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Report 399

Welfare assessment of gas filled foam as an agent for killing poultry

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Abstract
During outbreaks of notifiable diseases in poultry measures are taken to restrict the spread of the disease. Mass on-farm killing of birds using gas-filled foam is such a measure. This study examines the method and technologies involved using gas-filled foam and looks at the problems involved by scaling up the procedure. Methods and results are discussed in relation to poultry physiology and behaviour monitored during controlled studies.
Recommendations are made for system design and an operational protocol is provided for practical on-farm implementation.

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Preface

During outbreaks of notifiable diseases in poultry control measures require poultry to be killed on-farm, preferably in their production housing in order to reduce the risk of spreading the disease.

The Dutch governments' interest in the potential of delivering gas into sheds using high- expansion foam as an acceptable method of emergency killing of poultry is shared by the UK government (Defra). To maximise the benefits of complementary research a collaborative programme of research between the respective project leaders Dr Dorothy McKeegan and Dr Marien Gerritzen

(Livestock Research Wageningen UR, NL) was established. Results and conclusions of this complementary research, as well as practical recommendations based on this research project are presented in this report.

Samenvatting

Voor de bestrijding van besmettelijke dierziekten is het noodzakelijk dat de dieren worden gedood op het bedrijf en bij voorkeur in de stallen. Hierdoor wordt het risico van verspreiding van de ziekte tot een minimum beperkt. Het ontwikkelen van praktisch toepasbare methoden blijft noodzakelijk om snel en accuraat te kunnen reageren op een uitbraak van een besmettelijke dierziekte.

Voor het doden van grote groepen pluimvee in de stal is onderzoek naar toepassing van gas gevuld schuim als dodingmethode in ontwikkeling. Onderzoek waarbij schuim met een hoge dichtheid en dus kleine schuimbellen is toegepast is uitgevoerd in de USA. De structuur en samenstelling van dit met lucht gevulde schuim was dusdanig dat de dieren in deze experimenten werden gedood door een blokkade van de luchtwegen met verstikking tot gevolg. Ondanks de negatieve effecten van mechanische verstikking op het welzijn van de dieren is deze methode toegestaan in de USA (USDA/APHIS 2007). Mechanische verstikking is volgens Nederlandse en EU opvattingen echter een niet acceptabele methode voor het doden van dieren. Vanuit dit oogpunt is in een pilot studie onderzocht of het toepassen van een lage dichtheid schuim gevuld met stikstof of kooldioxide een acceptabele methode voor het doden van pluimvee zou kunnen zijn. Gebaseerd op gedrag parameters, hartslag metingen en pathologisch onderzoek is vastgesteld dat stikstof of kooldioxide gevuld schuim een acceptabele dodingmethode voor pluimvee kan zijn.

Het toepassen van stikstof of kooldioxide gevuld schuim zal de dieren doden door hypoxie en of anoxie. Zowel hypoxie en anoxie onder gecontroleerde omstandigheden kunnen leiden tot een acceptabele dodingmethode onder praktijk omstandigheden.

Project doelen:
1. Het ontwikkelen van een systeem waarmee gas gevuld schuim kan worden toegediend aan een kleine tot middelgrote groep pluimvee.
2. Het vergelijken van fysiologische en gedragsparameters bij pluimvee tijdens blootstelling aan schuim gevuld met stikstof of kooldioxide. Vergelijking van gas gevuld schuim met luchtgevuld schuim.
3. Ontwikkelen van een praktisch toepasbaar systeem voor het toedienen van stikstof of kooldioxide schuim in pluimveestallen, rekeninghoudend met parameters zoals distributie en stabiliteit van het schuim en met de effectiviteit van de methode.

Doel 1: Ontwikkeling van een system voor het toedienen van gas gevuld schuim

In Nederland heeft LST International BV een kleinschalige gasschuim generator ontwikkeld voor gebruik met kooldioxide en andere gassen. Het ontwerp bestaat een vat met een pre-mix oplossing van water en 3% schuimconcentraat. Het vat is verbonden met een schuimgenerator via een slang met een maximum operatiedruk van 20 bar. De schuimgenerator heeft een roestvrijstalen sproeikop waarin meerder roestvrijstalen roosters zijn geplaatst. De diameter van de gaten in deze roosters en de vorm van de roosters bepalen voor een belangrijk deel de schuimbel grote. Gas toevoer naar de generator gaat direct vanuit een hogedrukcilinder. Samenstelling en werkwijze van het systeem is beschreven in hoofdstuk 2.1 van dit rapport.

Doel 2. Fysiologische reacties van pluimvee op gasgevuld schuim

De fysiologisch- en gedragswaarnemingen bij kuikens, kalkoenen en eenden tijdens blootsstelling aan gasschuim zijn in hoofdstukken 2.2 (methodology), 3 (results) en 4 (conclusions) van dit rapport beschreven. Het doel van dit deel van het project was het onderzoeken van de geschiktheid van gasschuim verrijkt met koolstofdioxide of stikstof voor het doden van verschillende soorten pluimvee.

Vergelijkbare proeven waarbij kuikens werden blootgesteld aan schuim verrijkt met stikstof werden in Engeland en Nederland uitgevoerd. Proeven met kooldioxide schuim zijn alleen in Nederland uitgevoerd. Proeven met gasschuim gevuld met stikstof met leghennen zijn alleen in Engeland uitgevoerd.

Temperatuur van het gasschuim wordt bepaald door de temperatuur van het water waarmee het schuim werd geproduceerd. Dit resulteerde in een schuimtemperatuur tussen 8.9 en 14.8 ºC tijdens de verschillende proeven. In alle experimenten is een residueel zuurstof percentage van minder dan 1% gerealiseerd.

Een vergelijking van het effect van gastype (Stikstof of Kooldioxide) op gedrag is uitgevoerd bij vleeskuikens. Het was duidelijk dat "gasping" (lucht happen) kwam vaker voor kwam bij het gebruik van Kooldioxide schuim dan bij Stikstof schuim. Verder kwam "headshaking" niet voor bij Stikstof schuim en

"gasping" werd alleen bij één kuiken gezien tijdens het gebruik van Stikstof schuim. Er zijn geen significante verschillen in Loss of Posture (evenwichtsverlies), convulsies en vleugel klappen tussen Kooldioxide en Stikstof gevuld schuim waargenomen. Dit impliceert dat onder de huidige omstandigheden loss of posture, convulsies en vleugel klappen ontstaan door anoxische omstandigheden in plaats van door hypercapnie.

De periode tot aan het moment dat de dieren niet meer bewogen was, hoewel klein, significant korter voor kuikens in Stikstof schuim dan voor dieren in Kooldioxide schuim,

De eerste fysiologische respons van de dieren op schuim productie was een matig toename in hartritme, waarschijnlijk veroorzaakt door een schrik reactie op het geluid van de schuimgenerator.

De toename in hart ritme is waarschijnlijk ook veroorzaakt door verhoogde activiteit van de dieren om van het schuim weg te lopen.

Alle pluimvee species vertoonde een vergelijkbare fysiologische reactie op blootstelling aan het schuim. Een duidelijke bradycardie, verlaging van de hartslag, direct na te zijn bedekt met schuim is in alle dieren waargenomen. Gemiddeld was er geen verschil tussen kuikens, kalkoenen en eenden in hun respons op Kooldioxide schuim. Bradycardie werd eerder geconstateerd in kuikens bedekt met Kooldioxide schuim dan met Stiksof schuim.

Variatie in tijdsduur tot inductie van bradycardie (figuren 10 t/m 13) binnen proefgroepen (diersoort of gastype) is waarschijnlijk gerelateerd aan de tijd die nodig was om dieren te bedekken met schuim, deze tijd varieerde van gemiddeld 10 tot 80 seconden. Dit verschil is veroorzaakt door de verschillen schuimproductie snelheid en door de mate waarin schuim door heftige bewegingen van dieren wordt afgebroken.

De eerste verlaging van EEG complexiteit (transitionele fase) was significant langer in de Stikstof groep. Met transitoneel EEG wordt een eerste verlaging van de EEG complexiteit bedoeld die intreden van verminderd bewustzijn karakteriseert. In vergelijking tussen het effect van Stikstof- en Kooldioxide schuim bij kuikens treedt er een significant (p=0.024) vertraging op in aanzet van een transitioneel EEG met Stikstofschuim. Het effect van Kooldioxide in het schuim werd duidelijk na correctie van de EEG data voor moment van bedekt zijn met schuim (Tabel 4). Een transitioneel EEG in Kooldioxideschuim was zichtbaar voordat de dieren met schuim waren bedekt. Hieruit blijkt duidelijk dat Kooldioxide ook om het schuim aanwezig was. Bij Stikstofschuim werd een transitineel EEG gezien na kompleet onderdompeling. Stikstof rond het schuim heeft dus geen effect op bewustzijn niveau van de dieren.

Bij eenden blijkt een tendens tot het eerder optreden van een EEG suppressie (P 0.075) die karakteristiek is voor bewusteloosheid vergeleken met kuikens en kalkoenen. Er zijn geen significante verschillen tussen gastype (Stikstof en Kooldioxide) of diersoort (kuikens, kalkoenen of eenden) geconstateerd in tijdsduur tot een iso-electrisch EEG. Hoewel, er significante verschillen zijn in gedrag tussen gastype en diersoort is verondersteld dat deze verschillen geen of weinig invloed zullen hebben op de praktische toepassing. Blootstelling aan schuim gevulde met zowel Kooldioxide als Stikstof veroorzaakt een snelle aanzet van bradycardie die uiteindelijk resulteerde in hartstilstand en sterfte.

Blootstelling aan Kooldioxideschuim veroorzaakte een sneller inductie van een transitineel EEG dan blootstelling aan Stikstofschuim wat kan worden verklaard door de anesthetische werking van Kooldioxide, Het effect van CO_2 op het bewustzijn treedt op voordat de dieren volledig bedekt zijn met schuim. Dit geeft aan dat de Kooldioxide concentratie rondom de schuimbellen een positieve werking heeft op de reductie van bewustzijn van de dieren. Suppressie en de daaropvolgende inductie van een iso-electrisch EEG treedt in na onderdompeling door schuim. De suppressie van het EEG is gecorreleerd met bewusteloosheid, dit wordt bevestigd door een reductie in correlatie dimensie analyses.

Na bedekking met het schuim is er geen verschil in mate van reductie van bewustzijn tussen Kooldioxide- en Stikstofschuim. Daarom kan worden geconcludeerd dat het anoxisch effect van beide schuimtypen vergelijkbaar zijn. Vanuit dit oogpunt maakt het geen verschil of pluimvee aan Kooldioxide- of Stikstof– schuim wordt blootgesteld.

Doel 3. Ontwikkelen van een systeem voor het toedienen van gasschuim in grotere pluimveestallen.

Om tot een commerciële toepasbare uitvoering te komen is een upgrade van de apparatuur noodzakelijk. De belangrijkste aandachtspunten hierbij zijn verhoging van productie capaciteit, voorkomen van bevriezing van de spray nozzles en stabiliteit van het schuim als het schuim wordt toegediend bij pluimvee dat is gehuisvest bij een commerciële (40kg/m²) of relevante (50kg/m²) bezettingsgraad.

Methodologie, uitleg van de werking van de systemen en de effecten op kuikens onder semipraktijk omstandigheden zijn toegelicht in hoofdstukken 5 en 6.

Het schuim productie systeem bestaat uit een bulktank met vloeibare stikstof (6200 liter cap.) gekoppeld aan een vaporisator en een ventilator met een nominale 8 uurs capaciteit van 1750 Nm^3 per uur. De schuimgenerator heeft een toevoer behoefte van 2400 Nm^3 gas per uur. Deze toestand moet gedurende een maximum van 1 uur kunnen worden volgehouden. Daarna volgt een periode om herstel van de vaporisator naar omgevingstemperatuur te laten terug stijgen. Een geïntegreerde systeem met watertank, schuimconcentraat tank, pomp en elektromechanisch regulator wordt gebruikt voor het controleren van druk en gas flow. Om een 3% oplossing te bewerkstelligen werd de juiste hoeveelheid schuimconcentraat nog voor de pomp direct in de waterstroom. Er werden vier schuim generatoren (Figuur 2), elk met een nominaal capaciteit van 10 m^3 per minuut, gebruikt om de juiste schuimverdeling te realiseren

In een 6 m breed hok, bij schuim toediening met een snelheid van 42.5 m^3 /min, werd in gemiddelde 11 seconden een schuimhoogte van 60cm bereikt. Een schuimhoogte van 1 m werd in gemiddeld 30 seconden bereikt. Deze schuimkwaliteit en schuimproductie waren voldoende om de dieren bedekt te houden tijdens perioden van vleugel klappen en convulsies. Totale schuim productie was 7.1 m^3 per minuut per meter hokbreedte. De bezettingsgraad van dieren had geen significant effect op schuimafbraak, Hierbij kan worden opgemerkt dat als de dieren weglopen van de aankomende schuimgolf effectieve bezettingsgraad toeneemt als ze onder het schuim zijn bedekt. Hokbreedte heeft een groter effect op de hoeveelheid schuim die afgebroken werd dan hoklengte. Dit geeft aan dat de breedte en niet de lengte van de pluimveestal bepalend is voor de volumemetrische snelheid van het schuim. Voor praktijk toepassing is het daarom van belang aantal en plaatsing van de schuimgeneratoren kritisch te bepalen.

Fysiologische data gemeten bij dieren blootgesteld aan anoxisch schuim in de praktijkproeven lieten geen significante verschillen zien ten opzichte van proeven met individuele dieren.

Veranderingen in gedragspatronen en veranderingen in EEG karakteristieken kwam overeen met die van de eerdere proeven. Het blijkt dat schuim geïntroduceerd in groter groepen pluimvee een betrouwbare en acceptabel anoxisch effect induceert zelfs bij een maximaal bezettingsgraad.

Een onverwachte onderbreking van de schuim toevoer in 1 van de testen gaf een duidelijke indicatie voor eventuele consequenties voor welzijn bij een technische storing. Hernieuwd blootstelling aan lucht van dieren die het bewustzijn hebben verloren kan leiden tot recovery van deze dieren De onderbreking van de schuimproductieproces gedurende proef 4 was waarschijnlijk toe te rekenen aan een fout door de bediener en slechte onderlinge communicatie.

Uit de experimenten zowel in Engeland (Defra, gesubsidieerd) als in Nederland (LNV gesubsidieerd) kan worden geconcludeerd dat het gebruikt van anoxische schuim acceptabele is als dodingmethode voor grote groepen pluimvee in de stal. Echter, voor dat de methode kan worden toegepast in de praktijk moeten alle onderdelen verder worden ontwikkeld. Belangrijke uitgangspunten hierbij zijn dat het systeem flexibel en compact moet zijn en dat betrouwbaarheid, veiligheid en effectiviteit zijn gewaarborgd

Praktische aanbevelingen voor het ontwerpen en voorstellen voor een gebruiksprotocol zijn aangegeven in hoofdstuk 8.

Schuimproductie systemen voor het doden van pluimvee in praktijksituaties moeten voldoen aan een aantal belangrijke criteria. De volgende criteria zijn opgesteld op basis van de in dit project uitgevoerde experimenten:

1 Expansie ratio, ratio van schuimvolume tot de hoeveelheid water tussen 250 - 350:1
2 Snelheid van schuimproductie moet minimal 7.1 m^3 per minuut zijn, per meter hokbreedte bij een bezettingsgraad van maximaal 50 kg/m^2.
3 Volume en productie snelheid van het gas en schuim moeten worden gemeten.
4 Volume van water en schuimconcentraat moeten gemeten worden.
5 Watertemperatuur moeten worden gemeten.
6 Zuurstof percentage in het schuim moet worden gemeten.
7 Het system moet zonder onderbreking voldoende schuim kunnen produceren om de pluimveestal te vullen tot 4 keer het benodigde volume.
8 Operators moeten met elkaar kunnen communiceren tijdens de uitvoering
9 Een checklist moet worden bijgehouden

Criteria moeten worden opgesteld voor het evalueren van geschiktheid van de schuim methode voor verschillende omstandigheden.

Voorafgaand aan uitvoering in de praktijk moet de benodigde capaciteit worden berekend.

Apparatuur moet in een stand-by periode regelmatig worden gecontroleerd en worden getest.

