Perspex box.

**Table 4.** Behavioral responses exhibited by broilers, ducks, and turkeys in response to CO<sub>2</sub>-filled foam<sup>1</sup>

Item	Gasping (s)	Headshaking (s)	Ataxia/loss of posture (s)	Flapping onset (s)	Time to cessation of movement (s)
Broilers	25 ± 2	23 ± 0	54 ± 6	65 ± 5	54 ± 5
Ducks	13 ± 1	10 ± 1	38 ± 4	43 ± 2	69 ± 7
Turkeys	14 ± 1	10 ± 2	47 ± 2	63 ± 7	60 ± 7

<sup>1</sup>Data are shown as mean ± SE. All timings indicated are in relation to foam onset because some responses were seen before submersion.



This would also have been the case for N<sub>2</sub>, though we would not expect to see overt behavioral responses to this inert gas. Thus, the presence of CO<sub>2</sub> around the foam may argue for a welfare cost associated with using CO<sub>2</sub>, which can be avoided by using inert gases.

Submersion in either N<sub>2</sub>- or CO<sub>2</sub>-filled foam led to death, characterized by a pronounced bradyarrhythmia, vigorous wing flapping, and altered EEG pattern. It is interesting to note that time to isoelectric EEG was very consistent across species and foam types; therefore, it would appear that death was induced in both cases by anoxia, rather than by hypercapnia with CO<sub>2</sub>. Compared with previous reports of anoxic gas killing (in N<sub>2</sub>) under laboratory or commercial conditions, the timing of these events was particularly rapid. For example, McKeegan et al. (2007) reported that broilers undergoing anoxia exhibited wing flapping after 40 s, compared with an average 18 s here for hens and 15 s for broilers. Time to death in the former study was 95 s compared with a time to cessation of movement here of 65 s for hens and 51 s for broilers. Similarly, in a commercially relevant study using argon, time to cessation of movement in broilers was more than 180 s (Abeyasinghe et al., 2007). It is also important to note that the anoxic foam used here was approximately twice as rapid in inducing brain death than lower expansion foam applications reported elsewhere (mean time to EEG silence 134 s with air-filled foam and 120 s with CO<sub>2</sub>-enriched foam; Alphin et al., 2010). That N<sub>2</sub>- and CO<sub>2</sub>-filled high expansion foam applications result in an impressively effective anoxic death is supported by the very low oxygen concentrations measured at bird level. Foam is advantageous in this regard because it eliminates the possibility of air in the feathers of the birds increasing the oxygen concentration during introduction to the anoxic environment.

The maximum time to loss of consciousness can be defined by time to suppressed EEG: this is a conservative approach because the slow wave EEG pattern exhibited by all the birds in the transitional phase is also consistent with a reduction in vigilance state. Onset of vigorous wing flapping tended to occur in the transitional phase, after ataxia and loss of posture. The onset of isoelectric EEG and cessation of movement were closely associated, making time to cessation of movement a reasonable measure of time to death. Due to the anesthetic effect of CO<sub>2</sub>, exposure of birds to the CO<sub>2</sub>-filled foam led to an earlier induction of a transitional state of the EEG than with N<sub>2</sub>-filled foam. Moreover, the effect of CO<sub>2</sub> on consciousness began before submersion, indicating that the CO<sub>2</sub> concentration around the foam is having an effect on the conscious state of the birds. After submergence, there was little difference general response to birds to the CO<sub>2</sub>- and N<sub>2</sub>-filled foams.

During postmortem examinations, small bubbles were observed in the tracheas or tracheal openings of almost all birds. Because the foam used to deliver the

gas had bubble diameters of 10 to 20 mm, the presence of these small bubbles needs to be explained. During anoxic death, it was apparent that the vigorous flapping exhibited by the birds caused the foam in their immediate area to be whipped into a froth consisting of very small bubbles. It is likely that some of this froth was then inhaled in the later stages of euthanasia. However, some of these small bubbles were also seen in the tracheal openings of control birds, and it may be that the movement of birds in control foam (which sometimes included wing flaps) led to a similar froth. Nevertheless, it is important to note that in no case was there any evidence of occlusion of airways by foam.

An important finding (though not formally quantified) was that convulsions and wing flapping of the birds broke down the foam very rapidly. There was a visible decrease in height in the amount of foam in the apparatus during wing flapping. This implies that the height of foam above birds before flapping begins is crucial for the success of the technique, and sufficient capacity of foam generation is necessary to create foam faster than it can be destroyed. If the foam is destroyed, allowing reexposure to air before birds are in a deeply unconscious state, they could regain consciousness very rapidly. Destruction of CO<sub>2</sub> foam will still lead to an atmosphere that is saturated with a high level of CO<sub>2</sub>, which will form a carpet because it is denser than air. Therefore, from an efficacy point of view, CO<sub>2</sub> will lead to a more stable anoxic situation than if N<sub>2</sub> is used.

In conclusion, these trials show that submersion in anoxic (N<sub>2</sub>- or CO<sub>2</sub>-filled) foam provides a highly effective and rapid method of euthanasia. Initial aversion to the foam is not extreme, although submersion of conscious birds in air-filled foam for up to 60 s appeared to be unpleasant. The EEG pattern in birds submerged in anoxic foam began to change very rapidly (on average in less than 10 s or before foam submersion with CO<sub>2</sub>), and unequivocal unconsciousness (suppressed EEG) was apparent no later than 30 s after submersion. The rapidity of the response, physiological observations, and measurements of oxygen in the foam all show that the method of killing was anoxia, not occlusion of the airway. The results provide proof of principle that submersion in anoxic high-expansion foam is a humane method of euthanasia in a range of poultry species, which may have potential to provide humane emergency killing or even routine depopulation.

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