

# **ACCEPTABLE METHODS FOR LARGE SCALE ON-FARM KILLING OF POULTRY FOR DISEASE CONTROL**

**Marien Gerritzen**



# ACCEPTABLE METHODS FOR LARGE SCALE ON-FARM KILLING OF POULTRY FOR DISEASE CONTROL

**Methoden voor het grootschalig doden van pluimvee voor dierziekte bestrijding: Aanvaardbaar wat betreft ongerief en efficiëntie.**

(met een samenvatting in het Nederlands)

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## Abstract

Worldwide large numbers of birds are killed to stop the spreading of contagious diseases like Highly Pathogenic Avian Influenza (HPAI). From animal welfare and epidemiological point of view animals are preferably killed on the premises and if possible in their housings. Furthermore, applied methods should be effective, have large capacity and animals should be unable to escape from it.

In this thesis, animal welfare aspects, i.e. behavioural and physiological, and efficacy aspects of gas killing were assessed for individual birds and groups of poultry. Observations during the 2003 HPAI outbreak in The Netherlands made it clear that whole house gassing with carbon dioxide is a method with high capacity and is relatively safe. Also, birds don't have to be handled and transported to a killing device which is a major advantage for their welfare.

Questions that raised about the suitability of carbon dioxide to kill ducks and turkeys were answered in experiments where ducks and turkeys were exposed to increasing carbon dioxide concentrations. In these experiments blood gas values, EEG's and ECG's confirmed that both species are equally sensitive to these increasing carbon dioxide concentrations. Furthermore, the results found in ducks and turkeys are comparable with results found in chickens and confirmed that all farmed poultry species can be killed by increasing the carbon dioxide concentration up to 40-45%. However, it is unmistakable that killing animals always interferes with their welfare. Before loss of consciousness all animals express heavy breathing (gaspings) and headshaking during a period of 2 to 3 minutes. Convulsions, if they occur, are seen after loss of consciousness. Therefore, it can be concluded that the period of compromised animal welfare is not longer than 3 minutes. This period of compromised animal welfare however, is shorter than when animals are caught and handled when using mobile gas containers or mobile electrocution lines.

Large scale killing of animals for disease control purpose meets a lot of societal resistance, especially when alternatives like vaccination are available.

Furthermore, the visibility of the depopulation of poultry farms makes people aware that large numbers of birds are killed in our society every year. The moral attitude of people towards killing animals is strongly coloured by the reason why we kill animals; killing for food is more accepted than for disease control. However, we should realize that animal welfare is presumably more compromised in the normal slaughter process than when killed in their housings for disease control. Also, it is not realized that the non vaccination policy is an economical policy to protect our export position, something that benefits producers and consumers.

In respect of animal welfare and of the moral attitude of man the non- vaccination policy should be reconsidered. However, we must realize that there will always remain situations that farms must be depopulated and therefore we should provide maximum effort in further developing acceptable killing methods.



## Chapter 1

### **General introduction**

CHAPTER 1

## Background

Worldwide large numbers of animals are kept under commercial conditions for the production of food, i.e. meat, milk and eggs. In most cases, especially in Western Europe, the production of animal products under commercial conditions means large farms with large numbers of animals close together. High densities of animals that are susceptible for infectious diseases like foot and mouth disease, classical swine fever or avian influenza enlarge the change of a major disease outbreak. To control outbreaks of highly contagious animal diseases and to prevent viruses from spreading, infected farms as well as contact- and surrounding farms are depopulated by on farm killing of all susceptible animals. This implies the killing of large numbers of sick and healthy animals. Whereas the slaughter of animals for food production is in general socially accepted (Rutgers et al., 2003), the pre-emptive culling and destruction of ostensible healthy animals during disease control meets with far less social approval. The killing of healthy animals and destroying potentially high quality consumptive products meets a lot of resistance from animal owners and from rural communities; especially when alternative strategies as vaccination may be available (Swabe et al., 2005). The non-vaccination policy which is based on economical interests with as a result the killing of large numbers of healthy animals is widely criticized. The opinion that animals have the right to for fill their lives, the cruelty of unnecessary killing, and concern for animal welfare are the base for the societal attitude. It is commonly accepted that animals can suffer. The farm Animal Welfare Council stated that any animal kept by man, must at least, be protected from unnecessary suffering (FAWC 1968). In perspective of animal welfare this means that when we kill animals we should keep them free from pain, injury, fear and distress. Killing animals always involves animal welfare and negative effects or suffering should be minimized. In emergency killing however, animal welfare aspects are not number one priority of politics and producers. Because highly contagious diseases may harm large regions and affect export of animals and animal products the eradication of these diseases is impaired in international directives and regulations (EU 1993; AVMA 2000; OIE 2003). Stunning and killing methods for slaughter are well defined in legislation whereas in case of disease outbreaks killing is described in guidelines that are less strict. The OIE guidelines describe general principles concerning qualification of people involved, operational procedures, welfare aspects and a summary of available killing methods. For the European Union the regulation for the control of High Pathogenic Avian Influenza (HPAI) viruses is imposed in EU council directive 2005/94/EC. In this directive the killing and removal of infected and suspected flocks of poultry is the most important measure to stop spreading the disease. In case of HPAI depopulation often implies the killing of millions of birds. Difficulty with emergency killing is its large scale and that it must be performed on farm with a high capacity and requires high efficacy. At the start of this project the major question was if we are prepared to deal with an outbreak of an infectious disease in poultry in other words are we in disposal of acceptable

killing methods. In this context acceptable killing methods are considered to have high efficacy, are acceptable from animal welfare point of view and are save to use for the people involved. After all, at that moment control measures were only focused on controlling disease outbreaks by massive killing of all susceptible animals on infected and contact farms. This also included eradication of healthy, susceptible animals on surrounding farms to create a buffer zone to stop the spread of the disease. Major, or the only focus was to kill all animals on their farms or if possible in their housings.

### **Frequently used methods.**

Before starting this project (2002) for massive on farm killing only few methods were available or beyond experimental stage and these were all subject of discussion. The use of cyanide (HCN), first choice in practice till that moment, was questioned based on animal welfare and human safety grounds. Furthermore, HCN was no longer available at large scale and was in a number of countries already forbidden to use. Poisoning animals by addition of a killing drug to the feed or drinking water was questioned and advised not to be used because of the uncertain intake of food or water especially when it concern sick animals. Important criterion at that moment was, and still is, that it should be impossible for animals to escape from the killing procedure, something that could not be guaranteed when using poisoning drugs in the feed or water. Furthermore, poisoning was not tested on poultry on a large scale.

Most important methods used for on farm killing during recent HPAI outbreaks are mobile electrocution lines, mobile gas containers and whole house gassing. Mobile gas containers of different sizes, varying from larger disposal bans to shipping containers, can be filled with gasses or gas mixtures like CO<sub>2</sub>, N<sub>2</sub> or Ar. At the moment that the desired gas level is reached the containers are filled with animals. Implication when using pre-filled gas systems is that in most cases they have to be used outdoors because of human safety. Therefore, living animals must be caught and brought to the system. This means large pressure on labour, intensive contact between humans and animals and risks for animal welfare.

Electrocution lines operate comparable to electrical water bath stunners in slaughterhouses. Most important difference is the higher current on the water bath to make sure that all animals are killed due to the electrocution process. However, till now animals have to be caught and be shackled alive when using electrocution devices for on farm killing. This means again, intensive contact between humans and living animals. Furthermore, the capacity of gas containers and electrocution lines is limited due to their size and most of all due to labour capacity.

Whole house gassing enlarges the killing capacity enormously and also reduces the contact between living animals and humans. An important criterion in this is that the barn must be suitable for gassing. This means, leakage must be limited within certain margins i.e., can the barn be closed or can openings be covered so

that the desired gas concentration can be accomplished in the whole building. Distribution of the gas in the building is of major concern from efficacy point of view. Besides capacity and efficacy animal welfare and human safety are important criteria in the selection of methods. Furthermore, the availability and the possibility to use killing methods is an important criterion. The methods described above are used in Europe, USA and Canada. In other large poultry producing countries in Asia, Turkey and in African countries these methods are often not applicable. In these countries housing conditions, size and locations of the farms, and financial possibilities are not allowing these control measures. In these countries animals are killed by gassing in plastic backs, suffocation in plastic bags, buried alive, or killed by cervical dislocation. Besides the practical reasons cultural differences, like a different view on animal welfare are also important reasons for differences in approach.

### **Animal welfare implications.**

Judgement of animal welfare aspects of killing methods should not only be based on the killing procedure only but on the total procedure of the killing process including catching and handling of live animals. When using mobile equipment like gas containers or electrocution lines animals must be caught and walked to the killing device. Catching and handling of live animals is potentially stressful and animals can easily be damaged (Nijdam et al., 2004; Knowles & Broom 1990). Broken bones and painful bruises are common especially, when animals are caught under stressful conditions and under time pressure like during a disease outbreak. When using electrical water bath stunners poultry must be shackled alive which can be painful (Gentle & Tilson, 1999). Furthermore, in case of automatic de-shackling controlling if all animals died due to the electrocution needs special attention to prevent that living animals are transported to the destruction sites. Mobile gas containers are most times filled with 60% CO<sub>2</sub> at least. High levels of CO<sub>2</sub> are described as aversive, painful to inhale and causing breathlessness (Hari et al., 1997; Leach et al, 2002). Furthermore, gasses or gas mixtures do not introduce immediate unconsciousness or dead. Attention for the speed of introducing animals in gas containers is of major importance to prevent animals from suffocation due to layers of animals on top of each other. Advantage however, of mobile electrocution lines and gas containers is the controllability of these systems. Animals are handled batch wise and procedures can easily be stopped in case of malfunction. Control measures for well functioning can be built in into the equipment and the procedures.