Summary

Disease control measures require poultry to be killed on farm and preferably in their production housings to minimise the risk of spreading the disease.

Recent developments in emergency killing of poultry include killing with high density foam. Trials with foaming systems to date have used medium density fire fighting foam to create a blanket over the birds to restrict the availability of oxygen to the birds, so when all the available oxygen has been respired, the birds will die of hypoxia. The structure and size of the foam used in these trials was such that small bubbles were found in the airways of the birds. The conclusion was that the foam causes an occlusion of the airway and that the birds typically expire due to hypoxia, within 5 minutes of being covered with the foam. Despite the negative animal welfare aspects of this type of foam, the method has been approved in the United States (USDA/APHIS 2007). From our point of view killing animals by mechanical suffocation is unacceptable.

Possibilities for the use of CO_2-delivered by high expansion foam for killing of poultry have been examined in a pilot study (ASG report 37). Aim of the pilot study was to identify whether CO_2 foam has potential as an acceptable killing agent from the animal welfare point of view. Based on behavioural parameters, heartbeat measurements and pathological data it was concluded that CO_2 foam can become an acceptable killing method for poultry. The use of high expansion gas-filled foam containing CO_2 or an Anoxic gas such as Nitrogen presents a feasible alternative delivery method of hypercapnic or anoxic killing, because as the foam envelopes the bird, oxygen will be effectively eliminated and or carbon dioxide will be effectively presented and birds will die by hypercapnic-anoxia or by anoxia.

Objectives of this project are:
1. Development of a system to deliver gas filled foam to a small to medium group of poultry with similar specifications to that which would be used in the operational disease control situation.
2. Monitoring of the physiology and behaviour of poultry, i.e. chickens, turkeys and ducks, during exposure to carbon dioxide-filled foam in comparison to air filled foam and to anoxic gas-filled (Nitrogen) foam.
3. Development of a system to deliver gas-filled foam into large poultry sheds under consideration of parameters that influence the distribution of gas filled foam and its efficacy of usage as a practicable method for the humane killing of poultry within a shed.

Objective 1. Development of a system to deliver gas filled foam.
A small scale foam generator was developed in The Netherlands by LST International BV for use with carbon dioxide or another compressed gas. It consists of a pressure vessel containing a pre-mixed solution of water and 3% foam concentrate. The liquid tank is connected to the foam generator by a hose with a maximum working pressure of 20 bar. The foam generator itself consists of a spray nozzle and a pair of wire mesh screens mounted inside a stainless steel cylinder. The gas is supplied directly to the generator from a compressed gas cylinder through a separate hose to the generator. Working principle and how the components of the foam generation system fit together are described in chapter 2.1 of this report.

Objective 2. Physiological reactions of poultry to gas filed foam.
Physiological and behavioural reaction of broilers, ducks and turkeys to gas filled foam are described in chapter 2.2 (methodology) and chapters 3 (results) and 4 (conclusions) of this report. Aim of the objective was to investigate whether carbon dioxide or nitrogen filled foam could be an acceptable method of killing poultry. Experiments with broiler chickens exposed to nitrogen-filled foam were performed in comparable experiments in the UK and The Netherlands. Experiments with carbon dioxide foam were performed only in The Netherlands whereas experiments with Nitrogen-filled and air-filled foam on laying hens were only performed in the UK.

The temperature of the gas-foam was determined by the temperature of the water used to produce the foam more than by the temperature of the gas-source. As a result minimum temperature of the foam varied between 8.9 and 14.8 ºC in the different trials. Residual oxygen concentration in all experiments dropped to less than 1% in the foam.

The effect of gas source i.e. N_2 and CO_2, on the behaviour of animals was compared only for broilers. It is clear that gasping occurred more frequently and earlier using CO_2 filled foam than with N_2 filled foam. Moreover, headshaking was not observed in the N_2 filled foam and gasping was only observed in one broiler in the N_2 filled foam. No significant differences due to the gas source were observed on loss of posture, convulsions and flapping. This implies that under the present conditions loss of posture, convulsions and

flapping are induced due to the anoxic situation rather than due to the effect of CO_2. Absence of movement occurred significantly faster in broilers in N_2 filled foam than in the CO_2 filled foam although, the differences were small.

The initial animal response to foam generation was a moderate increase in heart rate, most likely associated with a fear response due to the loud noise generated by foam production. Heart rate increase was also likely to have been caused by increased exercise as the birds moved from resting positions to avoid the foam. All species demonstrated a consistent response to submersion in foam in the form of an almost immediate pronounced slowing of heart rate (bradycardia). On average there is no difference between broilers, turkeys and ducks in response to CO_2 filled foam. Although not significant, it looks that bradycardia was induced earlier in broilers exposed to CO_2 than those exposed to N_2 filled foam.

The variation in timing between birds, within an experimental group (species or gas source), in onset of bradycardia (Figure 10,11,12,13) is likely to be related to the different durations of submersion under foam waves (which took between 10 and 80s to cover all of the birds, depending on the trial).

The delay time to a transitional EEG was significantly longer in broilers in the N_2 group than those in other groups. Comparing the effect of N_2-filled foam with CO_2-filled foam in broilers there is a significant (p=0.024) delay in the occurrence of a transitional EEG for N_2-filled foam. The effect of CO_2 foam closure is clearly visible when correcting the EEG data for the moment of submergence into foam (Table 4). A transitional EEG in the CO_2-filled foam occurred prior to submergence. Only in the N_2-filled foam a transitional EEG occurred after complete submergence.

In ducks there appears to be a trend towards an earlier (P 0.075) suppression of the EEG compared to broilers and turkeys. No differences were observed in onset of an iso-electric EEG between gas sources i.e. N_2 and CO_2 or between species i.e. broilers, ducks and turkeys.

Although there are some significant behavioural differences due to the gasses used and poultry species. It can be concluded that these effects will have no, or only minor, relevance for practical application.

Exposure to both, carbon dioxide - and nitrogen-filled foam leads to fast bradycardia ending in heart failure and death.

Due to the anaesthetic effect of CO_2, exposure of birds to a CO_2-filled foam leads to an earlier induction of a transitional state of the EEG than is the case when exposed to N_2-filled foam. Additionally, the effect of CO_2 on consciousness starts before the birds are submerged, indicating that the CO_2 concentration around the foam has a clearly positive effect on the reduction in state of consciousness of the birds. Suppression and the following induction of an iso-electric EEG occurs after being submerged by the foam. The suppressed state of the EEG is correlated with unconsciousness, confirmed by the reduction in correlation dimension analyses.

After being submerged there is no difference in reduction of conscious state between CO_2- and N_2-filled foam, therefore it can be concluded that the anoxic effects of both gas filled foams are comparable. From this point of view it makes no difference if birds are exposed to CO_2 or N_2 filled foam.

Objective 3. Developing a system to deliver gas-filled foam into larger poultry sheds.

To achieve application for commercial conditions it was decided necessary to further upgrade the equipment. Main issues to be solved included increasing the capacity, prevention of freezing of the spray nozzles and stability of the foam when applied on birds at commercial ($40kg/m^2$) or relevant ($50kg/m^2$) stocking densities. Methodology, description of the systems and the effects on broilers in semi full scale experiments are described in detail in chapters 5 and 6 of this report.

The foam generating system consisted of a bulk tank of liquid nitrogen (6200 litre capacity) connected to a fan assisted ambient air vaporiser with a nominal 8 hour capacity of 1750 Nm^3 per hour. The gas flow requirement for the foam generation system was 2400 Nm^3 per hour that could be sustained by the unit for a maximum of 1 hour followed by a recovery period to allow the vaporiser to return to ambient temperature. An integrated system consisting of a water tank, foam concentrate tank, pump and electro-mechanical proportioning system was used for controlling both pressure and flow rate, this was used to pump the pre-mixed solution to the generators. The correct amount of foam concentrate was directly injected into the water flow downstream of the pump to achieve the 3% solution required, irrespective of flow rate. Four foam generators (Figure 2) were used, each with a nominal capacity of 10 m3 per minute.

In a 6 m wide pen, with foam delivered at a rate of 42.5 m^3 /min, the average time taken to reach depth of 60cm was 11 seconds and 30 seconds to reach a depth of 1 metre. This quantity and depth of foam was sufficient to keep the birds covered during bouts of wing flapping. The overall foam production rate is equivalent to 7.1 m^3 per min per metre width of pen. The initial stocking density of the birds did not make a great difference to foam destruction, although there was a tendency for the higher stocked birds to destroy

more foam. It is noteworthy, that movement of the birds away from the approaching foam meant that the actual stocking density at which the birds were submerged in the foam was greater than their initial stocking density and in some parts of the pen became maximal. This movement of birds may mask the true effect of the initial stocking density. The width of the pen had a greater effect on the amount of foam destroyed than the distance it had to travel over the birds through the pen. This suggests that it is the width of a poultry shed that will determine the volumetric flow rate of foam rather than the length of the shed. Physiological data recorded from birds exposed to anoxic foam in commercially relevant trials show no significant differences to the results of laboratory studies on single birds. Patterns of behavioural change and onset of changes in EEG characteristics closely matched those observed in laboratory trials. Foam, as deployed in these larger trials, delivered a reliable and humane anoxic kill which was robust even at maximal stocking densities (created as the birds moved to avoid the advancing foam). An unintended interruption in foam supply gave an indication of the welfare impact of a technical problem in the field situation, in the form of re-exposure of previously submerged birds. The welfare consequences of this depend on the duration of submersion prior to re-exposure, and will be an issue only for birds which have not yet started to wing flap (submerged for less than 15 seconds). This is unacceptable and should not occur during correct deployment of the system. Nevertheless, the nature of the foam system means that only a relatively small proportion of birds (those submerged under the leading edge of the foam with a height less than 80cm) are likely to be at risk of compromised welfare due to technical failure.

The interruption to the foam during trial 4 was most likely caused by operator error and a lack of communication.

From the results of the experiments in UK, funded by Defra, and the experiments in The Netherlands it can be concluded that using anoxic foam is an acceptable method of killing large groups of poultry on-farm. However, before the method can be applied in commercial practice the equipment should be designed and manufactured in such a way that it is versatile under different circumstances. Important criteria are reliability, safety of use, effectiveness and mobililily.

Practical recommendations for design including aspects for an operational protocol are given in chapter 8.

It is evident that any foam generation system that is to be used to kill birds in the field must meet a minimum design specification prior to selection or deployment. The following criteria are based on conclusions from these and previous experiments.

1. Expansion ratio, the ratio of volume of foam to the amount of liquid contained in the foam must be between 250 and 350 :1
2. Foam production must be a minimum rate of 7.1 m^3 per minute, per metre of pen width for birds that are initially stocked up to a maximum density of 50 kg/m^2.
3. Volume and flow rate of gas and foam solution must be measured.
4. Volume of water and foam concentration must be measured.
5. Gas and water temperature must be measured.
6. Residual oxygen level of foam must be measured.
7. The system must have a capacity to generate enough foam to fill the target shed to a volume equal to 4 times its floor area without refilling.
8. Operators must have contact through a 2-way radio and a check list should be used by the lead operator before commencing operations.

These design recommendations should be used as the minimum standards for assessment of the suitability of foam generation equipment prior to acquisition or deployment. For example, if the target for deployment are free range broiler sheds measuring 8.3 m x 15 m a foam flow rate of 58 m^3 would be required, it is expected that it will require about 250 m^3 of foam to complete the operation and the system should have a capacity of at least 500 m^3 without refilling.

Trials of the equipment should be carried out to ensure that it meets the requirements and annual tests should be performed to ensure that it is in working order. These could be incorporated into training exercises.

Table of contents

1 Introduction

1.1 Project background

Disease control measures require poultry to be killed on farm and preferably in their housings to minimise the risk of spreading the disease.

Recent developments in emergency killing of poultry include killing with high density foam. Trials of foaming systems to date (Dawson et al. 2006, Benson et al. 2007,) have used medium density[1] fire fighting foam to create a blanket over the birds to restrict the availability of oxygen to the birds, so that when all the available oxygen has been respired, the birds will die of hypoxia. The structure and size of the foam used in these trials was such that small bubbles were found in the airways of the birds. The conclusion was that the foam causes an occlusion of the airway and that the birds typically expire due to hypoxia, within 5 minutes of being covered with the foam. Despite the negative animal welfare aspects of this type of foam, the method has been approved in the United States (USDA/APHIS 2007).

Possibilities for the use of CO_2-enriched high expansion foam for killing of poultry have been examined in a pilot study (ASG report 37). Aim of the pilot study was to identify whether CO_2 enriched foam has potential as an acceptable killing agent from the animal welfare point of view. Based on behavioural parameters, heartbeat measurements and pathological data it was concluded that CO_2-enriched foam can become an acceptable killing method for poultry. The use of high expansion gas-filled foam containing CO_2 or an Anoxic gas such as Nitrogen presents a feasible alternative delivery method of hypercapnic or anoxic killing, because as the foam envelopes the bird, oxygen will be effectively eliminated and or carbon dioxide will be effectively presented and birds will die by hypercapnic-anoxia or by anoxia.

1.2 Collaboration between complementary projects in the UK and the USA

The Dutch governments' interest in the potential of high- expansion gas-enriched foam as an acceptable method for emergency killing of poultry is shared by the UK government (Defra). To maximise the benefits of complementary research a collaborative programme of research was established between the respective project leaders Dr Dorothy McKeegan and Dr Marien Gerritzen (Livestock Research Wageningen UR, NL).

1.3 Objectives of the joint project

1. Development of a system to deliver gas filled foam to a small to medium group of poultry with similar specifications to that which would be used in the operational disease control situation. Issues that need to be considered include expansion ratios, surfactant type, temperature of delivery, speed of delivery, method of gas delivery, bubble diameter and bubble composition.

2. Monitoring of the physiology and behaviour of poultry, i.e. chickens, turkeys and ducks, during exposure to Carbon dioxide filled foam and comparison of this to air filled foam and to anoxic gas-filled (Nitrogen) foam.

3. Development of a system to deliver gas-filled foam into large poultry sheds considering the parameters that influence the distribution of a gas filled foam and its efficacy of use as a practicable method for the humane killing of poultry inside a shed.

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[1] Expansion rate is defined as the ratio of volume of liquid to the volume of foam produced. Generally foam has a low expansion ratio up to 20:1, medium up to 200:1 and high to above 200:1. these definitions are related to foams used in fire fighting.

2 Material and methods

2.1 Objective 1: Development of a system to deliver gas filled foam

2.1.1 Foaming principle

Conventionally, fire fighting foam is described by the ratio of liquid to air volumes and may be divided into three groups (see Table 1). Foaming euthanasia methods developed in the United States have been based on medium expansion foam, the USDA (USDA APHIS, 2006) performance standards for foam require an expansion rate of between 40 and 135. The principle behind the proposed foam gassing equipment in the current work is to use high expansion foam as a gas delivery method.

Table 1   Descriptive names and typical uses of foam in relation to expansion ratio

Expansion ratio (Air:liquid)	Descriptive names used	Typical Uses
20:1	High density Low expansion Heavy foam	Inflammable hydrocarbon fires, creates foam blanket and has a long throw range. Also coverage of chemical spills.
20:1 to 200:1	Medium expansion Medium density Medium foam	For 3 dimensional fires (eg tyres) and filling oil bins.
200:1 to 1000:1	Low density High expansion Light Foam	Fire fighting in large enclosed spaces, (ships, hangers warehouses and mines) especially where inflammable vapours exist.

2.1.2 Generating foam

Many high expansion foam generators work by spraying foam solution onto a mesh though which air is driven by a fan. These facilities can be installed in the roof or walls of a building or as more compact portable systems. The fans are often driven by hydraulic flow, and therefore do not require an external power supply beyond that required to create the water pressure. These systems have the advantage that they can be used in closed environments where the foam is deployed against a back pressure, for instance in mines or inside oil storage tanks. An alternative system uses a high velocity jet nozzle to create a pressure differential across a mesh screen effectively sucking air through the unit and creating large volumes of high expansion foam, up to 1000 expansion rate, without the need for a fan. These systems have been developed to fill the air space in warehouses containing dangerous chemicals of several 1000 cubic metres within 3 minutes. There are also foaming systems that use compressed air or gas to develop foam, but these are limited to low expansion foam types. No systems have been developed to generate high expansion foam from compressed gas.