Whole house killing is less controllable compared to mobile devices. During whole house gassing animals can not be observed easily. Also, animals are not rendered unconscious or dead instantly. During the induction period aversive reactions, breathlessness and fear is not excluded. The time it takes to fill a whole poultry house depends on a lot of factors that are not always controllable. Inside and

outside temperature, leakage of the building are only few factors that are highly influencing the procedure and thus in potential have influence on animal welfare during the process. On the other hand whole house gassing prevents catching and handling of live animals what is of great advantage in respect of animal welfare. Major concerns in whole house killing are the controllability, efficacy and related animal welfare which all depend highly on correct proceeding of the method.

### **Aim and outline of this thesis**

Most important question at the start of the project was can we kill poultry on-farm in an acceptable way and preferable in their housings. Efficacy, animal welfare and epidemiological aspects were major focus at that moment. Assessment of the possibilities for whole house gassing with different gas mixtures and the use of mobile killing devices had to be examined on experimental scale. In case of whole house gassing the most important question was if it was possible to kill poultry of different ages with slowly increasing gas concentrations in an acceptable way. Till then, the killing or stunning of birds with gas mixtures was always performed in pre-filled systems with high concentrations of gas mixtures already present. In Chapter 2 aversion of broilers to different gas mixtures is described. Avoidance tests with different gas mixtures are performed and behavioural observations are described for broilers that entered a tunnel pre-filled with these gas mixtures. In these tests companion birds are used as stimulus for the broilers to enter the gas filled tunnel. The purpose of Chapter 3 was to assess the suitability of these gas mixtures for whole house gassing and thus the suitability of slowly increasing gas concentrations as killing agents. Furthermore, the suitability to kill young birds was assessed. Electro physiological and behavioural observations are accomplished for broilers at 3 and 6 weeks of age to assess efficacy as a killing agent and animal welfare aspects during exposure to slowly increasing gas concentrations. In Chapter 4 observations of the depopulation of poultry farms during the large outbreak of HPAI in The Netherlands in 2003 are described. The dimensions of this outbreak made it necessary to use killing methods that were still in an experimental phase for this purpose, not used before or even not yet examined on experimental scale. The conclusions of these observations and the results of the used depopulation methods were an important guideline for the further direction of this project. From here on, focus of the experiments was on efficacy and animal welfare during slowly increasing CO<sub>2</sub> concentrations comparable to the situation during whole house gassing. Physiological parameters and behavioural parameters are assessed to set criteria for whole house gassing and to examine the suitability of this method for different poultry species. In Chapter 5 we measured changes in blood gas values, electro-encephalograms and electro-cardiograms in ducks and turkeys during exposure to slowly increasing CO<sub>2</sub> concentrations. Motivation to concentrate on these two species was the discrepancy in efficacy during whole house gassing of duck and turkey houses in practise. In Chapter 6 the aim was to

measure how most important farmed poultry species, broiler chickens, laying hens, ducks and turkeys, react to increasing CO<sub>2</sub> concentrations and to set the criteria for gas concentrations for these different species. In Chapter 7 technical, animal welfare and ethical implications of large scale killing of birds for disease control purpose are discussed.

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## Chapter 2

# **Behavioral Responses of Broilers to Different Gaseous Atmospheres**

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## **Abstract**

This study was conducted to determine the differences in behavioral response of broilers when they come into contact for the first time with different gas mixtures that can be used for stunning.

The six test groups were divided into four experimental groups that were exposed to gas mixtures used for stunning and two control groups that were exposed to atmospheric air. The different gas mixtures and their concentrations were: a) air, no flow (control-); b) circulating air, flowing (control+); c) >90% Ar in air; d) 60% CO<sub>2</sub> in air; e) 40% CO<sub>2</sub> and 30% O<sub>2</sub> in air and f) 70% Ar and 30% CO<sub>2</sub>. The behavior of the broilers before entering the gas tunnel, the number of birds that moved into the gas mixture and the behavior in the gas mixture were recorded on video and analyzed afterwards.

No difference between the groups was observed in the number of broilers that walked into the gas tunnel or in the number of birds that tried to return to the cage. Exposure of broilers to the 70% Ar and 30% CO<sub>2</sub> mixture resulted in the fastest loss of posture. The number of broilers exhibiting headshaking and gasping was least in the >90% Ar in air mixture. Convulsions were rarely seen in the 40% CO<sub>2</sub> and 30% O<sub>2</sub> mixture, whereas the other gas mixtures resulted in severe convulsions.

The experiment did not indicate that broilers could detect or avoid increased CO<sub>2</sub> or decreased O<sub>2</sub> levels when they come into contact with such atmospheres for the first time.

**Key words:** *behavior, broilers, Ar, CO<sub>2</sub> and gas stunning.*





## Chapter 3

# **On Farm Euthanasia of Broiler Chickens: Effects of Different Gas Mixtures On Behavior and Brain Activity.**

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## Abstract

The Purpose of this study was to investigate the suitability of gas mixtures for euthanasia of groups of broilers in their housings by increasing the percentage of CO<sub>2</sub>%. The suitability was assessed by the level of discomfort before loss of consciousness, and the killing rate. The gas mixtures injected in the housing were, a) 100% CO<sub>2</sub>, b) 50% N<sub>2</sub>+50% CO<sub>2</sub> and c) 30% O<sub>2</sub>+40% CO<sub>2</sub>+30% N<sub>2</sub> followed by 100% CO<sub>2</sub>. At 2 and 6 weeks of age, groups of 20 broiler chickens per trial were exposed to increasing CO<sub>2</sub> concentrations due to injection of these gas mixtures. Behavior and killing rate were examined. At the same time 2 broilers per trial equipped with brain electrodes were observed for behavior and brain activity. Ten percent of the 2-week-old broilers survived the 30% O<sub>2</sub>+40% CO<sub>2</sub> +30% N<sub>2</sub>-mixture and therefore this mixture was excluded for further testing at six weeks of age. At 6 weeks of age 30% of the broilers survived in the 50% N<sub>2</sub>+50% CO<sub>2</sub>-group. Highest level of CO<sub>2</sub> in the breathing air was reached in the 100% CO<sub>2</sub>-mixture, 42% against 25% for the other two mixtures. In all three-gas mixtures head shaking, gasping and convulsions were observed before loss of posture. Loss of posture and suppression of electrical activity of the brain (n=7) occurred almost simultaneously. The results of this experiment indicate that euthanasia of groups of 2 and 6 weeks old broilers by gradually increasing the percentage of CO<sub>2</sub>% in the breathing air up to 40% is possible.

**Key words:** *Euthanasia, Broilers, gas mixtures, behavior, brain activity.*



## Chapter 4

# **Slaughter of poultry during the epidemic of avian influenza in The Netherlands in 2003**

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## **Abstract**

During an outbreak of avian influenza in the Netherlands in the spring of 2003, the disease was controlled by destroying all the poultry on the infected farms and on all the farms within a radius of 3 km. In total 30 million birds were killed on 1242 farms and in more than 17.000 hobby flocks, by using mobile containers filled with carbon dioxide. Observations of these methods were used to compare their effectiveness, and capacity, and their effects on the welfare of the birds. Gassing whole poultry houses had a much greater capacity than mobile equipment, and catching live birds to bring them to a mobile killing device caused extra stress and could cause pain due to injuries inflicted when catching and handling them. However, gassing whole poultry houses with carbon monoxide requires strict safety regulations and gassing with carbon dioxide was considered preferable. However, this method is not suited to all types of housing, and in these circumstances killing devices were a useful alternative.

## Introduction

In spring 2003 the Dutch poultry industry was plagued by an outbreak of avian influenza, the control of which, as with other notifiable diseases, is defined by EU Directive 92/40/EEC (CEC 1992). Article 5 of this directive states that once the disease has been officially confirmed all poultry on the holding shall, without delay, be killed on the premises and in a way which minimises the risk of spreading the disease. Until 1994, poultry flocks were culled in their houses with hydrogen cyanide. However, because of the risk to people and the severe convulsions suffered by the birds before they became unconscious this method is no longer recommended (Scientific Veterinary Committee 1997), and hydrogen cyanide is no longer available in the Netherlands.

All the birds on a premise can be killed either inside or outside their houses. Killing them inside their houses reduces the risk of spreading disease by carrying them to a killing device outside; moreover, killing them inside their houses with a lethal concentration of gas or with a toxic agent in the feed or drinking water avoids the stress produced by catching and transporting them. One problem when killing whole houses of birds simultaneously is the control of the killing process and the survival rate. Killing birds by bringing them to a killing device provides an opportunity to control the process for individual birds. Mobile killing equipment with a large capacity can be based upon gas or electricity. An advantage of electrocution is that death occurs immediately provided a sufficiently high current is applied; a disadvantage is that living birds have to be handled and shackled before they are electrocuted, a stressful process which can easily result in severe injuries such as broken bones (Knowles and Broom 1990). With a gas-killing device the birds do not have to be restrained individually, which is a major welfare advantage compared with electrocution, but they do have to be caught. Furthermore, the time required to induce unconsciousness and death, and the level of discomfort before loss of consciousness depends on the gassing method and the gas used. Gas killing methods are of two types; in the first, the birds are brought directly into contact with a high concentration of gas, resulting in the rapid induction of unconsciousness and death, which however, is often accompanied by excitation (Raj and others 1992, Danneman and others 1997). In the second approach the birds are brought into a relatively low concentration of an anaesthetic gas, the concentration of which is subsequently increased to a lethal level; by this method the induction of unconsciousness is prolonged but relatively smooth (Coenen and others 1995, Lambooi and others 1999).

During the 2003 epidemic of avian influenza in the Netherlands, contiguous culling by electrocution or different gassing methods was used to eliminate the disease. When large-scale on-farm culling is applied it is important not only that all the birds are killed, but also that any unpleasant, painful or frightening experiences before they lose consciousness should be minimised; the time needed to render the birds unconscious and thus the duration of these negative effects is therefore of major importance in terms of the birds' welfare. This paper describes observations on the

methods used to kill large numbers of birds with particular emphasis on gassing whole houses. The objective was to obtain an insight into the suitability of these methods, as judged by their efficacy in killing the birds and their effects on the birds' welfare.

## **Material and methods**

### *Killing birds outside their houses*

Gas killing devices several mobile types of gas killing devices were used during the epidemic. They differed in size and operating efficiency, but all of them were filled with a high concentration of carbon dioxide before the birds were placed in them. In the smallest systems, the birds were caught and crated in their houses, and the crates were placed in the prefilled gas chamber. Other systems used containers varying in size from approximately 2 m<sup>3</sup> to 35 m<sup>3</sup>. In these systems the birds were put into the container through a hatch in the top and plunged directly into a high concentration of carbon dioxide. The birds were kept in the gas containers long enough to ensure that they did not survive. All the mobile gas killing systems were easy to use and incorporated a unit for controlling the concentration of carbon dioxide.

### *Electrocution*

The mobile electrocution equipment consisted of a water-bath and a closed-loop shackling line. The capacity of the equipment varied with the length of the shackling loop and the size of the power supply used; lines with a 220 or 400 V ac power supply were used. The more power that was available to the water-bath the more birds could be immersed while ensuring that there was sufficient current to kill all of them.

### *Killing birds in their houses*

Different gasses and gassing methods were used to kill the birds in their houses. In all them the doors, windows and ventilation slats of the houses were covered with plastic and taped to prevent leakage. Tubes connected to the gas containers outside the houses were brought inside at different positions, and after the ventilation had been switched off and the lights had been dimmed the supply of gas was switched on.

### *Carbon monoxide*

Carbon monoxide was used in all the common types of housing, such as floor housing with litter and wire, battery-cage houses from three to seven cages in height, and houses with free-moving birds at different levels. The gas was supplied until a concentration of approximately 1.5 to 2 per cent carbon monoxide in the air was achieved. The concentrations of carbon monoxide were measured inside and outside the house by a fire brigade unit, and for safety reasons, access to within

25m of the house was forbidden. Six farms where this method was used were inspected after the safety officer had given permission.

### *Carbon dioxide*

Carbon dioxide was supplied either as a cold liquid or as preheated gas. The cold liquid was supplied either by direct injection through nozzles, or by allowing it to leak from holes in the supply tubes. Both methods were applied in different types of houses of different size, including floor housing, housing at different levels and battery-cage systems.

In the direct injections method, liquid carbon dioxide was pumped at a pressure of 20 bars through tubing fitted with specially shaped nozzles. The number and position of the injection nozzles depended on the type of housing; in large, tall housing such as multiple level systems, the nozzles were placed at the different floor levels; in high battery-cage systems they were located on the ceiling to allow downward injection; in floor and smaller battery-cage housing they were located on the floor injecting upwards. An opening was made in the roof to facilitate the escape of air. The high injection pressure and the environmental temperature within the house (30 to 35°) resulted in the carbon dioxide evaporating directly from the nozzles into the air.

In the second method liquid carbon dioxide was pumped through tubing with a diameter of 10 cm which had small holes in the metal connecting flanges, from which the gas vaporised due to the ambient temperature into the house. The concentration of carbon dioxide was not measured, but the quantity required to reach a concentration of 40 per cent was calculated by assuming that 1000 kg liquid carbon dioxide would provide 500 m<sup>3</sup> of gas.

In the method using preheated carbon dioxide, the cold gas was heated to 40 to 50°C and injected into the house through tubing 10 cm in diameter. Sound-muffling equipment was added to the end of tubing to reduce the possible stress to the birds from the noise.

### **Observations**

The following general observations were made to assess the efficacy of the different methods and their impact on the welfare of the birds. They were made after the atmospheric conditions in the houses had returned to normal.

The time taken to apply a method was measured either as the time from starting the introduction of the gas to reaching the required concentration, or as the time from catching the birds and taking them to the killing device until they had been killed. The percentage of birds surviving the procedure was recorded. When whole houses of birds were gassed the distribution of the dead birds in the house was recorded as an indicator of panic, fear or attempts to escape. In addition, the positions of individual birds were recorded as an indication of whether they had suffered convulsions, and the percentage of birds lying on their back was

calculated. Convulsive behaviour that starts after the loss of consciousness is not a welfare problem for the birds involved, but other birds in the house may be frightened by the behaviour. The convulsive behaviour may also affect the efficiency of the killing process by disturbing the concentration of the gas. The observations were related to the method of killing and the type of housing. For example, in battery-cage systems the distribution of birds through the building was irrelevant.

## Results

### *Killing birds outside their houses*

There were large differences in capacity due to the different types and sizes of the husbandry systems. The time taken to catch the birds and transport them to the killing device varied.

Crating the birds, as required for one of the mobile gas killing devices, also reduced the capacity. Moreover, capacity depended on the experience and the number of people that were available to catch and transport the birds to the killing device.

### *Gas killing devices*

The capacity of mobile gas killing devices depends on the size and number of gas containers available at the site. Wing flapping, jumping, and heavy breathing were observed in a large percentage of birds directly after placing them in the gas containers, and lasted for from 30 seconds to two minutes. The duration of these responses varied greatly depending on the number of animals that were brought into the killing device in a short time. No survivors were recorded in the mobile gas killing devices.

### *Electrocution*

The average current used for the electrocutions varied between 2 and 4 A depending on the voltage used and the number of birds entering the water-bath at the same time. With a maximum of five birds entering the water-bath at the same time the current ranged from 400 to 800 mA per birds. Although this current is normally sufficient to kill birds, not all of them were killed because some wing-flapped and raised their heads above the water and did not receive the full current; as a result, some of the birds were stunned but others remained fully conscious. No measures to reduce wing flapping were taken, but after an adjustment to the height of the water-bath all the birds were killed.

### *Killing birds in their houses*

Table 1 summarises the results of the observations made of the different methods.

Table 1: Observations of gassing whole poultry houses during the 2003 avian influenza epidemic in the Netherlands.

Methods	housing (n)	Housing type <sup>1)</sup>	Animals <sup>2)</sup> (n x1000)	Time taken <sup>3)</sup> (minute)	Laying on back <sup>4)</sup> (%)
CO	6	4batt, 2 floor	22 (4;35)	45 (30;120)	25 (1;50)
CO <sub>2</sub> -inj.	5	1batt, 2floor, 2level	15 (6;33)	30 (10;60)	1 (1;5)
CO <sub>2</sub> - flow	2	1floor, 1level	(6;12)	(60;60)	(1;2)
CO <sub>2</sub> - heated	5	2 batt, 2floor	9 (6;31)	30 (30;180)	3.5 ( 1;10)

<sup>1)</sup> Housing type: number of observations per type of housing: batt = battery cages, floor= floor housing on litter and wire, level = free moving animals at different levels.

<sup>2)</sup> Animals: median (lowest; highest) number of animals x1000 per farm.

<sup>3)</sup> time taken: median (lowest; highest) minutes taken from start of gas flow until reaching the required levels.

<sup>4)</sup> Laying on back: median (lowest; highest) percentage of animals laying on their back after gas killing.

### *Carbon monoxide*

In most cases the time taken to reach the required concentration of 1.5 per cent carbon monoxide varied from approximately 15 to 45 minutes but it occasionally took up to an hour. Concentrations of up to 0.2 per cent carbon monoxide were measured outside the buildings. When the houses were constructed of wood vocalisation and wing flapping against the walls were audible. Between 1 per cent and 50 per cent of the birds were found lying on their back, indicating that some birds had suffered convulsions. These convulsions were also visible on video recordings made during one of the trials, but it was impossible to determine whether the birds with convulsions were still conscious. However, it was clear that the first birds in a flock to be exposed had convulsions while most of the others were still conscious. At one farm with a house containing 35,500 birds, the concentrations of carbon monoxide did not exceed 1.0 per cent, and after an hour approximately 20 per cent of the birds were still alive. The surviving birds were mainly found close to the gas inlet points and in the top-cages of the battery. Immediately after this observation, the building was closed and the concentration was increased to 1.5 per cent and maintained for an hour. This resulted in 100 per cent mortality.