The gas-foam generator used in the current project was developed using a combination of the methods described above. A fan driven system would be useless if the gas would be supplied from a pressurised source, whether the gas was compressed or vaporised liquid. Simplification of construction, reduction of the complexity of effective cleansing and disinfection after deployment and price were all important considerations in the design.

2.1.3 Small scale generator

A small scale foam generator was developed in The Netherlands by LST International BV for use with carbon dioxide or any other compressed gas. It consists of a pressure vessel containing a pre-mixed solution of water and 3% foam concentrate (as per the manufactures recommendation, Figure 1). The liquid tank is connected to the foam generator by a hose with a maximum working pressure of 20 bar (Figure 2). The foam generator itself consists 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 (Figure 2 and 3). The gas is supplied directly to the generator through a hose from a compressed gas cylinder. The gas is released inside the closed body of the generator through an annulus drilled with 2.5 m holes on a 2 cm pitch. The holes are directed towards

the rear (sealed) end of the generator to reduce the distortion of the spray cone by the gas jets (Figure 2). Figure 3 shows how the components of the foam generation system fit together.

The larger tank holds pre-mixed foam solution, the small cylinder was originally used to hold nitrogen gas to pressurise the red tank.

Hand held foam generator. The black hoses supply the gas and the liquid is supplied down the central stainless steel tube. This generator was modified with openings on the side and rear to allow it to be run using atmospheric air in the control experiments.

Figure 1: Photographs and descriptions of the laboratory scale foam generator
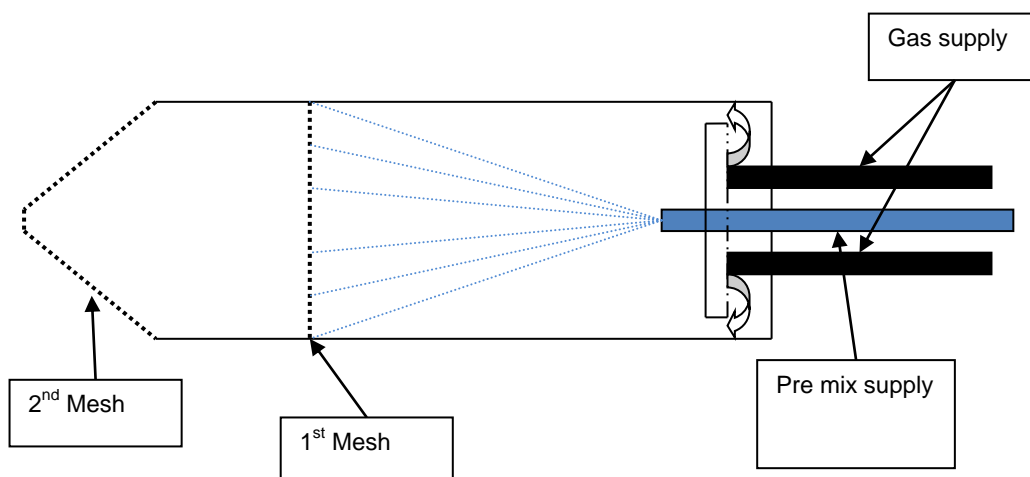

Figure 2:   Diagram showing the main components of the laboratory scale foam generator
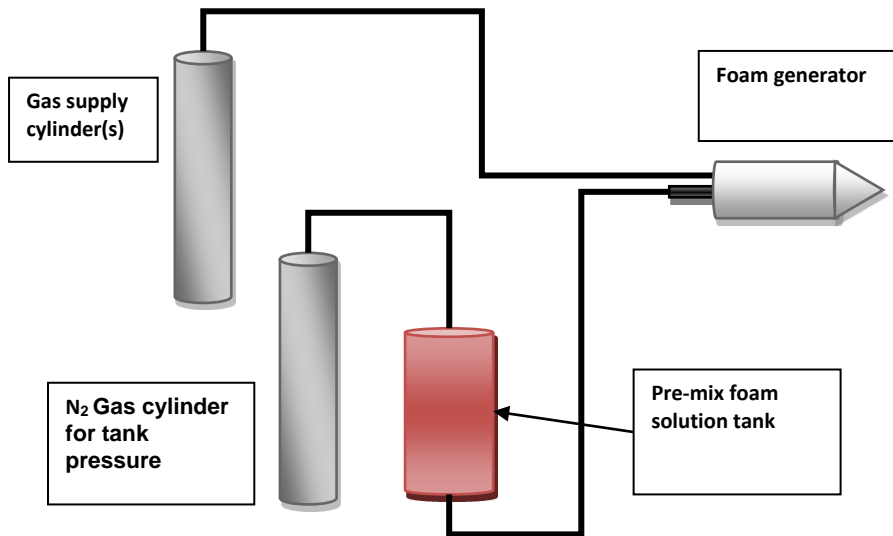
Figure 3:   Diagram of laboratory scale foam generator system

2.1.4 Liquid pre-mix supply

The pre-mix tank is pressurised by a separate compressed gas cylinder (Figure 1). The pressure required in the pre-mix tank depends on the required flow rate of solution. The nominal performance of the nozzle is 2.5 litres per minute at a pressure of 7 bar. Reducing or increasing the supply pressure will change the flow rate accordingly. Changing the pressure also alters the spray pattern of the nozzle, the idea being to generate a cone of spray that delivers a uniform flow of pre-mix across the first mesh. The characteristics of the cone are also changed by the flow of gas through the generator, which distorts the flow pattern. It is therefore necessary to balance the pressure and flow of the pre-mix with the flow of the gas through the generator while ensuring it is in the range of 3 to 8 bar.

2.1.5 Gas supply

For the small scale system, with a designed expansion rate of 300:1 and a nominal pre-mix flow rate of 2.5 m^3 per minute, the theoretical foam output rate is 0.75 m^3 per minute. The gas is supplied from compressed nitrogen cylinders through a high flow regulator. During pre-testing the outlet pressure varied between 4 and 18 bar during investigation of the optimum operating flow rate to balance with the flow of the pre-mix.

2.1.6 Foam concentrate

There are a number of foam concentrates available with different properties relevant to different fire fighting situations (Table 2). Concentrates designed for low or medium expansion foams were immediately discounted. Although several companies sell foam concentrates they ultimately belong to a small number of multinational organisations, and many foam concentrates with different brand names are in fact the same product. The two major companies producing fire fighting foam concentrate are UTC Fire & Security (represented by Kidde-Kerr Fire Fighting Chemicals in the UK and Ajax/Chubb in the Netherlands) and Tyco International/Ansul. The so-called "50% Drain time" is a standard test which measures the time for 50% of the liquid to drain from a one metre column of foam. High expansion foam has a faster draining time than low expansion, HTF 1000 was designed especially for high and very high expansion uses to build foam up to 12 metres high. The chemical formulation compensates for the faster draining time by maintaining its structure and a barrier between volatile vapour and the seat of the fire. The structure of the foam does not necessarily collapse but it becomes more brittle as the wall thickness of the bubble decreases. The foam can be easily and immediately dispersed with a fine water spray. HTF 1000 was selected based on the expectation that we would ultimately be required to generate a structurally strong foam to heights of 5 or

possibly 10 metres in large cage systems and to use gasses, other than nitrogen, that are heavier than air (e.g. Carbon dioxide or Argon). Dawson et al (2006) tried several different foam concentrates, including Ansul Jet-X high expansion foam concentrate, Playtex "Mr Bubble" and Procter and Gamble "Dawn Soap". However, the Ansul product provided the most consistent foam. During early attempts to generate foam we had used Antari Snow Liquid, designed for theatrical and disco foam generators, however this was not very stable especially at the production rate attempted with compressed gas.

Table 2   Currently available foam concentrates and their properties

Manufacturer	Ajax/Kidde	Ajax/Kidde	Ajax/Kidde	Ajax
Name	F-5	F-15	F-25	HTF-1000
Type	Standard Synthetic	Standard Synthetic	Standard Synthetic	Special synthetic
Expansion rate	6-1000	6-1000	6-1000	6-1000
Ph	6.5 – 8.5	6.5 – 8.5	6.5 – 8.5	6.5 – 8.5
Freezing point	-5 C	-10 C	-25 C	-10 C
50% Drain time	Low expansion - 20 minutes Medium Expansion - 15 minutes High Expansion - 10 minutes			High expansion - 10 minutes

2.1.7 Bubble size and expansion ratio

USDA performance standards for the use of water-based foam describe a bubble size not greater than 1.58 cm and note that bubble diameters exceeding 0.84 cm may not be appropriate for the depopulation of all types of poultry (USDA APHIS, 2006, see Figure 4). In addition they require expansion ranges from 25:1 to 140:1 and note that "foams exhibiting expansion ratios exceeding 120:1 may not be appropriate for depopulating all types of floor-reared poultry". For the current study, using foam as a gas delivery method, larger bubbles are appropriate and in successful pilot studies undertaken in Holland with carbon dioxide foam, the expansion rate was 300:1. This was therefore the target for the system modified for use with nitrogen (Figure 5). The bubble size in the pilot was 10-20 mm and these criteria minimised the chance of bubbles entering/occluding the trachea.

US Foam (North Carolina)
Expansion about 40:1

Comparison of foam.
Left: Kifco "Avifoamguard"
System (c.80:1)
Right: North Carolina
system (40:1)

Figure 4   Photographs illustrating the appearance of foam with different expansion ratios

2.1.8 Measuring bubble size

Measuring the actual diameters of individual bubbles in a sample of foam proved to be difficult. Methods that were attempted deformed the foam or modified the arrangement of bubbles and affected their size. During each individual trial a photograph was taken of the foam through the Perspex box against a millimetre scale with the intention of registering dimensions within a 10 cm square. However, on inspection, the depth of field of the photograph made it almost impossible to determine whether a particular bubble was on the plane of the Perspex wall or slightly deeper into the matrix, behind other bubbles. Adding dye to the foam did not improve matters as although the edges were slightly clearer, it was still not possible to determine whether one bubble was in front of or behind another. However some characteristics of the foam became evident. When the pressures of the gas and the foam pre-mix were well balanced in the generator the foam appeared to be very consistent, i.e. all the bubbles were of a similar size to those measured on the surface between 10 and 20 mm. Inconsistent foaming resulted in a large number of very small bubbles of 1 or 2 mm or below and some very large bubbles of 100-120 mm.
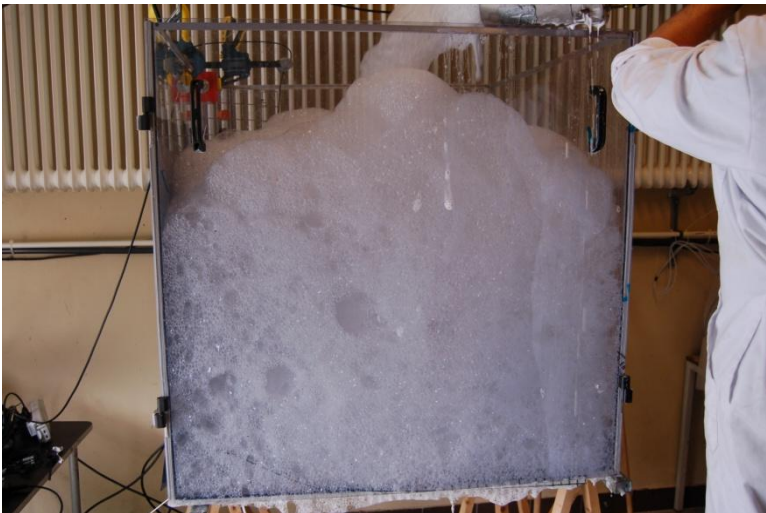
Figure 5   Initial tests showing appearance of high expansion gas filled foam

2.1.9 Measuring O_2 concentration

In earlier pilot studies with the foam generator, operating with carbon dioxide, measurements of gas concentration had been attempted with a Guardian CO_2 monitor (Edinburgh Instruments), which draws a sample of gas across a sensor with a pump. Unfortunately even a small amount of moisture from the foam caused the sensor to fail a number of times so an alternative method of sampling was explored. The

selected sensor was a zirconium dioxide dynamic oxygen sensor (J Dittrich Elektronic GmbH[2], Teda MF420-O-Zr) with a signal processing and recording system developed by Solutions for Research Ltd[3]. The sensor is mounted in a short stainless steel tube behind a sintered bronze plug to prevent introduction of moisture; in addition the probe was heated so that any moisture coming into contact with the sensors was evaporated. Three oxygen sensors were positioned at heights of 10, 30 and 90 cm in one corner of the box (see Figure 1). These were protected from bird movement by a wide metal mesh grid (aperture 20 mm, Figure 1).

Figure 6:   Oxygen sensor interface and positioning in test apparatus

2.2 Objective 2: Monitoring the physiology and behaviour of poultry during exposure to air filled and gas filled foam.

The primary aim was to investigate whether, in principle, Carbon dioxide gas-filled foam is an acceptable method of killing poultry. Secondly, the objective was to investigate if the effects of Carbon dioxide foam differ from those of anoxic Nitrogen-filled foam.
Experimental design and materials used (e.g. broilers, surgery, measuring devices) where the same in the UK and the NL experiments.

2.2.1 Subjects and husbandry

Twenty broilers (Ross 308) were obtained at 3 weeks of age from a commercial supplier and individually reared for two weeks in wire mesh pens. All broilers had visual and auditory contact with their neighbours. 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 were obtained at 6 weeks of age from a commercial supplier and individually reared for two weeks in wire mesh pens. All ducks had visual and auditory contact with their neighbours. The rearing pen was furnished with a deep litter of wood shavings. The birds had ad libitum access to food and water.

Ten Broad Breasted white turkeys were obtained at 6 weeks of age from a commercial supplier and individually reared for two weeks in wire mesh pens. All turkeys had visual and auditory contact with their neighbours. The rearing pen was furnished with a deep litter of wood shavings. The birds had ad libitum access to food and water.

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2 J Dittrich Elektronic GmbH & Co. Bahnhofstrsse 67 D-76532 Baden Baden Germany.

3 Solutions for Research, Building 42 , Wrest Park, Silose Bedford MK45 4EP.

All the experiments were carried out at the Livestock Research poultry farm "Spelderholt".
Broilers and hens were exposed to anoxic (nitrogen filled) or control (air filled) foam under standardised conditions while their behavioural and physiological responses were monitored.

2.2.2 Implantation of EEG electrodes

At 28 days of age, broilers underwent surgery to implant EEG electrodes. Ducks and Turkeys underwent surgery after a 7 day acclimatisation period. In all cases EEG electrode implantation methods were identical. After an analgesic pre-med (Buprenorphine 0.2mg/kg), general anaesthesia was induced and maintained with Isoflurane. The EEG implant consisted of two 0.35mm diameter Teflon insulated silver electrodes connected to a socket (DIN, RS components). 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 midpoints. 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 anaesthetic, all birds were housed in their home cages with ad libitum access to food and water and visual and auditory contact with their neighbours. All subjects were allowed to recover for a minimum of 6 days before undergoing any further experimental procedure.

2.2.3 Telemetry/logging units

In order to facilitate successful measurement of EEG and ECG from free moving birds we used a data logging system developed by the UK project team and the Royal Veterinary College in London. The data logging units had been used with success on hens during whole house gassing.
The main characteristics of the telemetric logging unit are:

- The logger is battery powered, and is small enough to be worn by a fowl in a Lycra backpack, thus requiring no wires and allowing freedom of movement;

- Three 'physiological waveform' input channels are provided. For this work, these channels were used to record ECG (from external non-invasive exercise electrodes) and EEG (from implanted electrodes).

- Logged data is recorded onto industry-standard 'micro-SD' memory cards;

- The logger has a radio communication facility, allowing bi-directional radio communication with a 'base station' connected to a standard laptop computer. This radio communication facility allows bursts of waveform to be requested from the waveform channels, and the current temperatures to be requested from the temperature channels, to be displayed on the computer screen. This greatly increases trustworthiness in the system, before logging commences, eliminating the need for trailing wires. If required, signal amplification of the waveform channels can also be adjusted through the radio link. Logging is started and stopped through the radio link.