### *Carbon dioxide*

Liquid carbon dioxide was injected until a concentration of 40 per cent had been reached in the air of the house, and it was maintained for at least 30 minutes before the house was ventilated. The concentration of carbon dioxide was measured at the different levels where birds were present during the procedure. In the largest system 5 per cent of the birds were found lying on their back after gassing, but in the other four houses less than 1 per cent were found lying on their back. Outside the buildings no sounds of agitation or convulsions could be heard. This technique creates a considerable amount of fog and reduces the temperature of the air in the building, but there were no visible signs that the reduced temperature affected the birds' behaviour. Because of the fog video recording was not possible. No survivors were observed in the five houses studies.

When liquid carbon dioxide was pumped into the houses through leaky tubes the time taken to reach the required concentration was approximately one hour. The gas concentrations were not measured but calculated on the basis of the size of the houses. In the two houses treated by this method, 1 and 2 per cent of the birds were found lying on their backs. No sounds of agitation or convulsions were heard outside the houses, and no survivors were observed.

Preheated carbon dioxide was injected up to a calculated concentration of 40 per cent and maintained for at least 30 minutes; the concentration was measured occasionally by introducing a tube through a small opening at a ventilation point. It took 30 minutes to reach the required concentrations in the houses with birds on the floor, and up to 60 minutes for the two battery-cage systems. In the floor housing between 1 per cent and 5 per cent of the birds were found on their backs, but in the battery-cage housing 10 per cent of the birds were found lying on their backs. In one of the battery-cage systems, one bird survived.

### **Discussion**

To control the spread of highly virulent viruses such as avian influenza it is essential to implement a policy of contiguous culling as quickly as possible. During the 2003 epidemic in the Netherlands all the poultry on infected and suspected farms were destroyed and all the flocks on the surrounding farms within a radius of 3 km were also killed. In total 30 million birds on 1242 farms and more than 17,000 backyard flocks were destroyed. It soon became evident that small mobile containers filled with carbon dioxide were inadequate, and to increase the killing capacity mobile electrocution lines and large 35m<sup>3</sup> containers prefilled with the gas were used. The capacity of these devices depends not only on the technology but also on the capacity of the operators to catch and transport the birds from their houses to the containers. The capacity was further enlarged by using either carbon monoxide or carbon dioxide to gas whole houses of birds.

Infections with highly pathogenic avian influenza in poultry are frequently accompanied by severe physical problems which can cause up to 100 per cent

mortality (Alexander 1995). For such infected flocks killing can provide 'a humane death' or 'mercy killing', because the impact of the killing method on the welfare of the birds may be less severe than the suffering caused by the disease. However, most of the birds killed during the epidemic were not infected and thus mercy killing did not apply to them. It was therefore important to choose a method with the least detrimental influence on the birds' welfare.

It is well known that catching and handling live birds can induce fear (Boissy 1995), and cause pain as a result of bruising and broken bones (Knowles and Broom 1990), particularly if the procedures are carried out by poorly trained staff. Furthermore, the shackling that is necessary with an electrocution line is potentially painful (Sparrey and Kettlewell 1994, Gentle and Tilson 2000). In addition, when birds are only stunned because they have not received sufficient current to kill them, some of them are probably still alive when they are placed in the killing chambers. It is therefore essential that the set-up and operation of an electrified water-bath is adequately supervised to ensure that all the birds are dead when they are de-shackled automatically. Exposure to high concentrations of carbon dioxide as in mobile containers, induces breathlessness and can be painful (Danneman and others 1997). These adverse effects can be ameliorated by introducing the birds to a low anaesthetic concentration of the gas, followed by higher, lethal concentrations (Hoen and Lankhaar 1999, Coenen and others 2000), but the birds still have to be caught and transported to the container. The advantage of mobile electrocution lines and mobile gas units is their controllability, but they have a relatively small capacity, and the live birds have to be caught and transported to them, with the attendant risk of spreading virus particles between the birds and human beings and potentially more widely on air currents. Killing the birds inside their houses would probably reduce the spread of virus particles because no virus would be spread from the respiratory tract of dead birds. The openings in the houses created for gassing would not increase the spread of virus more than the normal ventilation openings. Gassing whole houses provides a large killing capacity and eliminated the need to handle live birds.

The use of carbon monoxide provided no smoother induction of unconsciousness than carbon dioxide, and the large scale use of the gas requires strict safety regulations and procedures to minimise risks to personnel. During the gassing of whole houses the gas concentrations increased gradually and the birds tended to lose consciousness before they inhaled high and painful concentrations; the observations indicated that a gradual increase in the concentration of carbon dioxide induced few signs of severe convulsions. Danneman and others (1997) found that rats could be anaesthetised and euthanized humanely by a gradual increase in the concentration of carbon dioxide so that conscious animals were not exposed to concentrations above 70 per cent. Rhesus monkeys did not respond to noise and remained motionless in an atmosphere containing 17 per cent carbon dioxide, behaviour which was considered to indicate the onset of anaesthesia (Mattson and others 1972). Under experimental conditions broilers lost consciousness at 15 per cent carbon dioxide, and concentrations of 35 to 40 per

cent, maintained for 30 minutes, led to 100 per cent mortality (Gerritzen and others 2004). In practice 40 per cent carbon dioxide maintained for 30 minutes appeared to be effective in killing all the birds.

The characteristics and availability of the different methods made it possible to choose the most suitable method for each poultry farm. The choice of the method was based on the size of the farm, for example, high capacity equipment for large farms, and on the type of housing, because not every poultry house is suitable for in-house gassing techniques. In addition to the capacity of the method and the type of housing involved, the birds' age and the safety requirements are essential in making the decision. Although whole-house gassing appeared to be effective there are points for concern. During the epidemic there were few controls on the procedure. There is little information about the efficacy and survival rate of the birds', or about the gas flows and build-up of its concentration. To reduce the birds' suffering to a minimum it is essential that the procedure applied to the chosen method is carefully controlled. This control should be based on physiological and behavioural data derived from birds' at the most critical places in the system or animal house.

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## CHAPTER 5

### **Susceptibility of ducks and turkeys to severe hypercapnic hypoxia.**

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## Abstract

Large groups of poultry, including ducks and turkeys, are killed for disease control purposes with CO<sub>2</sub>. In this study we examined the physiological reaction of White Pekin ducks and turkeys to increasing CO<sub>2</sub> concentrations. Additionally, we examined the suitability of killing both species with increasing CO<sub>2</sub> concentrations. Blood gas values showed similar reaction patterns for both species: a strong increase in pCO<sub>2</sub> from approximately 40 mmHg to 200 mmHg, decreasing pO<sub>2</sub> and O<sub>2</sub> saturation, a decrease in pH from 7.4 to 6.7 and a strong shift in acid base equilibrium (averaging 0 to -23). On the EEG,  $\theta$  and  $\delta$  waves occurred at 21-23% CO<sub>2</sub> and suppression to a near iso-electric EEG between 41.8 and 43.4% CO<sub>2</sub> in inhaled air. Heartbeat declined from approximately 300beats per minute (bpm) at the start to 225bpm at loss of posture to 150bpm one minute before heartbeat ceased. During the last phase of heart activity an irregular rhythm and fibrillation were observed in addition to a decline in bpm. Blood gas values and electrophysiological data confirmed that ducks and turkeys lose consciousness before a level of 25% CO<sub>2</sub> in inhaled air is reached and that both ducks and turkeys die within 13 min in 45% CO<sub>2</sub> in inhaled air.

**Key words:** *Carbon dioxide, Blood gases, Electrophysiology, Duck, Turkey.*



## Chapter 6

# **Behaviour of poultry exposed to increasing carbon dioxide concentrations**

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## **Abstract**

Whole house gassing with carbon Dioxide (CO<sub>2</sub>) to kill poultry is an important tool in disease control. Behaviour of ducks, broilers, laying hens and turkeys was observed to assess differences in susceptibility between species and to assess animal welfare implications. All birds were individually exposed to CO<sub>2</sub> concentrations which increased from 0 to 45% with a speed of 14l/min. Heavier breathing, gasping and headshaking were observed in all species before loss of posture. Convulsions were observed in 3 out of 8 ducks, 8 out of 9 broilers, and 6 out of 8 laying hens but not in turkeys. Convulsions occurred mainly after loss of posture only 1 broiler and 2 ducks showed convulsions before loss of posture. At approximately 24% CO<sub>2</sub> loss of posture occurred in ducks and at approximately 19.5% CO<sub>2</sub> in the other groups. The duration of the period between the first behavioural changes and loss of posture was approximately 3 minutes. It can be concluded that there are slight but sometimes significant differences between species or due to age. However, these differences are not large enough to make it necessary to set different criteria in relation with whole hose gassing for the examined species.