2.2.4 ECG sensors and Lycra harness

As in previous work, birds already fitted with permanent EEG electrodes were also fitted with ECG electrodes immediately before each trial (see experimental procedure, below). These were commercially available disposable self-adhesive ECG electrodes (Blue Sensor, Ambu), 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) was applied to the ECG electrodes before placement on the skin to improve binding capacity. Each bird was also fitted with a re-usable Lycra harness which was secured using velcro fastenings behind the bird's head and incorporating a pocket positioned on the bird's back which contained the telemetry/logging unit. The harness incorporated a respiration sensor which was an adapted piezoelectric paediatric respiratory effort transducer (Grass Technologies Model F-RCTP).

2.2.5 Test apparatus

The foam trials were carried out in a large Perspex box (1m x 1m x 1m, see Figure 6). 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 behavioural observations a video camera (JVC) was positioned to view one complete side of the box, and a web cam (Logitech) in a waterproof box was positioned under the box to view the base.

<u>Figure 7:</u>　　　Photograph of test box with one wall (to the front) removed

2.3 Experimental procedure

Identical experimental procedures were used with broilers, ducks and turkeys. Individual broilers were assigned randomly to nitrogen or carbon dioxide filled foam treatments. Ducks and turkeys were assigned to the carbon dioxide filled foam in random order. Immediately prior to 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 if necessary. Signal logging was triggered and a 2 min baseline period was allowed during which the bird was placed in an open cardboard carrier in a room adjacent to the test area.

<u>Figure 8:</u>　　　Duck fitted with data logger in lycra harness

After baseline recording, the bird was carried to the test area and placed in the centre 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 was replaced and a further 2 min of baseline was recorded. Foam was introduced from the top of the box and care was taken not to aim foam delivery directly at the test subject. Timings were made of the start, foam touching bird, complete bird submersion and foam off (when the box was full) and were later confirmed with web cam recordings. Synchronisation of timings of

telemetry recordings and the web cam recordings ensured behavioural changes could be related to physiological responses. All measurements continued for 3 minutes after birds became motionless.

2.4 Behavioural observations

Visual obscuration by the foam limited the extent of detailed behavioural measurements. Nevertheless a number of observations were carried out from the video recordings of each trial. As foam was introduced, counts of headshakes, gasping, foam avoidance and escape attempts were noted. After submersion, ataxia, loss of posture, wing flapping (flapping onset, number of bouts, total flapping duration) and onset of motionless were recorded.

2.5 Post-mortem examination

After removal from the apparatus all birds were subjected to a post-mortem examination of the mouth, oesophagus and airways for the presence of foam or any other abnormalities. Furthermore, eyes, nasal mucosa, internal organs and brain tissue were examined for abnormalities that could be related to high levels of carbon dioxide or to suffocation.

2.6 Analysis

The logged data files were uploaded into a data acquisition and analysis program (Labchart pro 7, AD Instruments) and a combination of automated and manual analysis techniques were used to produce dedicated event channels representing heartbeats per minute (10s bins) from the raw traces during baseline and after foam application. Where clear waveforms were present, heart rate was calculated every 10 s. Visual inspection of the EEG traces allowed estimation of the timing of onset of different types of EEG activity: baseline, transitional, suppressed and approaching isoelectric. Furthermore, fourier transformation to compare percentage of total power in the alpha, beta, theta and delta frequency bands as well as Correlation Dimension (CD) (van den Broek et al., 2003) analyses were used to support the interpretation of the EEG data.

Visual inspection of the EEG traces allowed estimation of the timing of onset of different types of EEG activity. Four phases with particular characteristics are; 'transitional': high amplitude, low frequency activity or high frequency but reduced amplitude signal; 'suppressed': a greatly suppressed EEG but containing some slow wave activity; and 'isoelectric": residual low-level noise indicating lack of EEG activity.

3 Results

The experiments with broilers were performed on two consecutive days. The animals were randomly assigned to the treatments. A total of 8 broilers were successfully exposed to N_2 foam and another 8 broilers to the CO_2 foam. Experiments with ducks and turkeys where performed on two other consecutive days in a random order. A total of 9 ducks and 10 turkeys were successfully exposed to the CO_2 foam.

3.1 Oxygen concentration measurements

Trends in temperature and O_2 concentration at different levels during the foaming process from one typical bird are presented in Figure 8. It is clear that the gas concentrations decrease rapidly as the gas-foam reaches the sensor. Furthermore, this graph shows that the O_2 concentration at the different heights (10, 30 and 90cm) decreases rapidly, indicating the rapid increase in foam levels. The temperature remains constant from start to completion of the foaming process. This clearly indicates the stable temperature of the gas-foam mixture. The temperature of the gas-foam is determined by the temperature of the water used to produce the foam more than by the temperature of the gas-source.
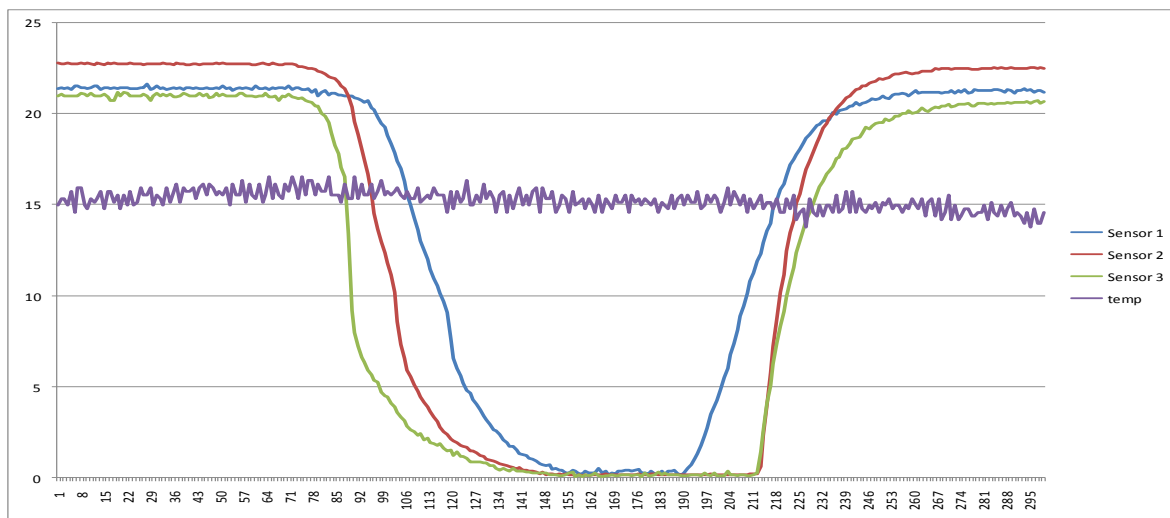
Figure 9:   Graphical example of O_2 depletion and temperature development before, during and after the foaming process.

Mean measurements of oxygen concentration in N_2 and CO_2 filled foam are shown in Table 1 (trials with broilers). Mean values were taken 1 minute after each sensor was submerged in foam and calculated over the subsequent 5 minutes. It became apparent that very low oxygen concentrations were achieved in the foam (regularly below 1% and the majority below 2%) at heights of 10 and 30 cm. However, at the 90 cm sensor the oxygen concentration was generally high and close to ambient air. This was probably due to the fact that the upper sensor was regularly not submerged in the foam. Oxygen concentrations in the air filled foam (data not shown) were very similar to ambient temperature, falling in some cases to a reading of 15%, most likely due to occlusion of the sensor.

Table 3: Oxygen concentrations[1] measured at 3 different heights during the different N_2 and CO_2 foam trials. Reported values measured when sensors were fully submerged[2] in the foam.

Trial	Sensor 1	Sensor 2	Sensor 3	Min Temp	Max temp
	90 cm	30 cm	10 cm		
	% O2	% O2	% O2	Deg. C	Deg. C
CO_2 1 [3]					
N1	0.07	0.43	2.22	13.2	16.3
N2	1.74	1.54	0.18	13.4	16.3
N3	0.07	0.12	0.05	13.4	17.5
CO_2 2	8.48	8.22	0.18	8.9	17.3
CO_2 3	0.14	0.12	0.05	13.8	16.5
CO_2 4	0.14	0.12	0.05	14.0	16.1
CO_2 5	0.07	0.12	0.05	14.0	16.5
CO_2 6	0.14	0.31	0.02	14.8	17.1
N4	0.07	0.12	0.05	14.2	17.9
N5	5.27	0.74	0.36	13.4	17.9
N6	[4]	0.43	0.36	13.6	16.9
N7	[4]	0.25	0.02	14.4	17.7
N8	0.20	0.68	0.05	14.6	17.7
CO_2 7	4.09	0.31	2.28	13.8	16.1
CO_2 8	3.60	0.12	0.92	14.4	16.5

[1] Oxygen concentration was recorded every 2 seconds for 10 minutes (300 readings). Values represent end concentration en temperatures when fully covered with foam.

[2] When the sensors are submerged in the foam there is a delay in getting a true reading due to the amount of moisture therefore it was not possible to measure the time that the atmosphere was below a certain level of oxygen.

[3] Data of this trial was lost

[4] Foam didn't reach sensor 1.

3.2 **Behaviour**

Animal behavioural events are presented in Tabel 3 The large variance within the N_2-broilers group is explicit in all events. It is most likely that the variance in the time elapse before animals are in contact and submerged with the foam is related to the variance on the behavioural events.

Table 4: Onset of behavioural events in seconds (mean±sd) measured from the start of foaming.

Group	Gasping	Headshake	Ataxia/ loss of posture	Convulsions	Flapping	Motionless
Broilers N$_2$	87[c]	-	48±23	50±24	61±34	106±16[b]
Broilers CO$_2$	25±6[b]	23±1[b]	50±10	54±16	65±15	54±15[a]
Ducks CO$_2$	13±4[a]	10±3[a]	38±10	41±13	43±7	69±21[a]
Turkeys CO$_2$	14±2[a]	10±7[a]	47±7	48±8	63±19	60±20[a]

[1]) Empty cells indicate that the specific behaviour was not expressed in that group.
[2]) Significant differences ($p<0.05$) within columns indicated with a different superscript.

The effect of gas source i.e. N$_2$ and CO$_2$, on the behaviour of animals is compared only for broilers. It is clear that gasping occurred more frequently and earlier using CO$_2$ filled foam than in N$_2$ filled foam. Moreover, headshaking was not observed in the N$_2$ filled foam and gasping was only observed in one broiler in the N$_2$ filled foam. No significant differences due to the gas source are observed on loss of posture, convulsions and flapping. This implies that under the present conditions loss of posture, convulsions and flapping are induced due to the anoxic situation more than due to the effect of CO$_2$. Absence of movement was induced significantly faster in broilers in the N$_2$ filled foam than in the CO$_2$ filled foam although, the differences are small.

Comparing the effect of CO$_2$ filled foam on behavioural events between poultry species displayed significant differences in gasping and headshaking. Both gasping and headshaking started earlier in ducks and turkeys than in broilers.

3.3 Electro-physiology responses

3.3.1 ECG responses

Figure 9 shows mean heart rate responses of the different species to exposure to carbon dioxide or nitrogen filled foam. Individual and mean heart rate responses in the four experimental groups are presented in figures 10 to 13.
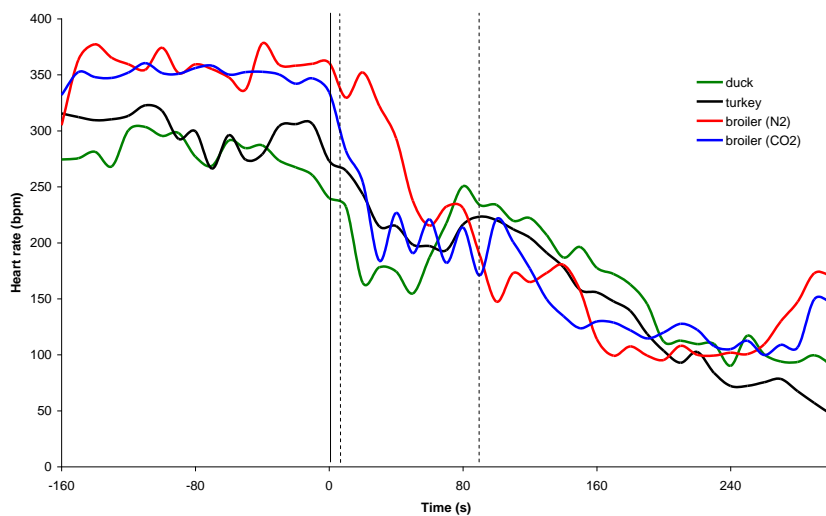

Figure 10: Average heart rate response to exposure to carbon dioxide or nitrogen filled foam

The initial bird response to foam generation was a moderate increase in heart rate, most likely associated with a fear response to the loud noise generated by foam production. Heart rate increase was also most probably caused by increased exercise as the birds moved from resting positions to avoid the foam. Basic heart rate level in broilers is higher than in turkeys and ducks. However, all species demonstrated a consistent response to submersion in foam in the form of an almost immediate pronounced slowing of heart rate (bradycardia). On average there is no difference between broilers, turkeys and ducks in response to CO_2 filled foam. The extended mean time before heartbeat ceased in ducks is greatly influenced by a single duck of which the heart rate ceased after a significantly longer duration than for any of the other birds. Although not significant, it would appear that bradycardia was induced earlier in broilers exposed to CO_2 than to N_2 filled foam.

The between-bird variation, within an experimental group (species or gas source), in onset of bradycardia (Figure10,11,12,13) is likely to be related to the differing timing of submersion under the wave of foam (which took between 10 and 80s to cover all of the birds, depending on the trial). Data show low variation between individuals in time to onset of bradycardia after submersion. The pattern of pronounced bradycardia followed by varying degrees of recovery and/or stabilisation before a final decline, seen in all birds, was exactly the same as that seen in individually exposed broilers in earlier studies.

Strong variation in the presented heart rate and more specifically, the irregular, short period, increase and decrease is also due to the analyzed periods. An analyzed period reflects a real time period of 10 seconds. As soon as heart rate becomes irregular due to heart failure and or fibrillation, the beats per minute fluctuate over very short periods. This implies that in a 10 second period there can be a strong increase or decrease in heart rate.
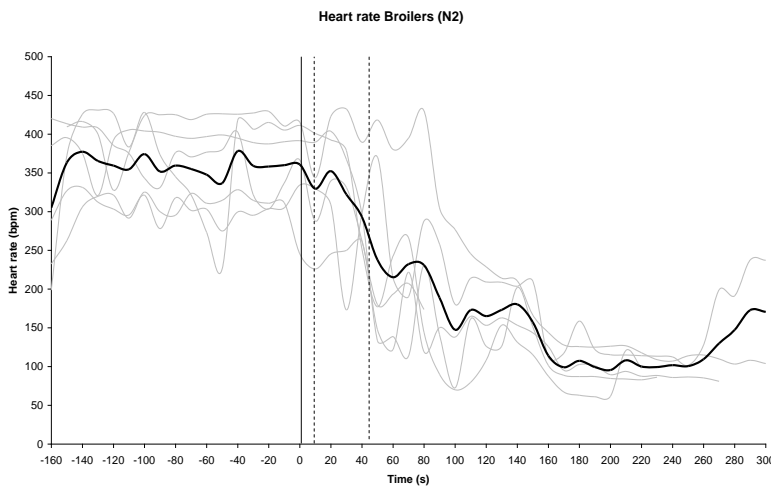
Figure 11: Mean (black line) and individual (grey lines) heart rate response of broilers to N_2 filled foam.

Figure 12: Mean (black line) and individual (grey lines) heart rate response of broilers to CO_2 filled foam.
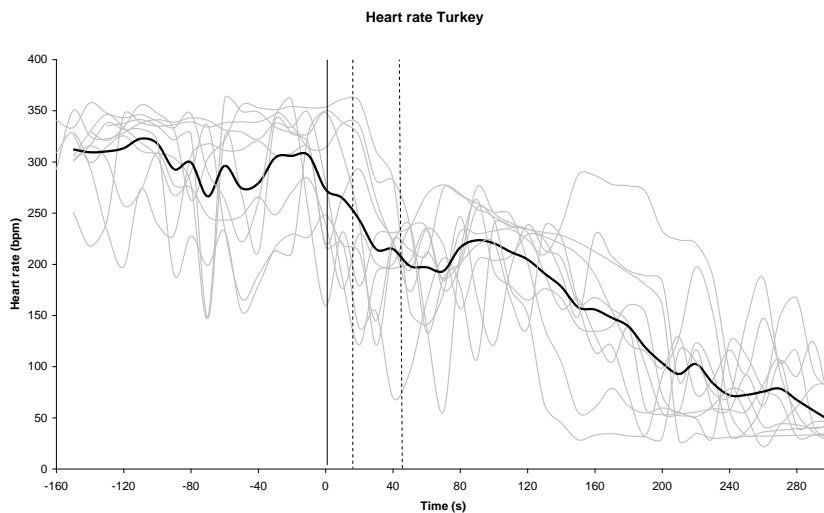
Figure 13:   Mean (black line) and individual (grey lines) heart rate response of turkeys exposed to CO_2 filled foam.

3.3.2 EEG response

To detect the moment of unconsciousness EEG traces are analysed visually for changes in frequency patterns. Changes are classified as 'transitional' characterized as a high amplitude, low frequency phase or a low frequency with a reduced amplitude. The 'suppressed' phase is characterized as a greatly suppressed signal containing slow wave activity. The 'iso-electric' phase presents a signal containing residual noise without EEG activity. Furthermore, correlation dimension analyses (CD) are performed on the EEG traces to identify the moment that the CD score is reduced to at least 60% of the baseline score.

Table 4:   Time elapsed in seconds (mean±s.e.m) from onset foaming to a transitional, suppressed and iso-electric EEG and time elapsed to a 60% reduction in the correlation dimension (CD)of the EEG signal.

Group	Transitional	Suppressed	Iso-electric	CD analysis
Broilers N_2	47±13[b]	51±12	92±6	69±9
Broilers CO_2	32±4[a]	51±6	102±26	63±23
Ducks CO_2	25±2[a]	35±4	94±22	*
Turkeys CO_2	30±3[a]	48±2	96±8	40±5

Table 5: Time elapsed in seconds (mean±s.e.m) corrected from being submerged by foam to a transitional, suppressed and iso-electric EEG and time elapsed to a 60% reduction in the correlation dimension (CD)of the EEG signal.

Group	Transitional	Suppressed	Iso-electric	CD analysis
Broilers N_2	9±2	13±2	48±11	26±11
Broilers CO_2	-2±4	16±5	66±25	25±22
Ducks CO_2	-11±2	1±2	58±24	*
Turkeys CO_2	-2±3	15±4	60±8	13±4

The delay time to a transitional EEG was significantly longer in broilers in the N_2 group than in the other groups. Comparing the effect of N_2-filled foam with CO_2-filled foam in broilers there is a significant ($p=0.024$) delay in the occurrence of a transitional EEG for N_2-filled foam.

The effect of CO_2 enclosure of the foam is clearly visible when correcting the EEG data for the moment of submergence into foam (Table 4). A transitional EEG in the CO_2 filled foam had already occurred prior to the animals are being submerged. A transitional EEG only occurred in the N_2-filled foam after submergence.

In ducks there appears to be a trend towards an earlier (P 0.075) suppression of the EEG compared to broilers and turkeys. No differences were observed in onset of an iso-electric EEG between gas sources i.e. N_2 and CO_2 or between species i.e. broilers, ducks and turkeys.

CD analyses were strongly obscured by noise and movement artefacts on the EEG traces. As a result, only a limited number of traces (broilers N_2: 5 of 8, broilers CO_2: 4 of 8, ducks: 1 of 10 and turkeys: 8 of 10) could be assessed using CD analysis. Because of the irregularity of the number of animals that could be used for CD analysis the outcome of this analysis can only be used as indicative support to the visual assessment of the traces. However, the reduction CD values for those individual animals analysed followed the induction of a suppressed EEG.

4 Conclusions on individual bird trials

4.1 Conclusions on behavioural events

Broilers exposed to CO_2-filled foam displayed more gasping and headshaking than broilers exposed to N_2 filled foam.

Loss of posture, the behavioural indication, for loss of consciousness occurred after the same time had elapsed in CO_2- and N_2- filled foam.

Since loss of posture, convulsion and flapping started after the same time elapse in CO_2- and N_2- filled foam it would appear that under the present conditions these behavioural events are more likely to have been induced by anoxia (low O_2 levels), than by hypercapnia (high CO_2).

Gasping and headshaking started earlier in ducks and turkeys than in broilers. The difference between these species however is small and it is not clear if this is due to a difference in sensibility of the broilers or due to differences in the quality of foam production. These aspects cannot be separated in these experiments.

Convulsions and wing flapping of the birds break down the foam very rapidly. Therefore sufficient capacity of the foam generators is necessary to create foam faster than it can be destroyed due to vigorous movements of birds. If the foam is destroyed before birds are in a deeply unconscious state the birds could regain consciousness very rapidly especially when exposed to N_2. Destruction of the CO_2 foam will still lead to an atmosphere that is saturated with a high level of CO_2. Therefore, from an efficacy point of view, CO_2 will lead to a more stable anoxic situation than if N_2 is used.

4.2 Conclusions on electro-physiological response

4.2.1 Heart activity

The initial response to foam generation in the birds was a moderate increase in heart rate, most likely associated with a fear response to the loud noise generated by foam production and the increased exercise as the birds moved from resting positions due to alert behaviour.

All species demonstrated a consistent response to submersion in foam in the form of an almost immediate pronounced slowing of heart rate (bradycardia). However, it seems that bradycardia occurs more rapidly in birds exposed to CO_2 filled foam than to N_2 filled foam.

The pattern of pronounced bradycardia is followed in all birds by varying degrees of recovery and/or stabilisation before a final decline. During the final decline heart rate becomes very irregular resulting in fibrillation, short periods of recovery alternating with short periods of absence of heart beat, resulting in heart failure and death.

4.2.2 Brain activity

Due to the anaesthetic effect of CO_2, exposure of birds to a CO_2-filled foam leads to an earlier induction of a transitional state of the EEG than when birds are exposed to N_2-filled foam. Moreover, the effect of CO_2 on consciousness begins prior to submergence indicating that the CO_2 concentration around the foam has a clearly positive effect on the reduction of the conscious state of the birds. Suppression and subsequent induction of an iso-electric EEG occurs after submergence in the foam. The suppressed state of the EEG correlates with unconsciousness which is confirmed by the reduction in correlation dimension analyses.

After submergence there is no difference in the reduction of conscious state between CO_2- and N_2-filled foams. Therefore, it can be concluded that the anoxic effects of both gas_filled foams are comparable. From this point of view it makes no difference whether birds are exposed to CO_2 or to N_2 filled foam. However, the positive effect of CO_2 enveloping the foam on the induction of unconsciousness can be seen as a safeguard against destruction of the foam structure by wing flapping or convulsive spasms.

4.3 **Recommendation based on individual bird trials**

Further development towards a reliable method that can be used under practical conditions can be justified based on these experiments. Areas of concern for further development of the foam method are the stability of the foam when applied on larger groups of birds taking into account the risks of convulsive spasms. The capacity of the system should supply sufficient foam to compensate for the breakdown of foam by convulsing birds. This is a point of special attention when using N_2 instead of CO_2.
Foam produced with CO_2 can easily result in freezing of the injection nozzle and thus to malfunctioning of the system. Preventing freezing of a large scale system when using CO_2 will be a challenge.

5 Foam trials without birds aimed at increasing foaming capacity

5.1 Background

Previous trials with individual hens, broilers, ducks and turkeys provided strong evidence that submersion in nitrogen or Carbon dioxide filled foam is a highly effective and rapid method of euthanasia (see sections 2 and 3). Based on the results from these experiments it was decided to upgrade the gas foam method to a more commercially applicable scale. To do so we identified two objectives.
Objective 1: Capacity of the foam generators must be increased significantly Furthermore, stability of the foam and possible solutions for use of high carbon dioxide and or nitrogen flows without freezing the spray nozzles must be investigated.
Objective 2: Determination of whether or not the humane euthanasia achieved with nitrogen or carbon dioxide filled foam in the laboratory could be repeated under more commercially relevant circumstances.
Foam was applied to groups of birds at differing high commercial stocking densities in which a subset of individuals were equipped with physiological monitoring devices. Factors such as stocking density and distance travelled over birds by foam (pen configuration) were examined for their effects on the efficacy of the euthanasia process.

5.2 Material and methods

5.2.1 Equipment and facilities

The work was carried out at the research facility of the Animal Science Group of Wageningen UR, The Netherlands in June 2009 in collaboration with Dr Dorothy McKeegan Jules Sparrey and LST international. The UK (Defra), and Dutch (LNV) governments co-funded the project. The trials were carried out in a research building normally used for poultry rearing. The room measured 13 m x 10.5, with a height of 6 m to the eaves and 9.5 m to the gable, ventilation although fully mechanized was not activated during the trials. A pen was built within this space to contain the foam during the trials, the different pen configurations are described below.

The foam generating system consisted of a bulk tank of liquid nitrogen (6200 litre capacity) connected to a fan assisted ambient air vaporiser with a nominal 8 hour capacity of 1750 Nm^3 per hour[4] (Figure 14). The gas flow requirement for the foam generation system was 2400 Nm^3 per hour and it was confirmed by the manufacturer that this could be sustained by the unit for a maximum of 1 hour followed by a recovery period to allow the vaporiser to return to ambient temperature. Since each run would last only a few minutes the planned use of the vaporiser was well within these parameters. The foam solution was mixed in a 2 m^3 open tank (Figure 14) prior to each run and consisted of a 3% solution of Ajax HTF1000 in water. HTF1000 is a special synthetic very high expansion foam concentrate (which does not contain fluoro-carbons) which can achieve an expansion ratio of up 1000:1. The choice between alternative foam surfactants was discussed in the report on the previous project. A specialist fire fighting pump, capable of controlling both pressure and flow rate, was used to pump the pre-mixed solution to the generators. Four foam generators (Figure 15) were used, each with a nominal capacity of 10 m^3 per minute. The generators were positioned in a group in the centre towards the end of the pen, 350 mm above ground level.

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[4] Nm^3 Normal m^3 at normal temperature and pressure.

Figure 14   Liquid nitrogen tank, vaporiser, pump and pre-mix tank

5.2.2 Experimental trials

Three different pen widths (four, six and eight metres) were used to investigate three different forward speeds of foam, from a constant source. The length of the pen was eight metres. One long side of the pen was constructed from Perspex to provide a clear view through which to video and photograph the foam bow wave (Figure 16). Eight cameras, at 1 m intervals, were focused on the side wall and a further two were hung in the roof space covering the pen from above. The outputs of the cameras were passed through three multiplexors to enable records of up to four images onto each of three analogue video recorders. The height of the pen sides was a minimum of 1.6 metres. The concrete floor was covered with a thin layer of bedding material (wood shavings, Figure 16) to ensure the correct (commercially relevant) friction between the foam and the floor. Each pen width was filled twice and the volume of foam produced each time was calculated by measuring the final depth of foam at four points along the length of the pen. The time taken to fill the pen was measured from the moment foam entered the pen to the moment it stopped. The floor was marked with tape at 1 m intervals and the Perspex wall marked horizontally at 20 cm intervals to enable the progression of the foam to be measured. In a couple of cases the foam overflowed the pen and the volume of this was measured and included in the final volume.

Figure 15 Pre-mix manifold and pressure gauge   Foam generators

<u>Figure 16</u> Test pen set up, showing Perspex wall, ariel view and camera positioning

5.3 Results and discussion

The video recordings taken from above the pen were analysed by measuring the time taken for the foam front to cross each of the floor markers down the centre line of the pen. Additionally, footage from the side cameras was used and the depth of foam with time was measured at fixed points along the Perspex wall. Table 5 shows the basic data for each of the runs including the volumetric flow rate and the average speed of the foam along the whole length of the pen. Table 6 shows the cumulative average speed of the foam as it progresses down the pen and also the speed over each 1 m section.

Table 5 Summary of flow rates recorded in Phase 1 runs

	Pen width (m)	Final Foam volume (m^3)	Fill time (s)	Volume flow rate (m^3/min)	Average forward speed over 8 m (ms-1)	Flow rate per metre pen width (m^2/min)
Run 1	4	51	163	18.8	0.09	4.7
Run 2	4	51	122	25.2	0.14	6.6
Run 3	6	72	133	32.5	0.11	5.4
Run 4	6	82	150	32.6	0.12	5.4
Run 5	8	115	212	32.6	0.09	4.1
Run 6	8	99	183	32.5	0.10	4.1

Runs 1 and 2 had lower foam production rates which may have been due to the poor balancing of the gas flow and pre-mix. Observations of foam flow, especially during the first two runs, showed periods when gas escaped from the top of the foam above the generators. This indicated that gas flow did not sufficiently match liquid flow, allowing too much gas through the generators and reducing their efficiency. The gas flow was simply controlled by a valve on the liquid nitrogen tank and there was no way of knowing the actual gas flow rate out of the vaporiser. Additionally the high ambient temperature (26 ºC) during the trials meant that it was initially difficult to control the pressure in the liquid nitrogen tank despite opening the pressure relief valve.

Each generator was producing 8.1 m³/min of foam in runs 3 to 6, which was below their expected flow rate of 10 m³/min. The spray nozzles inside the generators have a specific flow rate of 33 litres/min at 7 bar pressure; this flow was checked and found to be correct therefore the reduced performance was attributed to poor gas flow control for the reasons mentioned above. It was recommended that potential pressure losses in the gas distribution manifold should be addressed and a flow meter should be installed in the gas line and this was completed for the second phase in October 2009 (see below).

Table 6   Velocity of foam front in forward direction

Run	1		2		3		4		5		6	
	4 m		4 m		6 m		6 m		8 m		8 m	
	Ave.	Inst.	Ave.	Inst.	Ave.	Inst.	Ave.	Inst.	Ave.	Inst.	Ave.	Inst.
1 m	0.50	0.5	0.50	0.50	1.00	1.00	0.50	0.50	0.50	0.50	0.50	0.50
2 m	0.29	0.20	0.33	0.25	0.40	0.25	0.40	0.33	0.50	0.50	0.50	0.50
3 m	0.17	0.09	0.23	0.14	0.25	0.14	0.25	0.14	0.43	0.33	0.43	0.33
4 m	0.13	0.08	0.18	0.11	0.17	0.09	0.19	0.11	0.19	0.07	0.24	0.10
5 m	0.11	0.06	0.16	0.11	0.14	0.08	0.16	0.09	0.14	0.07	0.16	0.07
6 m	0.10	0.08	0.15	0.13	0.11	0.06	0.14	0.10	0.11	0.05	0.12	0.06
7 m	0.10	0.07	0.15	0.11	0.11	0.08	0.13	0.08	0.09	0.06	0.11	0.06
8 m	0.09	0.07	0.14	0.09	0.11	0.10	0.12	0.07	0.09	0.05	0.10	0.06

[Inst. = "instantaneous" velocity over 1 m, m/s; Ave. = Average velocity from start of run, m/s]

The foam speed in a forward direction close to the generators is much greater than that towards the end of the pen driven by the pressure of the gas in the generators. As foam is produced it follows the path of least resistance and therefore pushes up over the preceding foam, quickly building height. This effect is increased when the foam hits the side walls and can only flow forwards.