**Key words:** *Disease control, Carbon Dioxide, Killing, Broilers, Laying hens, Ducks, Turkeys*

## Introduction

In the European Union (EU), the regulation for the control of HPAI viruses is imposed by EU council directive 92/40/EEC. Killing and removal (culling) of infected poultry flocks as well as uninfected flocks in the surrounding area is the most important control measure to stop spreading the disease. In areas with a very high poultry density outbreaks of HPAI viruses can probably only be controlled by vaccination or by depopulation of such an area (Stegeman et al., 2004). For disease control purpose carbon dioxide (CO<sub>2</sub>) is often used in on farm killing of large groups of poultry, especially in mobile gas containers and for whole house gassing.

The majority of birds killed during the Avian Influenza epidemic of 2003 in The Netherlands were killed by gassing whole houses with carbon dioxide (Gerritzen et al., 2006<sup>a</sup>). Compared to mobile killing devices whole house gassing enlarges the killing capacity enormously enabling to stamp out infected farms more rapidly. Observations in practice during whole-house gassing of broilers and laying hens demonstrated that 40% carbon dioxide in inhaled air is sufficient to kill all chickens within 30 minutes. Experiments with broilers (Gerritzen et al., 2004) resulted in less than 0.05% survivors at concentrations of 35% CO<sub>2</sub> in inhaled air. However, at these relative low concentrations of carbon dioxide it takes 15-30 minutes to kill all chicken. The use of carbon dioxide for stunning or killing of animals meets a lot of resistance from an animal welfare point of view (leach et al., 2002; Raj et al., 1998). Inducing unconsciousness with CO<sub>2</sub> is associated with breathlessness, hyperventilation (Raj & Gregory 1995, Ludders et al., 1999) and irritation of the nasal mucosa (Ewbank 1983; Iwarsson & Reh binder, 1993). Furthermore, CO<sub>2</sub> at high concentrations (>60%) is found to be highly aversive in humans (Paton 1983; Gregory et al., 1990) in laboratory animals (Hackbarth et al., 2000; Leach et al 2004) and in farmed animals (Raj & Gregory, 1994). However, exposure of broiler chickens to mixtures of 30% CO<sub>2</sub> with 30% O<sub>2</sub> in air was not found to be aversive (Gerritzen et al., 2000). Also, the incidence of gasping and headshaking was reduced when adding 30% O<sub>2</sub> to a CO<sub>2</sub> air mixture (Lambooj et al., 1999). The suitability of carbon dioxide for killing ducks is questioned based on the assumption that diving birds, including ducks, are less sensible to asphyxia and hypoxia and that they possess physiological mechanisms enabling them to withstand hypercapnia (Hawkins et al., 2001). However, electro-physiological measurements and blood-gas values confirmed that white pekin ducks and turkeys lose consciousness and die at comparable CO<sub>2</sub> concentrations as broilers and laying hens (Gerritzen et al., 2006<sup>b</sup>). Despite the physiological comparability it is not clear if effects on animal welfare between different species can be compared as well. Replacement of CO<sub>2</sub> by other gas mixtures is not an alternative yet; carbon monoxide is dangerous to use and has no welfare benefits (Gerritzen et al., 2006<sup>a</sup>) and application of argon or nitrogen for whole house gassing is not developed till now. Furthermore, the application of inert gasses as developed for controlled atmosphere stunning is advised to be used with less than 2% residual oxygen (Raj

et al, 1993; Raj et al., 1998) which will be hard to realize in poultry houses. Other applicable methods all involve with handling of living animals and therefore introducing welfare problems like fear (Boissy, 1995; Cashman, 1987) and pain due to bruising and broken bones (Knowles and Broom, 1990; Gentle and Tilson, 2000). Furthermore, handling of live animals that are possibly infected with HPAI viruses can be a potential risk for human health. Koopmans et al. (2004) reported transmission of H7N7 avian influenza virus from chicken to people directly involved in handling infected poultry.

The aim of the study reported here was to examine differences in behavioural reactions of different poultry species i.e., chickens, ducks and turkeys, and of different age i.e. broilers versus laying hens, to slowly increasing carbon dioxide concentrations. Behavioural observations of individual animals are conducted to assess the effects on animal welfare.

## Materials and methods

### *Animals*

At the poultry facilities of the Animal Sciences Group (ASG) 25 two-week-old White Pekin ducks (*Anas platyrhynchos*), 25 three-week-old turkeys (*Meleagris gallopavo*), 25 one-week-old broilers (*Gallus Domesticus*) and 25 forty week-old laying hens (*Gallus Domesticus*) were raised under conditions compared to those in the poultry industry. All animals were housed on litter and had ad libitum access to feed and water. Experiments started when the animals were 6-7 weeks of age and for the laying hens at 42 weeks of age. Ethical aspects of the experiment were judged and approved by the animal ethical committee of the ASG.

### *Experimental design*

From all 4 groups 10 animals were selected for these experiments. Individual animals were placed in a Perspex box i.d. 0.8x0.8x0.8m (see Figure 1) and exposed to slowly increasing CO<sub>2</sub> concentrations. The test box was fitted with a 1-cm Ø CO<sub>2</sub> inlet 5 cm above the base of the box, a CO<sub>2</sub> measuring tube placed in the centre of the box at sitting head height of the animals and an overflow valve situated at the top of the box. From a 100% CO<sub>2</sub> source CO<sub>2</sub> was injected with a flow of 14 litres per minute until 40%CO<sub>2</sub> in the inhaled air was reached at the measuring point. The birds remained in this CO<sub>2</sub> concentration until they died. Death of the animals was judged visually based on behavioural observations, i.e. absence of breathing movements of the chest and erection of the feathers accompanied by a tonic cramping of the muscle that is followed by complete relaxation.

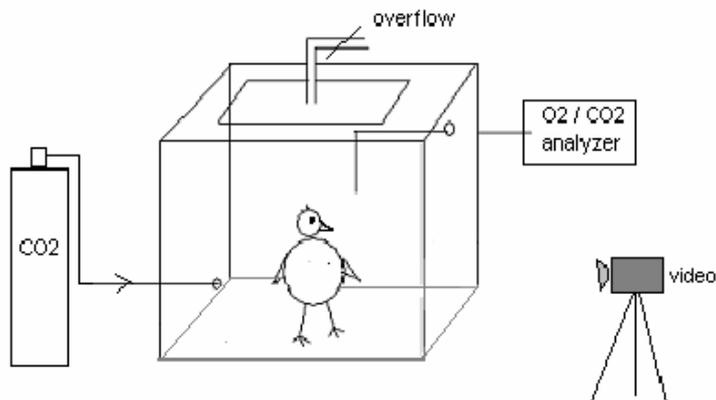


Figure 1: Schematic overview of the test situation.

### *Measuring Behaviour*

During the experiments behaviour of the birds was recorded continuously using a digital video camera (Sony camcorder, IP7E). The video recordings were analyzed afterwards (Table 1) using behavioural observation analysis software (Etholog, version 2.2). The total number of behavioural events, at which CO<sub>2</sub> level these events started and the number of events while the birds were still conscious, were used to judge impact on animal welfare.

### *Statistical Analysis*

All data were analyzed using an analysis of variance model (ANOVA). In this model the response variable is equal to the population mean plus the effect of species. The assumption of normality of the model was visually tested using plots of between and within session residuals. Differences between groups were declared significant when their probability levels, based on the residual errors, were below 0.05. Differences between pairs were analyzed using regression analyses and were visualized using ppair and rpair procedures.

All analyses were carried out using the Genstat statistical software package. (Genstat Committee 2000).

*Table 1: Description of the recorded behaviours*

Behaviour	Description
Notice	Alerting reactions like restless movements of the head, restless moving trough the test box, “tasting” movements with the beak.
Headshake	Rapid shaking of the head, most times accompanied by stretching and or withdrawal movements of the neck.
Breath	Deep breathing mostly with open mouth.
Gasping	Very deep breathing accompanied with width open mouth, stretching movements of the neck or bending of the neck to back.
Sit	Sagging trough there legs to sitting position
Jump	Explosive movement from sitting position to standing go together with a jump. Often followed by directly returning to sitting position.
Convulsions	Severe wing flapping together with tense up of neck and legs.
Loss of posture	Unable to maintain position, fall aside or backwards.

## Results

The behavioural variation between groups in association with CO<sub>2</sub> concentration in the breathing air is presented in Table 2. Layers notice increasing CO<sub>2</sub> concentrations at 6,6% which is at a higher concentration than broilers, ducks and turkeys. Heavier breathing also started at a higher level of CO<sub>2</sub> (8.0%) in the breathing for layers than for the other groups although, this difference was not significant between layers and broilers. The same pattern is seen for the parameters headshaking and gasping, both started at a higher CO<sub>2</sub> concentration for layers than for the other three groups. Significant differences in changing to sitting position is only seen between broilers ( 10.2% CO<sub>2</sub>) and layers (16.0% CO<sub>2</sub>), ducks and turkeys changed to sitting position at respectively 11.8% and 10.4% CO<sub>2</sub>. Jumping is seen short after going to sitting position in all groups of animals and occurred at the highest CO<sub>2</sub>% for layers. Convulsions, when they occurred, started at equal CO<sub>2</sub> concentrations for all animals, in all groups this was after loss of posture. Loss of posture occurred for broilers at 19.0% CO<sub>2</sub> which is a significant lower concentration than for ducks at 23.8% CO<sub>2</sub>. Loss of posture occurred for layers and turkeys at 19.9% and 19.3% CO<sub>2</sub>, respectively.