The bow wave of the foam rose to between 0.5 and 0.6 m within 1 m and 0.70 – 1.0 m within 4 m, measured from the leading edge. Although the initial bow wave was similar in each case, the following height of the foam was 10 cm lower for each wider pen. If this is related to the speed of the bow wave and it is assumed to travel at an average speed of 0.1 m/s then the birds will be covered with 0.5 m of foam in 10 seconds and up to 1.0 m in 40 seconds. This period covers the onset and duration of wing flapping when it is expected that most foam destruction will occur.
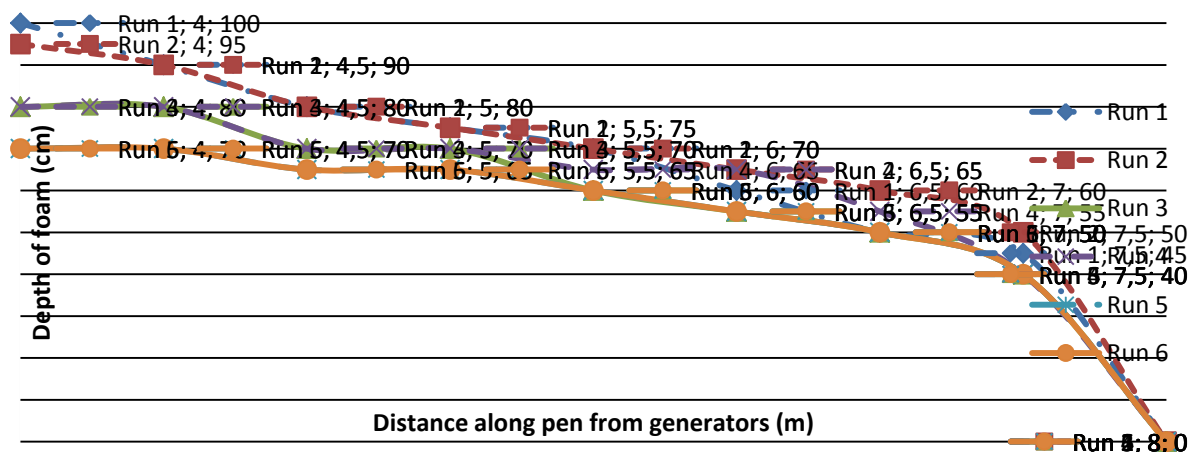
Figure 17   Height of foam as front edge touches the end wall, 8 m distance from the generators

5.4 Conclusions

Despite the foam generation rate not being quite as high as expected in these trials, the equipment was able to create a foam bow wave of sufficient depth that it could be expected to completely cover birds

during their bouts of wing flapping. It is recommended that the quickest way to achieve a high consistent bow wave is that the foam should be generated across the whole width of the pen, rather than in one place, so that it reaches the side walls as quickly as possible. The initial 50 cm of the foam front was consistently above 40 cm and if moving faster than 0.1 m/s will completely cover birds in about 3 seconds. The height of foam will then build up over the birds to be 50-60 cm when the bird starts flapping at about 15 seconds after submersion.

It was decided that for the live bird trials in Phase 2 the pen width adopted should be 6 m, maintaining the length at 8 m, with the 4 foam generators spaced equally across the width of the pen. This would give a depth of foam over the birds of 65 cm within 16 seconds increasing to 80 cm by 34 seconds.

6 Foam trials with groups of birds at commercial stocking density

6.1 Introduction

The aim of this part of the study was to determine the influence of critical, commercially relevant factors such as the rate of foam destruction by flapping birds and effect of stocking density on the effectiveness of the foam killing technique. A pilot study on a small group of broilers, previously carried out in the Netherlands, had indicated that there was less foam destroyed when birds had less space to move around, however the limitations of this effect were not examined. To investigate this, a series of trials were carried out in which groups of broiler chickens were exposed to the gas filled foam at different stocking densities and pen configurations. During these trials some birds were equipped with telemetry logging devices to allow their physiological responses to be monitored (see Objective 2, below).

6.2 Equipment and facilities

The work was carried out at the same facilities and location as the Phase 1 trials (research facility of Livestock Research, Wageningen UR, The Netherlands) in October 2009. Some changes were made to the foam gas system based on the outcome of the Phase 1 trials. An ultrasonic portable gas flow meter (GE Panametrics TransPort® PT878GC) was installed on a 1.5 m length of 75 mm diameter pipe connected downstream of the vaporiser. This also meant that an improved gas distribution manifold (Figure 18) was built reducing pressure loss in the gas system. The open tank of pre-mixed foam solution was replaced by an integrated system consisting of a water tank, foam concentrate tank, pump and electro-mechanical proportioning system (Figure 18). This allowed the correct amount of foam concentrate to be directly injected into the water flow downstream of the pump to achieve the 3% solution required, irrespective of flow rate. Passive flow proportion regulators which rely on the venturi effect to draw foam concentrate into the water steam had previously been rejected as they are not very accurate and require a steady state flow which would not be achieved in the very short duration operational runs.

Figure 18   Gas flow meter and improved manifold   Integrated foam mixing and pumping unit

6.3 Subjects and husbandry

A commercial supplier provided 1000 broiler chickens obtained at one day-old that were reared in a single group under commercially relevant conditions. The rearing area was furnished with deep wood shavings litter and was equipped with heat lamps. The birds had ad libitum access to food and water. The experiments were carried out at the research facility of Livestock Research, Wageningen UR in the Netherlands with permission from the animal ethical committee of the Animal Sciences Group.

6.4 *Implantation of EEG electrodes*

At 28 days of age, 17 broilers underwent surgery to implant EEG electrodes under general anaesthesia (induced and maintained with Isoflurane), in exactly the same way as previously described (see section 2.2.2). Briefly, the EEG was recorded by two 0.35mm diameter Teflon insulated silver electrodes, placed

on the dura through small holes drilled in the skull. An indifferent electrode placed between the skull and the overlying tissue under the comb. The EEG implant was secured to the skull with dental cement and the surrounding skin was closed with sutures. After initial recovery from the anaesthetic, all birds were housed in individual cages with ad libitum access to food and water and visual and auditory contact with their neighbours. The birds were allowed to recover for a minimum of 4 days before undergoing any further experimental procedure.

As in previous trials (described in section 2.2.3), birds already fitted with permanent EEG electrodes were also fitted with ECG electrodes immediately before each trial procedure These were commercially available disposable self-adhesive ECG electrodes (Blue Sensor, Ambu), 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) was applied to the ECG electrodes before placement on the skin to improve binding capacity. Each bird was also fitted with a reusable Lycra harness which was secured using velcro fastenings behind the bird's head and incorporated in a pocket positioned on the bird's back which contained the telemetry/logging device. The telemetry/logging units were protected from physical damage and water ingress by being wrapped in foam and bound with tape.

6.5 *Experimental procedure*

Immediately prior to each trial, each monitored bird to be added to the test pen was fitted with ECG electrodes and a Lycra harness containing a telemetry unit. The telemetry function was used to verify of the existence of high quality physiological signals on each channel, and adjustments made if necessary. Signal logging was triggered for a duration of 60 minutes and the logging start time was noted. Monitored birds were placed in the test pen at a range of locations to represent the length and width of the pen (although we could not control their movement after this). The birds were allowed a few minutes to settle and data logged at this time provided a baseline for each monitored bird. Once the trial began, timings of onset of foam production, foam entering the pen and all birds submerged were noted on the time synchronised with the loggers.

6.6 **Trials**

Initially, three stocking densities were selected, 40, 50 and 60 kg/m^2 to test the hypothesis that restricting space by increasing the stocking density would alter the effect of wing flapping on the foam. Unfortunately, the Animal Science Group's ethics committee decision a few days before the trials would not allow the use of the highest stocking density, so only densities of 40 and 50 kg/m^2 were examined. Based on the number of birds available, four trials were carried out, two at 40 kg/m^2 and two at 50 kg/m^2. In order to reduce the number of birds used in each trial, smaller pen areas 2 m x 6 m within the main trial pen were devised. Two different pen configurations were selected, laterally across the end of the pen exposing the whole of the foam front to the birds and a longitudinal pen exposing a section of the foam front to continual challenge over the length of the pen (Figure 19, Figure 20). The longitudinal pen was built adjacent to the Perspex wall in order to give the best view to the cameras.

Figure 19   Lateral pen (2 m x 6 m)   Longitudinal pen (2 m x 6 m)
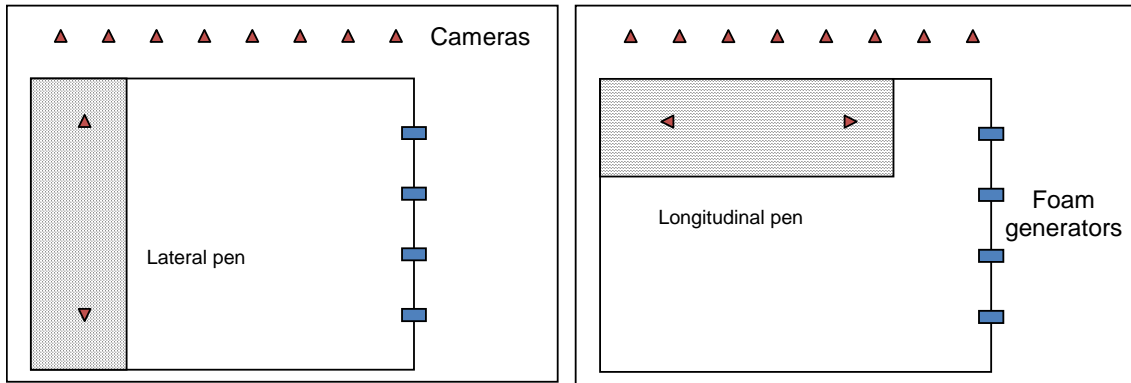
Figure 20   Experimental pen configurations

Eight video cameras were placed along the length of the Perspex wall at 1 m intervals and connected to an 8 channel digital video recorder to allow observations of the foam and bird responses. A digital camcorder filmed the pen of birds from above and two water proof digital video cameras were placed in the bird pen at a height of 300 mm to record a 'bird level view' (see also Figure 20). Both the long sides of the pen were marked with horizontal tape at 20 cm intervals and a series of 5 poles, again marked at 20 cm intervals were positioned down the centre line of the pen to enable assessment of foam depth (figure 19).

During each trial, residual oxygen was measured by a hand held oxygen sampling metre (MX40, Industrial Scientific Corporation) which had a small integrated pump which sucked a sample down a 3 mm diameter PVC hose via a glass jar moisture trap to prevent liquid damaging the sensor. The unit did not record a time history and the intention was to test whether or not the unit would be a suitable instrument to use in the field.

A preliminary run without birds was carried out to establish a base line for foam production volume flow rate. In trial 1, 192 birds were placed in the lateral pen configuration, to achieve 40 kg/m^2. In trial 2, the same number of birds was placed in the longitudinal configuration, again at 40 kg/m^2. In trial 3, 240 birds were placed in the lateral pen configuration, to achieve 50 kg/m^2. In trial 4, the remaining 360 birds were placed in the longitudinal configuration, again at 50 kg/m^2, the width of the pen was increased slightly from 2 m to 2.4 m to maintain the correct stocking density. , Foam generation protocol was identical in all trials.

The duration of the run was timed from the moment foam entered the pen to the moment the pump was turned off. The decision as to when to turn off the foam generator was made based on the cessation of movement of the birds. The gas was turned on 10 seconds before the pump was engaged to allow liquid nitrogen to begin to vaporise and the flow rate to build up. At the end of the run although the liquid nitrogen is turned off at the same time as the pump, excess gas has to be allowed to vent out through the generators to prevent a dangerous built up of pressure within the vaporiser. The amount of water and foam concentrate used was recorded by flow meters in the foam proportioning unit and the gas meter was set to record the total volume flow and also displayed the instantaneous gas flow rate during the run. The video recordings of the preliminary run were used to take measurements of the speed of the foam front and depth of foam as in phase 1. For trials 1 to 4 with birds they were used to make observations on the behaviour of the birds and the rate of foam destruction.

6.7 *Analysis*

The logged data files were uploaded into a data acquisition and analysis program (Spike 2 Version 4.2, Cambridge Electronic Design) and a combination of automated and manual analysis techniques were used to produce dedicated event channels representing heartbeats per minute (2 s bins) from the raw traces during baseline and after foam application. Analysis was performed by Dr. Dorothy McKeegan of Glasgow University. Where clear waveforms were present, heart rate was measured every 10 s. Where possible, visual inspection of the EEG traces allowed estimation of the timing of onset of different types of EEG activity: transitional, suppressed and isoelectric. Vigorous body movement was visible as a characteristic artefact on the ECG trace, this was used to determine onset of flapping, number of bouts and total flapping duration. Some simple comparisons of timings of EEG changes and behavioural responses in this trial were made to laboratory results using T-tests.

6.8 *Results*

6.8.1 Foam Generation without birds

The foam production rate increased from the phase 1 of 32 m^3/min to 42 m^3/min (Table 7). This was due to improved control of the gas flow rate, reductions in pressure drops in the gas supply pipe and improvements in foam solution mixing. The average speed of the front edge of the foam also increased, being 0.15 m/s compared to 0.12 m/s in the equivalent trial in phase 1 (this effect is also related to the increased generation rate).

Table 7 Characteristics of foam generation in the preliminary run (without birds)

Run time	120 s	
Time to end of pen	53 s	
Time to 1 m at end of pen	89 s	
Average speed to end of pen	0.15 m/s	
Total foam produced	85 m^3	
Vol. Flow rate	42.5 m^3/min	
Pre-mix used	244 litre	
Expansion rate	348:1	
Gas used	112 m^3	

Figure 21   shows that, compared to the equivalent trial in phase 1, the foam front was about 10 cm higher at the leading edge and nearly 20 cm higher 3-4 metres back towards the foam generators (Figure 21).
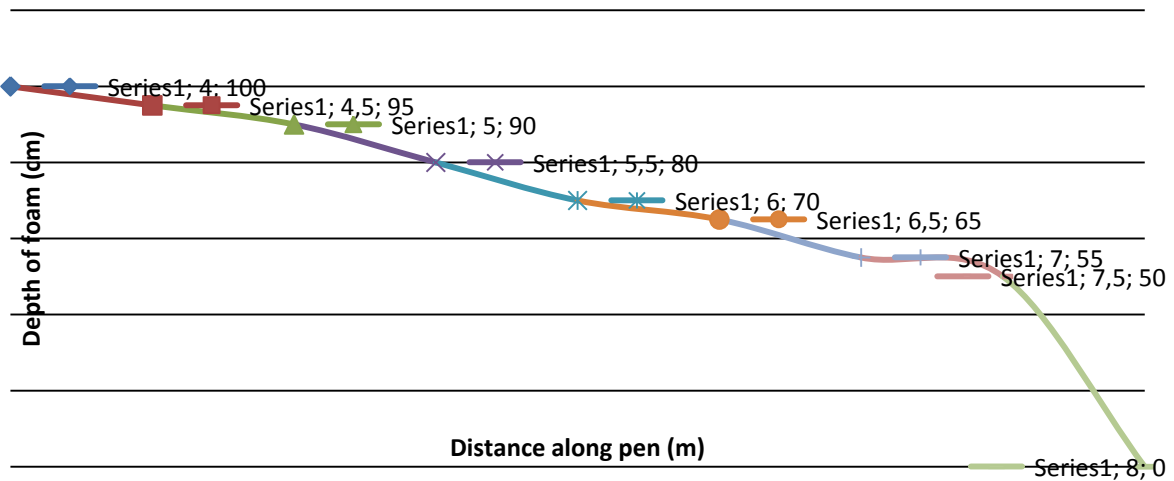

Figure 21   Profile of the foam front in the preliminary run (without birds) as it reached the end wall of the pen, furthest from the foam generators.