*Table 2: CO<sub>2</sub> concentration (%) in the breathing air at the start of behavioural events*

Parameter	broiler	duck	layer	turkey	P(F) <sup>2</sup>	SED
notice	2.4 <sup>a</sup>	1.5 <sup>a</sup>	6.6 <sup>b</sup>	2.3 <sup>a</sup>	0.001	1.1
breath	5.6 <sup>ab</sup>	2.8 <sup>a</sup>	8.0 <sup>b</sup>	4.1 <sup>a</sup>	0.034	1.7
headshake	8.3 <sup>a</sup>	5.1 <sup>a</sup>	13.0 <sup>b</sup>	5.5 <sup>a</sup>	0.001	1.9
gasp	9.2 <sup>a</sup>	9.5 <sup>a</sup>	14.0 <sup>b</sup>	6.4 <sup>a</sup>	0.004	1.9
sit	10.2 <sup>a</sup>	11.8 <sup>ab</sup>	16.0 <sup>b</sup>	10.4 <sup>ab</sup>	0.143	2.6
jump	13.2 <sup>a</sup>	11.5 <sup>a</sup>	18.6 <sup>b</sup>	9.2 <sup>a</sup>	0.002	2.2
convulsions	30.0 <sup>a</sup>	24.0 <sup>a</sup>	28.0 <sup>a</sup>	26.0 <sup>a</sup>	0.447	4.7
loss of posture	19.0 <sup>a</sup>	23.8 <sup>b</sup>	19.9 <sup>ab</sup>	19.3 <sup>a</sup>	0.023	2.3

<sup>a-c</sup> Means within a row with no common superscript are significantly different at  $p < 0.05$  using students t-test

<sup>1</sup> Data presented are ANOVA means

<sup>2</sup> ANOVA significance levels of the F-test for the factorial effects; df 3,32

Average numbers of behavioural events are presented in Table 3. Layers showed a significant lower number of headshakes than broilers, ducks and turkeys. Ducks changed more often to sitting position than the other three species although, this was not significantly different from broilers. Birds jumped mainly from sitting position and returned immediately to sitting position again. Therefore, the incidence of jumping was also highest in ducks. The average number of convulsions for ducks and turkeys (0.8 and 0, respectively) was significantly lower than for broilers (1.9) and for layers (3.6). Convulsions didn't occur in turkeys and only in 3 out of 8 ducks whereas convulsions occurred in 8 out of 9 broilers and in 6 out of 8 layers.

Headshaking, heavier breathing, gasping and jumping occurred for all species mainly before loss of posture. Convulsions however, occurred only in 3 cases before loss of posture, 1 broiler and 2 ducks.

*Table 3: Total number of behavioural events during increasing CO<sub>2</sub> concentrations<sup>1</sup>*

Parameter	broiler	duck	layer	turkey	P(F) <sup>2</sup>	SED
headshake	5.8 <sup>b</sup>	8.5 <sup>b</sup>	2.4 <sup>a</sup>	7.6 <sup>b</sup>	0.005	1.6
breath	15.3 <sup>a</sup>	14.8 <sup>a</sup>	17.4 <sup>a</sup>	22.2 <sup>a</sup>	0.360	4.5
gasp	14.8 <sup>a</sup>	16.5 <sup>b</sup>	10.6 <sup>ab</sup>	13.9 <sup>ab</sup>	0.207	2.7
sit	3.3 <sup>ab</sup>	4.5 <sup>b</sup>	2.4 <sup>a</sup>	2.6 <sup>a</sup>	0.018	0.7
jump	2.3 <sup>ab</sup>	3.8 <sup>b</sup>	2.9 <sup>ab</sup>	1.4 <sup>a</sup>	0.099	0.9
convulsions	2.8 <sup>b</sup>	0.8 <sup>a</sup>	2.6 <sup>b</sup>	0.0 <sup>a</sup>	0.003	0.8

<sup>a-b</sup> Means within a row with no common superscript are significantly different at  $p < 0.05$  using students t-test

<sup>1</sup> data presented are ANOVA means

<sup>2</sup> ANOVA significance levels of the F-test for the factorial effects; df 3,32

The order in which behavioural events occurred and the time (min.) and CO<sub>2</sub> concentration (%) at which they were observed is schematically presented in fig. 2. Since the order of appearances of the behavioural parameters was the same for all groups they are presented as an average of all birds of the different groups. In this schedule it is visualized that birds in this experiment show alerting reactions (notice) from approximately 3.2% CO<sub>2</sub> in the breathing air. Deeper breathing (breath) starts at a CO<sub>2</sub> concentrations round 5% followed by headshaking around 8% and gasping at 9.8%. Short after gasping birds turned to sitting position, from this position they jumped up and return to sitting position again. Loss of posture occurred at approximately 20.5% CO<sub>2</sub> in the breathing air, at some time followed by convulsions of a small number of the birds at approximately 27% CO<sub>2</sub>. The duration of the period between first notice and loss of posture is approximately 3 minutes. The duration of the period of headshaking and gasping before loss of posture is approximately 2 minutes.

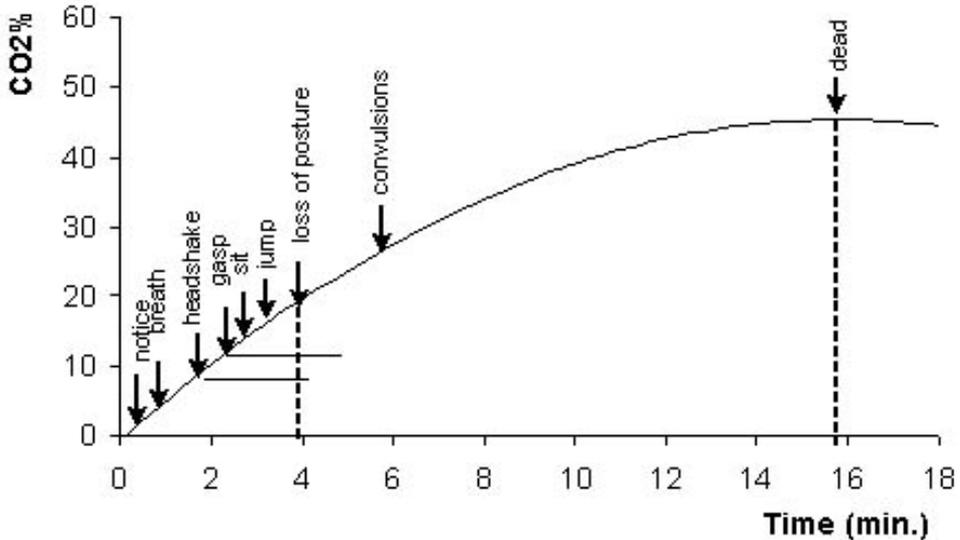


Fig 2: Schematic overview of the increase of the CO<sub>2</sub> concentration in time and the onset of the behavioural parameters ( average of all animals from the 4 groups).

- 1) the first vertical dotted line indicates the moment of loss of consciousness.
- 2) the second vertical dotted line marks the moment all animals are dead.
- 2) lines parallel with the x-axis indicates the period in which the behaviour repeats.

## Discussion

In this experiment we investigated the effects of slowly increasing (from 0-45%) CO<sub>2</sub> concentrations on the behaviour of broilers, laying hens, ducks and turkeys. Purpose of the observations was to determine the effects on animal welfare and to determine if there are differences between ducks, turkeys, broilers and layers. Furthermore, if there are differences in sensitivity between age (broilers versus layers) and or species (ducks, chickens, turkeys) this would implicate the need to set different conditions for different groups of poultry during whole house gassing. Physiological parameters i.e. eeg, ecg, blood gasses, (Gerritzen et al., 2006<sup>b</sup>) confirmed that white pekin ducks and turkeys are equally sensitive to hypercapnic hypoxia as chickens. Onset of unconsciousness although, is not instant when using gasses for stunning or killing and is therefore potentially conflicting with animal welfare. Loss of posture indicating loss of consciousness occurred at approximately 20% CO<sub>2</sub> in the breathing air which is reached within 4 minutes. A first sign that animals notice a change in their environment, i.e. an increasing CO<sub>2</sub>%, is seen from 2% CO<sub>2</sub> in the breathing air. It manifests as a more alert attitude, i.e. looking around and walking to and fro. Although, this is obviously a sign of detecting changes in the breathing air, it does not prove that it is a detection

of CO<sub>2</sub>. For chickens it has been reported that they can detect CO<sub>2</sub>% at a 10% level (McKeegan et al., 2005). First behavioural signs of physiological effects of CO<sub>2</sub> which shows as heavier breathing started between approximately 3 and 8% CO<sub>2</sub> depending on species. Carbon dioxide is the most important chemical stimulator for respiration, increasing CO<sub>2</sub> % will increase pCO<sub>2</sub> in the blood which is detected by the “respiratory centre” in the medulla oblongata that will react by increased breathing to lower the pCO<sub>2</sub> (Guyton and Hall, 2000). Heavier breathing therefore, is not necessary associated with reduced welfare but is a physiological reaction. Short after the start of heavy breathing, between 5 and 8% CO<sub>2</sub> first handshaking’s occurred followed by gasping that started at approximately 10% CO<sub>2</sub> in the breathing air. Interpretation of the effect of headshaking on animal welfare is controversial; it has been described as indicative for an aversive reaction to CO<sub>2</sub> and respiratory distress (Webster and Fletcher, 2001); or as an alerting response or to regain an alert state (Hughes, 1983). Gasping is in many cases, especially when birds are sitting, almost directly followed by headshaking. It is likely that when birds are feeling dizzy and start fainting they want to regain alertness with headshaking as a result. Furthermore CO<sub>2</sub> appears to have a stimulating effect on headshaking (Gerritzen et al., 2000) and is dose dependent (McKeegan et al., 2005 in press). Therefore, it can not be exclude that headshaking is related with reduced welfare.