Figure 22 shows the increase in depth of foam with time at certain points along the length of the pen. The data indicate that the foam reaches a depth of 60 cm in an average of 11 seconds and 1 m in an average of 30 seconds.
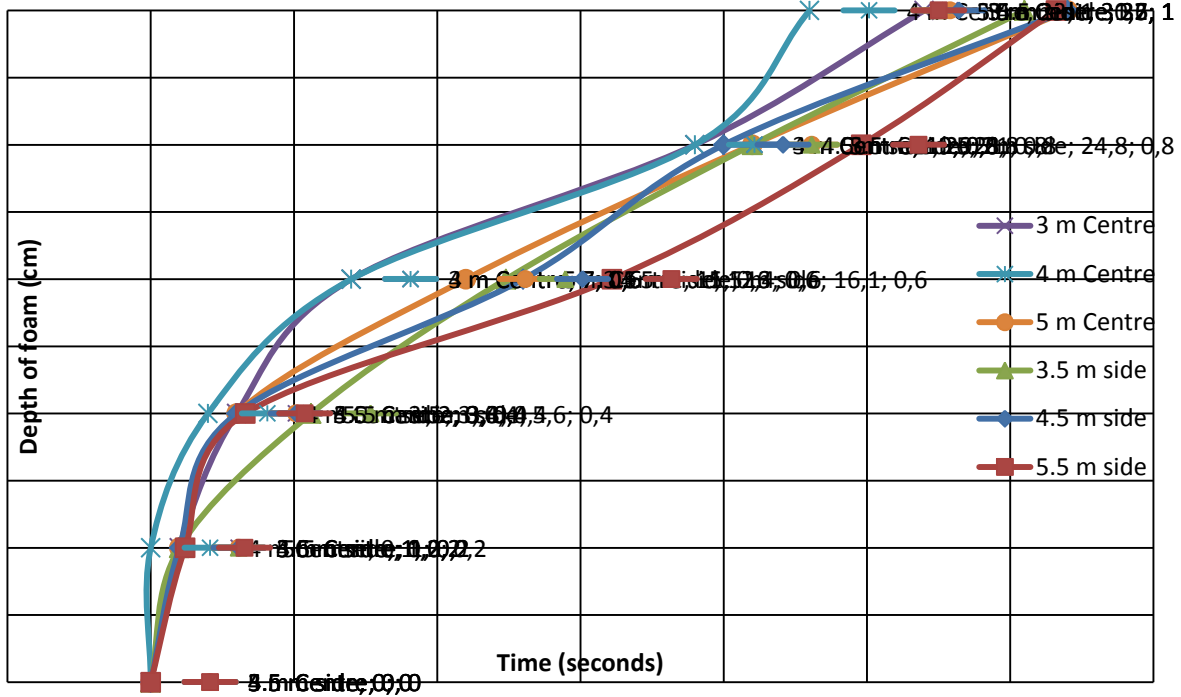
Figure 22   Depth of foam at fixed points in the pen against time

6.8.2 Trials with birds

Since foam production could not be measured directly it was calculated from the duration the system was running and assumed the foam production rate was maintained at 42.5 m^3 /min as was achieved in the preliminary trial. The height of the foam could not be measured accurately when passing over the birds as either flight reactions from a number of birds at the leading edge tended to lift the foam and after the onset of wing flapping the surface of the foam moved up and down. Expansion rate was calculated from the estimated total volume of foam produced and the measured quantity of foam solution used. Table 8 shows a summary of foam production data for the 4 trial with birds.

Table 8   Summary of foam production results for trials 1-4 (with birds)

	Trial 1	Trial 2	Trial 3	Trial 4
Date	14/10/2009	14/10/2009	14/10/2009	15/10/2009
Starting time	10:11	12:59	16:13	11:58
Stocking density (kg/m^2)	40	40	50	50
Number of birds	192	192	240	360*
Pen arrangement	Lateral	Longitudinal	Lateral	Longitudinal
Run time (s)	139	144	151	165/130*
Time to end of pen (s)	63	91	72	104
Time to 1 m at end of pen (s)	113	112	129	145
Average speed to end of pen (m/s)	0.13	0.09	0.11	0.08
Gas Volume (m^3)	124	154	125	206
Pre-mix used (litres)	284	294	317	281
Calculated foam generated (m^3)	98	102	107	96
Final foam at end of run (m^3)	86	96	89	86
Estimated foam destroyed (m^3)	12	6	18	10
Foam destroyed (m^3 per 100 birds)	6.2	3.0	7.5	3.3
Expansion rate	346:1	350:1	337:1	342:1

Note: During trial 4 there was an interruption to the generation of foam for 35 seconds, the reasons for this are discussed below (and welfare implications discussed in Objective 2). The total run time was 165 seconds, the actual time when foam was generated was 130 seconds.

6.8.3 Effect of stocking density on foam destruction

Lateral pen configuration: Trials 1 and 3

Foam production rates may have differed slightly between these two trials based of the time it takes the foam front to travel the first 6 metres across clear ground. For the first 4 m their speeds are similar then in trial 3 the foam appeared to slow down for around 10 seconds before speeding up again. This results in a possible overestimation of the total foam production for trial 3. The amount of foam destroyed by the birds in trial 1 was 12 m^3. There were 192 birds in the pen, and so the destruction of foam is equal to 6.2 m^3 per 100 birds. In trial 3 the foam destruction rate was 7.5 m^3 per 100 birds. Although, the data show that more densely stocked birds destroy more foam, it is difficult to determine significance of these differences due to the suspected overestimation of foam generation in trial 3..

Longitudinal pen configuration: Trials 2 and 4

Comparisons between the two runs are difficult due the interruption of foam supply in trial 4 which is discussed below. In both cases the forward speed of the foam was reduced once the birds started flapping and flow became less consistent as different sections of birds started and stopped flapping. The calculated foam destruction rate was 3.0 m^3 per 100 birds in trial 2 and 3.3 m^3 per 100 birds in trial 4. It should be noted that although the trials were designed to compare two stocking densities, the behaviour of the birds in response to the foam (moving away, see Objective 2, below) meant that in real terms, the stocking densities of the birds in trials 2 and 4 were maximal by the time the foam submerged them in areas of the pen furthest from the foam generators. This suggests that there is unlikely to have been difference in the absolute stocking densities as the foam moved over the last 3 metres of the pen.

Effect of distance travelled over birds by the foam

There is a large difference in the foam destruction rate by the birds when comparing the two different pen configurations, by around a factor of 2. This strongly suggests that the width of the pen has a greater impact on the rate of foam destruction than the length.

6.8.4 Problems encountered during Trial 4

In trial 4, foam production stopped after 35 seconds and resumed after a break of 35 seconds, making the overall run time 165 seconds with foam being generated for 130 seconds. Gas was flowing during the whole period. One possible reason for interruption of foam production could be that the nozzles in the four foam generators had simultaneously frozen caused by low gas temperature. However analysis of the data and video recordings do not support this theory. The problem occurred on day two of the trials yet ambient temperatures on both days of the experiments were similar; a minimum of 0 ºC overnight air temperature on both preceding nights and a day time high of 10 ºC for trials 1-3 and 12 ºC for trial 4. If freezing were the problem then it could have been expected to happen in trial 1, as for both preceding nights foam pre-mix had stood in the hoses overnight. One of the video views showed the foam generators and it was observed that a considerable amount of liquid began flowing out under the generators four seconds after foam production stopped, when prior to this point this had not been the case. This flow of liquid continued until 10 seconds after the foam restarted, which indicated that liquid was flowing through the foam generator nozzles throughout the whole period. The pipes connecting the regulator to the generators have a total volume of approximately 75 litres which is sufficient for the system for run for about 35 seconds. Deductions based on the available evidence, it would appear most likely that the break in foam production was the result of a lack of foam concentrate being injected into the line when the system was starting up. The pre-mix residue in the hoses from the previous trial the day before was sufficient to generate foam for the first 33 seconds after which there was a break of 35 seconds. It is possible that the regulator had been accidentally reset just before the trial, which sets the proportioning rate back to zero, it then has to be increased in 0.1% increments to reach the desired 3.0% rate. This would have taken approximately 30 seconds which could feasibly account for the duration of interruption in foam production.

6.8.5 Conclusions

In a 6 m wide pen, with foam delivered at a rate of 42.5 m^3 /min, the average time taken to reach a depth of 60cm was 11 seconds and 30 seconds to reach 1 metre. This quantity and depth of foam was sufficient to keep the birds covered during bouts of wing flapping. The overall foam production rate is equivalent to 7.1 m^3 per min per metre width of pen. The initial stocking density of the birds did not make a great difference to the foam destruction, although there was a tendency for the higher stocked birds to destroy more foam. It is noteworthy that movement of the birds away from the approaching foam meant that the actual stocking density at which the birds were submerged in the foam was greater than their initial intended stocking density and in some parts of the pen became maximal. This movement of birds may mask the true effect of the initial stocking density and could be reduced if the operation was carried out under different lighting conditions (see below). The width of the pen of birds had a greater effect on the amount of foam destroyed than the distance it had to travel over the birds down the pen. This suggests that it is the width of a poultry shed that will determine the volumetric flow rate of foam rather than the length of the shed.

The interruption to the foam supply during trial 4 was most likely caused by operator error and a lack of communication as it took 3 people to start the system. In future operators must be able to maintain contact using a 2-way radio and follow a check list read by the lead operator before starting. It is also recommended that the temperature of the gas and foam solution should be monitored.

6.9 **Results of physiological measurements**

Logging of physiological data was achieved throughout the euthanasia process for 12 birds. Unfortunately data from others was lost due to a logger unit power failure caused by vigorous movement or, in one case, memory card failure. In one individual (Bird 10), only the ECG trace was useable as the EEG trace was contaminated with electrical interference of unknown origin. However, the success rate of logging was

reasonable given the challenging circumstances and extensive data was collected from a minimum of two birds per trial.

6.9.1 ECG responses

Figure 23 shows individual and mean heart rate responses to exposure to nitrogen filled foam in birds exposed at stocking densities of 40kg/m^2 or 50kg/m^2.

40kg/m^2
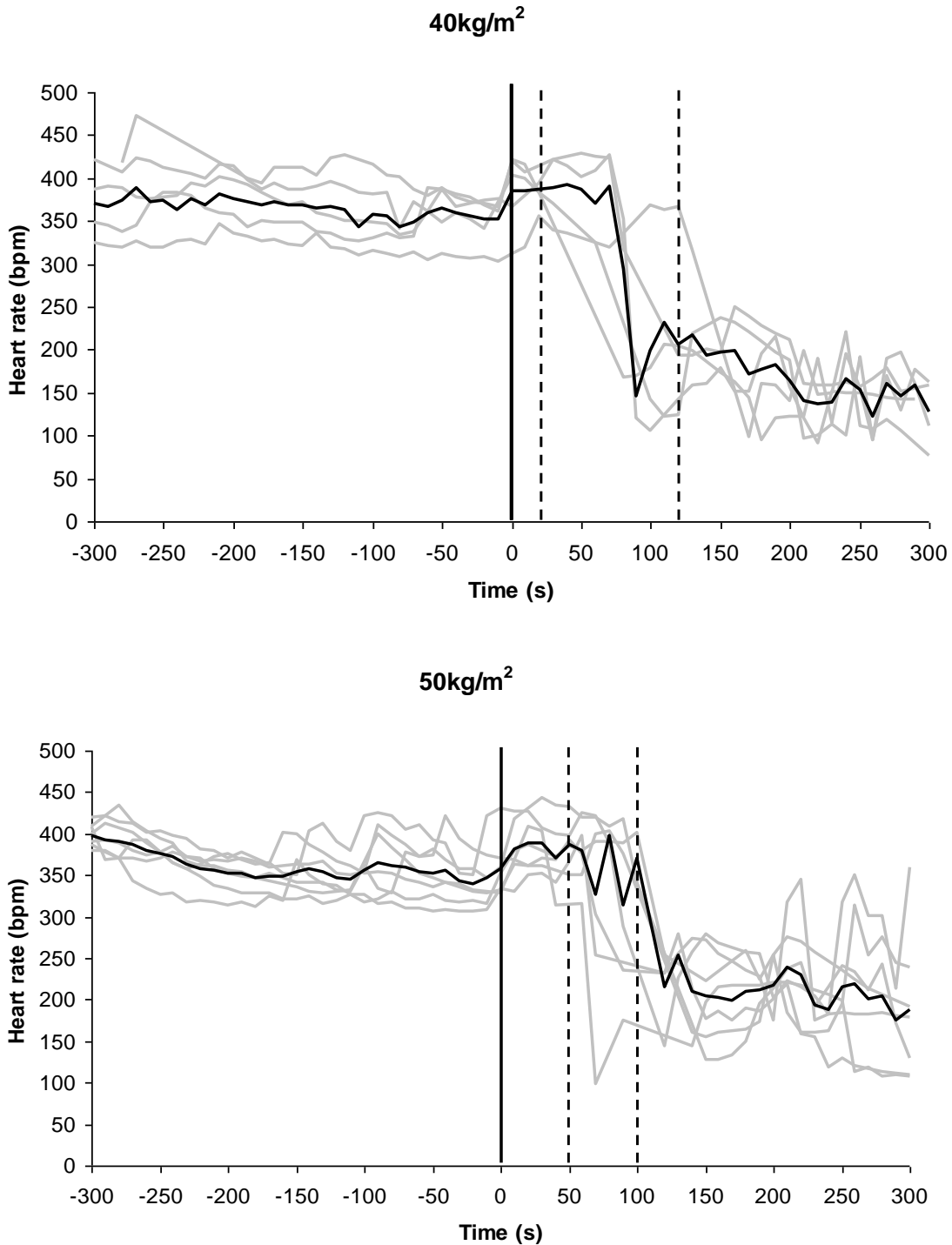

50kg/m^2

Figure 23   Graphs showing changes in individual and mean heart rate in broilers exposed to anoxic foam (grey lines represent individuals, black line represents the mean) at 40 or 50kg/m^2. Missing values have been interpolated. Vertical line markers indicate timings of foam generation onset (solid line) and earliest and latest submersion/bradycardia (dashed lines).

The initial response to foam generation in the birds was a moderate increase in heart rate, most likely associated with a fear response to the loud noise generated by foam production. Heart rate increase was also likely to be caused by increased exercise as the birds moved from resting positions to avoid the foam. Previously, broilers exposed to anoxic foam demonstrated a consistent response to submersion in foam in the form of an almost immediate pronounced slowing of heart rate (bradycardia). This occurred within 10s of submersion in foam and thus serves as a reliable indicator of submersion. Exactly the same response was observed here. Since the design of the trials meant that we could not track exactly when each monitored bird became submerged, all other responses (EEG changes, flapping onset) were analysed relative to bradycardia onset, at which time we could be certain of submersion. The variation in timing between birds in onset of bradycardia (Figure 23) is likely to be related to the differing timing of submersion under foam wave (which took between 50 and 100 s to cover all of the birds, depending on the trial) as previous data show low variation between individuals in time to onset of bradycardia after submersion. The pattern of pronounced bradycardia followed by varying degrees of recovery and/or stabilisation before a final decline, seen in all birds, was exactly the same as that seen in individually exposed broilers in earlier studies.

6.9.2 EEG responses

Many of the EEG traces were difficult to interpret due to obscuration by substantial movement artefacts before, during and even after the euthanasia process (due to interference from the movements of neighbouring birds). Nevertheless, as in previous work in which birds were exposed to anoxic foam, a series of consistent changes in the appearance of the EEG were apparent and were assigned to one of 4 phases with particular characteristics; '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. Because sometimes significant portions of the EEG trace were obscured by artefact, caution in visual interpretation was exercised by omitting the timings of phase transitions when these were not clearly visible. As found previously, where transitional EEG could be discerned, it was characterised by slow wave (high amplitude, low frequency) activity. Table 9 shows the timings of phase changes in broilers exposed to foam at stocking density of 40 or 50kg/m^2.

Table 9 Individual and mean timings of EEG phase changes in broilers in each trial. Data loss due to movement artefact is indicated by a dash.

Trial	Broiler	Transitional	Suppressed	Isoelectric
Trial 1	1	5.4	30.9	40.4
	2	8.0	-	-
Trial 2	3	-	17.0	39.5
	4	4.1	23.0	36.0
	7	-	25.4	43.3
Trial 3	9	8.4	20.0	-
	11	-	14.4	31.8
	12	0.3	15.6	46.0
Trial 4	14	4.2	28.7	48.7
	16	3.9	-	62.5
	17	-	-	69.5
40kg / m^2 Mean ± SD		**5.8 ± 2.0**	**24.1 ± 5.8**	**39.8 ± 3.0**
50kg / m^2 Mean ± SD		**4.2 ± 3.3**	**19.7 ± 6.5**	**51.7 ± 14.8**

Mean time to transitional EEG onset ranged from 0.3 to 8.4s (mean 5.8 ± 2.0 and 4.2 ± 3.3s at $40kg/m^2$ and $50kg/m^2$ respectively), while suppressed EEG onset ranged from 14.4 to 30.9s (mean 24.1 ± 3.0 and 19.7 ± 6.5s at $40kg/m^2$ and $50kg/m^2$ respectively). Onset of isoelectric EEG ranged from 31.8 to 69.5s (mean 39.8 ± 3.0 and 51.7 ± 14.8s at $40kg/m^2$ and $50kg/m^2$ respectively). There was evidence that the latencies of these phases were been affected in the final $50kg/m^2$ trial (Trial 4) in which there was a failure of foam generation (see 6.8.4 above). This was evident in the longer times to isoelectric EEG in birds 16 and 17, although there were no statistically significant differences in timings of phase changes in relation to stocking density (but note sparse data made numbers of data points for these comparisons very small). Comparison of pooled data with equivalent timings in broilers exposed to anoxic foam individually in previous work (mean onset transitional phase 8.3s, suppressed phase 17.6s and isoelectric phase 46.9) also showed no significant differences. As outlined in previous studies, onset of suppressed EEG is a reliable indicator of loss of consciousness. On this basis, the maximum measured time that consciousness was a possibility during euthanasia with nitrogen filled foam was 31s at $40kg/m^2$ and 29s at $50kg/m^2$.