Gasping or very deep breathing is by others described as a respiratory distress associated with breathlessness (Raj and Gregory, 1995; Ludders et al., 1999) and interpreted as a state that can be qualified as at least very unpleasant and can therefore be associated with reduced welfare. Furthermore, headshaking and gasping are repetitive up to, or even till after, the moment of loss of consciousness and thus not an instant event. This is implicating a period of reduced welfare.

*Table 4: Number of behavioural events before loss of posture*

Parameter	broiler	duck	layer	turkey	P(F) <sup>2</sup>	SED
headshake	7.0 <sup>b</sup>	6.8 <sup>b</sup>	2.3 <sup>a</sup>	7.6 <sup>b</sup>	0.010	1.6
breath	12.1 <sup>a</sup>	10.0 <sup>a</sup>	12.9 <sup>a</sup>	16.5 <sup>a</sup>	0.523	4.3
gasp	13.3 <sup>a</sup>	114.8 <sup>a</sup>	9.8 <sup>a</sup>	12.6 <sup>a</sup>	0.317	2.6
jump	1.3 <sup>a</sup>	3.3 <sup>b</sup>	1.8 <sup>a</sup>	1.4 <sup>a</sup>	0.024	0.7
convulsions	0.3 <sup>a</sup>	0.5 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.572	0.4

<sup>a-d</sup> Means within a row with no common superscript are significantly different at p<0.05 using students t-test

<sup>1</sup> Data presented are ANOVA means

<sup>2</sup> ANOVA significance levels of the F-test for the factorial effects; df 3,32

Animals change to sitting position, which can be related with a reduced level of balance, at approximately 11-16% CO<sub>2</sub>. In most cases, after a short time this sitting position is followed by a strong jump followed by returning to the sitting position again. Most likely the jumps are attempts to keep control over their body position. In this period animals are still considered to be aware of their environment and thus this period should be classified as at least having impact on animal welfare. At on average 20% CO<sub>2</sub>, which was reached within approximately 4 minutes, animals lose posture and thus their consciousness (Gerritzen et al., 2004). Behavioural changes that can be associated with breathlessness like deep breathing and gasping, with aversion or unpleasant experiences like headshaking, and with reduced control like sitting-jumping all occurred in a period of 2.5 to 3 minutes before loss of consciousness. The duration of potentially reduced welfare is therefore in this experiment at maximum 3 minutes long. After loss of posture, between 25-30% CO<sub>2</sub> approximately, few convulsions occurred in a small number of animals. But, these convulsions have no impact on animal welfare as they started amply after loss of posture and thus after birds lose consciousness.

The resistance to use CO<sub>2</sub> for stunning or killing is based on experiences with bringing animals in an existing CO<sub>2</sub> concentration which is for killing at least 60%. High concentrations CO<sub>2</sub> are irritating to the nasal mucosa (Ewbank, 1983) and are found to be highly aversive in humans (Paton, 1983; Hari, 1997) and different animal species (Hackbart et al., 2000; Leach et al., 2004; Raj & Gregory, 1994). During gassing whole houses, as simulated in this experiment, CO<sub>2</sub> concentrations increase gradually and thus animals never contact high concentrations while conscious. When using low or slowly increasing CO<sub>2</sub> concentrations the time to loss of posture and thus to lose consciousness will therefore be lengthened but the induction will be smoother (Coenen et al., 1995; Coenen et al., 2000; Lamboojij et al., 1999; Gerritzen et al., 2000). From an animal point of view it is important to reduce the period in which its welfare can be affected in a negative way. Although, during whole house gassing, used for emergency killing of chicken flocks, the CO<sub>2</sub>-flow is limited due to the maximum flow from the lorry. This implicates that for practical purpose it is hard to reduce the period between starting the CO<sub>2</sub> inflow and loss of consciousness. Furthermore, in practice CO<sub>2</sub> concentration build-up depends on conditions like; temperature insight and oversight the building, conformation and leakage of the building.

It is obvious that killing animals always conflicts with animal welfare. To make a decision in acceptability of a killing method all aspects have to be considered. Advantages of whole house gassing are the large capacity and the fact that animals don't have to be handled alive. These advantages are important enough to make whole house gassing, if executed properly, an acceptable method in emergency disease control. It is unmistakable that during slowly increasing CO<sub>2</sub> concentrations heavy breathing, gasping and headshaking occur during a period of approximately 2-3 minutes. However, considering the duration and level of possibly

reduced welfare whole house gassing is especially on larger farms, preferable above methods that involve handling of living animals.

The results presented in this paper show that there are slight but sometimes significant differences between species or due to age. However, the differences as measured in this experiment between animals of different age or species were not of dimensions that make it necessary to set different conditions for chickens, turkeys or ducks in relation with whole house gassing.

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## Chapter 7

### **General Discussion**

CHAPTER 7

## Background considerations

Eggs and poultry meat are an important source of food for many people worldwide. Furthermore, the poultry industry is a major economical activity in many countries. To protect the production of poultry products it is important to deal with diseases especially with highly contagious diseases like avian influenza (AI). Therefore, the control of this disease is laid down in legislation and guidelines. (OIE 2003, EU 2005, WHO 2006, FAO 2006). In case of highly pathogenic avian influenza (HPAI), depopulation of infected and suspected flocks is so far an important measure to stop the spread of this disease. General aspects of massive killing of animals like qualification of people involved, operational procedures, animal welfare matters, human safety aspects, and available methods are described in these legislation and guidelines. Important criteria in killing of poultry are efficacy, e.g. all animals have to be killed, animal welfare, e.g. preventing animals from avoidable pain, fear and suffering, and human safety, e.g. procedures should not endanger humans and the chance for humans to become infected is minimized.

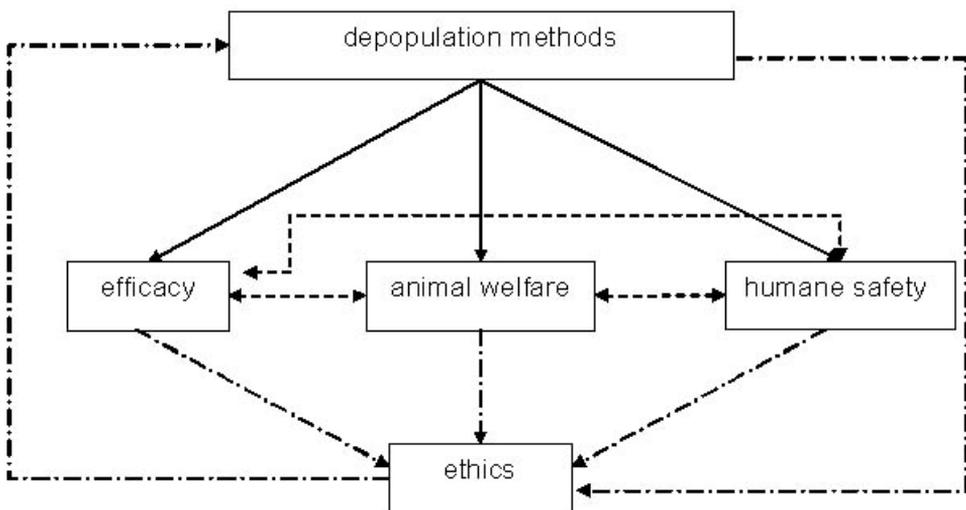


Figure 1: Factors or criteria (→) of depopulation methods and relations or conflicts (- ->) between the different criteria.

Different from slaughter, for disease control animals are generally killed on the farms and preferable in their housings. Depopulation of whole flocks is complex, because huge numbers of animals have to be killed on sites which are not designed for this purpose and as a consequence, efficacy, animal welfare, and humane safety are often in conflict with each other. Furthermore, killing animals for disease control is frequently subject of social and ethical discussions.

The reason why animals are killed is probably the most important issue in these discussions; after all it is generally accepted to kill animals for human consumption (Rutgers et al. 2003, Swabe et al. 2005). Societal discussions, furthermore, depend strongly on factors like animal species (dairy cattle versus poultry), animal welfare, and human safety (are humans susceptible for the disease?). As guideline in the societal discussion the ethical matrix of Mepham can be very useful. In this matrix important principles for the different parties involved are arranged under the moral values, benefit (do no harm), autonomy (rights and freedoms), and justification (intrinsic value and legislation).