6.9.3 Behavioural responses

In this trial the behaviour of the monitored birds could not be observed directly, so movement artefacts and EMG activity on the ECG trace were used as an indirect indicator of wing flapping and convulsion. Table 10 shows the time to flapping onset, number of flapping bouts and total duration of flapping obtained from analysis of the traces. Mean time to flapping onset was 3.8s (range 0.8-7.0s) for $40kg/m^2$ and 5.1s (range 0.8-15.8) for $50kg/m^2$. Mean number of bouts was 3.2 and 3.3 for $40kg/m^2$ and $50kg/m^2$ respectively. Mean total duration of flapping 14.6s (range 9.5-22.9s) for $40kg/m^2$ and 13.9s (range 7.6-19.6) for $50kg/m^2$. Statistical comparisons between these means did not significantly differ in relation to stocking density, nor did the number of flapping bouts and total flapping duration significantly differ from behavioural parameters recorded in previous work in individually exposed broilers (mean number of flapping bouts 3.9 and total flapping duration 13.7s). Mean time to flapping onset in the current trial was significantly shorter than that recorded previously in individual broilers (mean onset 15.3s) which is related to the fact that the timings used here are expressed in relation to onset of bradycardia which occurs slightly later than visual confirmation of submersion which was used previously.

Table 10   Individual and mean time to onset of flapping bouts relative to onset of bradycardia, number of bouts and total flapping duration in each trial and by stocking density.

Trial	Broiler	Flap onset (s)	Number of bouts	Total duration (s)
Trial 1	1	0.8	4	18.7
	2	2.4	4	22.9
Mean ± SD	**1.6 ± 1.1**	**4.0 ± 0.0**	**20.8 ± 3.0**	
Trial 2	3	4.5	2	13.4
	4	7.0	3	9.5
	7	4.1	3	9.5
Mean ± SD	**5.2 ± 1.6**	**2.7 ± 0.6**	**10.8 ± 2.3**	
Trial 3	9	2.1	4	11.0
	10	3.6	4	19.6
	11	2.4	2	12.5
	12	6.2	2	16.5
Mean ± SD	**3.6 ± 1.9**	**3.0 ± 1.2**	**14.9 ± 3.9**	
Trial 4	14	4.6	1	19.5
	16	15.8	4	10.6
	17	0.8	6	7.6
Mean ± SD		**3.6 ± 7.8**	**3.7 ± 2.5**	**12.6 ± 6.2**
40kg / m² Mean ± SD		**3.8 ± 2.3**	**3.2 ± 0.8**	**14.8 ± 5.9**
50kg / m² Mean ± SD		**5.1 ± 5.0**	**3.3 ± 1.7**	**13.9 ± 4.7**

In this trial there was an interruption of foam production after 33 seconds which resumed after a break of 35 seconds (see Objective 1 for explanation). The foam had travelled 5.5 m along the length of the pen (longitudinal configuration) before it stopped at which point a 3.5 m section of birds were submerged in the foam. In the remaining 2.5 metres of the pen length the birds were not affected but remained alert. The birds that had been submerged were initially under between 40 cm of foam at the leading edge and 80 cm, one metre further back. When the birds under the foam began to flap they destroyed the covering foam quickly, and although some foam flowed in from the deeper section, a section (approximately 2.5 m²) of birds was re-exposed to the atmosphere. It was not possible to count the number of birds but based on the stocking density and the fact that they had bunched up when they moved away from the approaching foam, it is estimated that between 40 and 50 birds were re-exposed. Within this group four were seen to be on their feet and a further 8 were having bouts of vigorous wing flapping. The remaining birds were either motionless or exhibiting body tremors (muscle contractions observed in the final stages of the euthanasia process). As noted above in the EEG results section, birds 16 and 17 had longer than usual times to isoelectricity, but no later onset of transitional EEG, so the foam interruption resulted in a longer overall time to death (62.5 and 69.5s compared to the mean of 51.7s). During the transitional phase consciousness is a possibility, but the extent of movement artefact on the traces for these two birds did not allow identification of the onset of the suppressed phase (a reliable indicator of unconsciousness). Bird 16 also exhibited an unusually long delay between bradycardia and the onset of flapping (15.8s compared to the mean of 5.1s) which suggests it was submerged (causing bradycardia) then re-exposed to air (delaying the onset of convulsive wing flapping). Based on video evidence, the birds were re-exposed for a maximum of 20 seconds, and when foam generation recommenced this area of the pen was covered in seven seconds.

6.9.4 Discussion

Successful capture of physiological data during the challenging but commercially relevant circumstances created in these trials allowed comparison with previously generated laboratory data on the killing of individual birds with anoxic gas foam. Heart rate data support general behavioural observations of fright in response to initial foam generation and the advancing foam front (though these are confounded by heart rate increases caused by exercise as the birds moved away from the advancing foam). Because accurate timings of the submersion in foam of individual birds were not available, onset of bradycardia (a reliable physiological response to submersion) was used as the reference point for the timing of physiological and behavioural events. Bradycardia occurs after but within 10s of submersion and for this reason, time to onset of wing flapping was significantly shorter than reported previously, although the patterns of behavioural response in terms of number of bouts and total duration of wing flapping were not significantly different. Despite the foam production problems encountered in trial 4, the timings of EEG phase changes did not differ significantly in relation to stocking density, nor did they differ from those recorded in individual birds in the laboratory. This suggests that submersion in anoxic foam results in a reliable and consistent euthanasia that is robust in relation to scaling up of the system and is unchanged even at maximal stocking density.

While the foam production problems encountered in trial 4 were unfortunate, they allow an examination of the welfare consequences of re-exposure to atmospheric air which is a relevant risk with the foam killing process. Information from both the video records and the physiological data suggest that birds that were re-exposed were either (a) dead – motionless birds which had already convulsed; (b) convulsing – these birds had been submerged for more than 15 seconds and were undergoing death by anoxia; or (c) conscious – these birds must have been submerged for less than 15 seconds – the time taken for onset of wing flapping. Based on EEG records associated with anoxic death, it is very unlikely that any of the birds observed wing flapping regained consciousness, as the cessation of wing flapping is usually closely linked in time with the onset of suppressed EEG which is incompatible with consciousness. Clearly, those birds that had been submerged and re-exposed while conscious are likely to have been distressed by the experience, and when the system is properly employed this should never happen. A notable advantage of the foam system is that birds near the leading edge (which the foam has not yet reached) are unaffected, limiting the welfare impact of this type of technical failure.

6.9.5 Conclusions

Physiological data recorded from birds exposed to anoxic foam in commercially relevant trials show no significant differences to the results of laboratory studies on single birds. Patterns of behavioural change and onset of changes in EEG characteristics closely matched those observed in laboratory trials. Foam as deployed in these larger trials delivered a reliable and humane anoxic kill which was robust even at maximal stocking densities (created as the birds moved to avoid the advancing foam). An unintended interruption in foam supply gave an indication of the welfare impact of a technical problem in the field situation, in the form of re-exposure of previously submerged birds. The welfare consequences of this depend on the duration of submersion before re-exposure, and will be an issue only for birds which have not yet started to wing flap (submerged for less than 15 seconds). This is unacceptable and should not occur during correct deployment of the system. Nevertheless, the nature of the foam system means that only a relatively small proportion of birds (those submerged under the leading edge of the foam with a height less than 80cm) are likely to be at risk of compromised welfare due to technical failure.

7 General discussion and conclusions.

The trials with individual birds show that submersion in anoxic (N_2-filled) or hypercapnic (CO_2-filled) high expansion foam provides an effective and rapid method for euthanasia. Initial aversion to the foam is not extreme, there were only minor no escape attempts from the foam. Using CO_2 in the foam will lead to gasping and headshaking before birds are submerged and thus while conscious. Since there is no agreement on the severity of the effect of gasping and headshaking on animal welfare we should prefer to prevent periods of gasping and headshaking. Although, in contrast to the N_2-filled foam birds are affected by CO_2 prior to submersion, which is an advantage of using CO_2 especially when the foam is destroyed by convulsive spasms of the birds.
Although it is evident that there are some differences due to the type of gas used and between poultry species, it can be concluded that these effects will have no or only a minor relevance for practical application.
Comparing the results of the individual bird trials using N_2 foam performed in the Netherlands, to trials executed by Dr. Dorothy McKeegan in the UK it became obvious that there are no marked differences in induction of unconsciousness in broilers between these two independent experiments. Therefore, it can be concluded that the experiments we executed under comparable conditions and are repeatable and thus it is valid to extrapolate the results to different situations and poultry species.

Upgrading the system to a larger capacity for practical application showed CO_2-filled foam will lead to major problems. Vaporisation of CO_2 requires a high level of energy requiring a large heating capacity to prevent the foam from freezing up. Since the major benefit from using CO_2 is the anaesthetic effect, which remains effective for some time after foam collapse, it is decided to focus on N_2-filled foam together with increasing the foam production capacity.
In trials without birds, the equipment was able to create a foam bow wave of sufficient depth that would be expected to completely cover birds during their bouts of wing flapping. It is recommended that the quickest way to achieve a consistent bow wave of sufficient depth is to generate foam across the whole width of the pen, rather than in one place, so that it reaches the side walls as quickly as possible. The initial 50 cm of the foam front remained consistently above 40 cm and if moving greater than 0.1 m/s will completely cover birds in about 3 seconds. The height of foam will then build up over the birds to be 50-60 cm when the birds start flapping about 15 seconds after submersion.

For trials on a (semi) commercial scale it was decided that for the live birds a pen width of 6 m should be adopted, maintaining the length at 8 m, with the 4 foam generators spaced equally across the width of the pen. The pen size was chosen based on small scale field situations that can be found under free range farming conditions.
Successful capture of physiological data during the challenging but commercially relevant circumstances created in these trials allowed comparison with previously generated laboratory data on the killing of individual birds with anoxic gas foam. Heart rate data support general behavioural observations of fright in response to initial foam generation and the advancing foam front (though these are confounded by heart rate increases caused by exercise as the birds moved away from the advancing foam). Because accurate timings of the submersion in foam of individual birds were not available, onset of bradycardia (a reliable physiological response to submersion) was used as the reference point for the timing of physiological and behavioural events. Bradycardia occurs after but within 10s of submersion and for this reason, time to onset of wing flapping was significantly shorter than in individual bird tests and as reported by Dr McKeegan et al (Defra report MHO 143). However, the patterns of behavioural response in terms of number of bouts and total duration of wing flapping did not differ significantly. Despite the foam production problems encountered in trial 4, the timings of EEG phase changes did not differ significantly in relation to stocking density, nor did they differ from those recorded in individual birds in the laboratory. This suggests that submersion in anoxic foam results in a reliable and consistent euthanasia that is robust in relation to scaling up of the system and is unchanged even at maximal stocking density.
Physiological data recorded from birds exposed to anoxic foam in commercially relevant trials show no significant differences to the results of laboratory studies on single birds. Patterns of behavioural change and onset of changes in EEG characteristics closely matched those observed in laboratory trials. Foam as deployed in these larger trials delivered a reliable and humane anoxic kill which was robust even at maximal stocking densities (created as the birds moved to avoid the advancing foam).   An unintended interruption in foam supply gave an indication of the welfare impact of a technical problem in the field situation, in the form

of re-exposure of previously submerged birds. The welfare consequences of this depend on the duration of submersion before re-exposure, and will be an issue only for birds which have not yet started to wing flap (submerged for less than 15 seconds). This is unacceptable and should not occur during correct deployment of the system. Nevertheless, the nature of the foaming system means that only a relatively small proportion of birds (those submerged under the leading edge of the foam with a height less than 80cm) are likely to be at risk of compromised welfare due to technical failure.

Results from the experiments in UK, funded by Defra, and experiments performed in the Netherlands indicate that anoxic gas delivered into sheds by high expansion foam is an acceptable method of killing large groups of poultry on-farm. However before such a method can be applied in commercial practice the equipment should be designed and manufactured in a robust manner to facilitate versatility of use under different circumstances. Important criteria are reliability, safety of use, effectiveness and mobility.
Practical design recommendations including aspects for an operational protocol are given in chapter 8.

8 practical recommendations for design including an operational protocol.

8.1 *Design standard*

It is evident that any foaming system that is to be used to kill birds in the field must meet a minimum design specification prior to selection or deployment. The following criteria are based on conclusions from these and previous experiments.

1. The expansion ratio, the ratio of volume of foam to the amount of liquid contained in the foam must be between 250 and 350 :1
2. Foam must be produced at a minimum rate of 7.1 m^3 per minute, per metre of pen width for birds that are initially stocked up to a maximum density of 50 kg/m^2.
3. Volume and flow rate of gas and foam solution must be measured
4. Volume of water and foam concentrate must be measured
5. Gas and water temperature must be measured
6. Residual oxygen level of foam must be measured
7. The system must have the capacity to generate enough foam to fill the target shed to a volume equal to 4 times its floor area without refilling

These design recommendations should be used as the minimum standards in assessing the suitability of foam generation equipment prior to acquisition or deployment. For example, if the target for deployment were free range broiler sheds measuring 8.3 m x 15 m a foam flow rate of 58 m^3 would be required. This would require about 250 m^3 of foam to complete the operation and the system should have a capacity of at least 500 m^3 without refilling.

All equipment should be tested prior to use to ensure that it meets requirements and annual tests should be performed to ensure that it is in working order. These could be incorporated into training exercises.

8.2 *Application protocol*

The foam should be delivered to the pen across the entire width to ensure that a straight foam front is developed as quickly as possible, rather than spreading out in a semi-circle, as this will encourage the front to develop in height. If the foam is generated from multiple units then these should be evenly spaced across the pen. If the foam is generated from a single source then the output may have to be channelled though a number of parallel ducts into the shed.

One target deployment of the system is in free range poultry houses where stocking density is low (e.g. 25 kg/m^2 for broilers). The conclusion drawn from this work is that stocking density is not a major influence on the efficacy of the technique so it is not necessary to confine birds to increase stocking density prior to foam application. However it may be of benefit to move floor-reared broilers away from the immediate vicinity of the generators to reduce the initial flight reaction of the birds. This can be done by using hurdles made of parallel horizontal bars placed about 2 m from where the foam enters the pen. These will not impede the flow of the foam and will also allow it to spread out across the width of the pen and build up before encountering the birds. It is also recommended to reduce light levels to a minimum. The wall behind the generators should be at least 1.8 m high to prevent the foam flowing backwards away from the birds therefore barriers may have to be erected across doors or ventilation openings.

8.3 *Operating routine*

The correct set up of the equipment should be verified before use against a check list supplied by the equipment manufacturer. The performance test described in Objective 3 should be carried out after initial set up and then again if the equipment has stood idle for a number of hours.

There may be a number of operators involved in running the equipment due to its size and the number of components: they should be in constant contact via a 2-way radio. An additional lead operator should be

able to see the inside of the shed, either directly or where safety may be a problem via a CCTV link to direct operations and indicate when foam should be turned on and off.
Monitoring points.
During foam application the following should be monitored:

> Gas flow and temperature
> Pre-mix flow and temperature
> Residual oxygen content
> Progression of the foam inside the shed.

8.4 *Foam depletion*

Foam should be left for at least 1 hour to allow liquid to drain from it before attempting to break it down. If the foam has been left to dry for this time it is easily destroyed using a fine water spray from a hosepipe. It may be beneficial to incorporate a disinfectant into the spray, whilst this will have little effect on virus attached to organic matter it may reduce virus that is present as an aerosol in the atmosphere within the shed. This method will leave the minimum amount (about 10 cm) of dense foam on the floor from which birds can be collected. If water is sprayed onto the foam too soon a thick layer of dense foam is created (30-50 cm) completely covering the birds and making it more difficult to remove them.

8.5 *Bird Removal*

Floor reared birds could be removed using a small mechanical handler with a tined bucket, such as a bobcat or equivalent piece of equipment. If mechanical clearing is not an option then the birds will have to be picked up in the same way as would be the case for whole house gassing. If the foam depletion protocol has been followed then it is relatively easy to locate and pick up the carcases.

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