Respect for	benefit (do no harm)	Autonomy	justification
Animals	well-being	right to exist	Intrinsic value
Farmers	survival profit	management freedom	legislation rival position
Consumers / society	safety, product costs, social acceptance	freedom of choice	legislation human safety

Figure 2: Ethical matrix of Mepham

In this final chapter, the most important results of this thesis and the implications of the results will be discussed. Following Figure 1, the discussion is organised taking three important criteria into account; efficacy, animal welfare, and humane safety. However, these criteria are not independent of each other. The total complex of killing for disease control is subject of moral or societal discussion and so are also the different criteria on their own subject of these discussions. A utilitarian approach is followed to discuss the moral and societal aspects. The interests of animals and man and different interests of different groups of man or even sometimes different values of the same people are weighed to have an optimal outcome meeting as many moral concerns as possible.

## Efficacy

With regard to disease control efficacy of the measures is the most important criterion in stopping the disease from spreading. Killing the animals will stop the virus production and consequently the probability that the virus will be transmitted to another farm will be sharply reduced. As a consequence, the aim from this perspective is to kill all animals quickly, while minimizing the probability that the virus is transmitted to other flocks. Besides disease control, important reasons to stop the disease as fast as possible are based on economical grounds (financial consequences for producers and community), human safety (reduce the risks for people getting infected) and animal welfare reasons (preventing animals from

suffering). Efficient disease control, therefore, means that all susceptible animals on a farm have to be killed and preferable by the initial method with a high capacity. As presented in Figure 3, efficacy depends on different factors and these factors may also affect animal welfare and human safety.

At the beginning of this project an important reason to focus on gas mixtures for killing was the need for methods with high capacity, from which animals could not escape, that are acceptable from animal welfare point of view, and that are safe for humans. To reach high efficacy it is thought that whole house gassing is an important tool to kill animals in high numbers and without handling living animals which could be a great advantage from animal welfare and labour point of view. Furthermore, minimizing contact with living animals and not bringing live animals in the open is presumed to reduce the spreading of viruses.

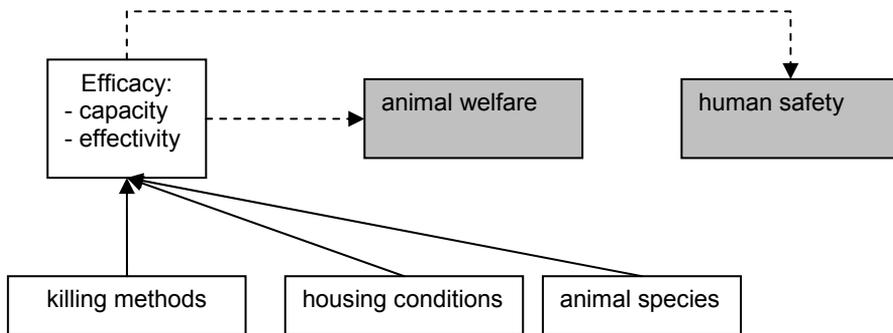


Figure 3: important factors determining efficacy.

In Chapter 3 the suitability to use slowly increasing concentrations of different gas mixtures to kill broiler chickens of different age is described. Till then these gas mixtures were developed and used in practice for stunning animals at slaughter and not judged on their capacity as killing agents. Furthermore, till then major focus when using gas mixtures was on pre-filled systems and not on using them for whole house gassing. The results from this experiment made it clear that for whole house gassing a 100% CO<sub>2</sub> source is much more efficient than using CO<sub>2</sub> mixtures containing N<sub>2</sub> or O<sub>2</sub>. When using a CO<sub>2</sub>/N<sub>2</sub> mixture it takes a long time to reach anaesthetic levels and it is hard to create an atmosphere that is acceptable for killing animals quickly. The addition of O<sub>2</sub> prolonged the time to reach these levels even more and as a consequence unconsciousness was postponed with detrimental effects on animal welfare. Based on this experiment and despite it was not tested under practical conditions before, the use of CO<sub>2</sub> from a 100% source was advised to test for whole house gassing during the HPAI outbreak of 2003 in The Netherlands. Observations of killing methods in practice (described in Chapter

4) made it clear that whole house gassing either with CO<sub>2</sub> or CO enlarges the capacity enormously. The capacity of mobile gas containers appears to be too small for efficient disease control on a large scale. Therefore, whole house gassing became the most important control measure at that moment and more than 60% of all farms were depopulated using this method (Berenschot 2004). Besides gas-filled containers and whole house gassing, mobile electrocution lines are used for on-farm killing as well. Most important limitation of this mobile equipment is the necessary catching capacity. Animals are caught manually and walked to the killing devices located outside the sheds. Despite the limited capacity, mobile devices play an important role especially on farms where sheds are not suitable for gassing and on smaller farms. For effective whole house gassing leakage of the building must be limited or be controlled by closing or covering the most important openings. In this regard very open housing types where birds have open access to outdoor space are difficult to fill with gas. Also, the leakage of old wooden sheds is difficult to control.

Besides capacity the rate of successful killing is part of the efficacy. Different from normal slaughter, where animals are stunned prior to killing by exsanguinations, the methods to control infectious diseases are meant to kill all animals without additional measures. Therefore, it should be impossible for animals to escape from the killing procedure and killing methods should have great accuracy. Additional measures like lethal injections to kill survivors will reduce the capacity considerably and require extra labour. Killing turkeys and especially ducks by whole house gassing with CO<sub>2</sub> was not always successful during the 2003 HPAI outbreak in The Netherlands. However, it was not clear if this was because of the procedures and housing conditions or due to fact that these species are less susceptible to CO<sub>2</sub>. Especially the susceptibility of ducks to CO<sub>2</sub> is questioned based on the assumption that diving birds are highly tolerant to hypercapnia and hypoxia (Hawkins 2004, Powell et al., 2004). Based on these assumptions official guidelines often advise not to use CO<sub>2</sub> for stunning or killing waterfowl (Scientific Veterinary Committee, 1997). However, although ducks are generally seen as diving birds, white Peking ducks are related to the wild mallard ducks which are dabblers (Belrose 1981), who find their food in low shallow waters and don't dive very deep. In Chapter 5 blood gas values, EEG's and ECG's measured in turkeys and ducks during exposure to slowly increasing CO<sub>2</sub> concentrations are described. The results of these experiments show that there are no significant differences in sensitivity for CO<sub>2</sub> between the different poultry species. Furthermore, as presented in Chapter 6 there are no differences in behaviour of ducks, turkeys and chicken that are exposed to slowly increasing CO<sub>2</sub> concentrations. The general opinion, as reworded in the scientific veterinary Committee (1997), that CO<sub>2</sub> is unsuitable to use for killing ducks is not confirmed and therefore needs reconsideration.

## Animal welfare aspects

An important aim of this project was to examine the possibility to kill animals on farm taking animal welfare into account. The first definition of animal welfare, proposed by the Brambell Committee states that; “*Welfare is a broad term that embraces both the physical and mental well-being of the animal*” (Brambell Committee 1965). To protect the welfare of animals it is important to do them no harm what implies that we should prevent them from unnecessary pain, fear and suffering (Fraser et al. 1997) During on farm killing animal welfare can easily be compromised. The chosen method for killing and the related procedure of catching and handling animals is highly influencing animal welfare (Figure1). Especially in birds, handling and crating frequently accomplice with fear, painful bruises and broken bones (Sparrey and Kettlewell 1997, Knowles and Broom 1990). Electrocution is, when well performed, causing instant unconsciousness and, when a sufficiently high current is used, resulting into death. However, birds must be caught and transported to the killing device, which in on farm killing means walk them to electrocution lines located outside the barns. The handling of live birds till now can not be prevented and is in case of electrocution the major compromising factor in bird welfare. Furthermore, the shackling on an electrocution line is potentially painful (Sparrey et al. 1997).

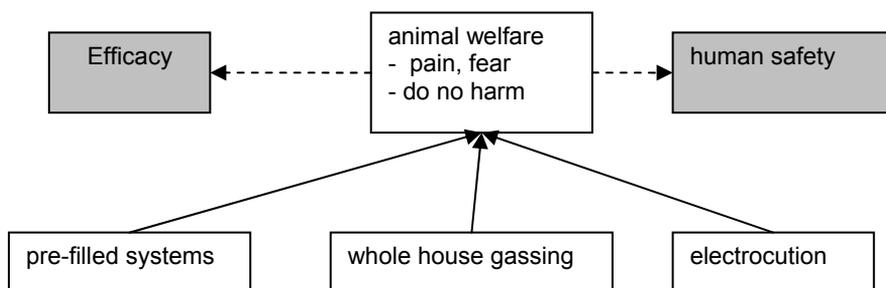


Figure 4: important factors determining animal welfare in on-farm killing

When using mobile gas containers the birds' welfare is affected by handling as well as by the gas killing procedure itself. Gasses used for killing are not inducing immediate unconsciousness and especially the use of high concentrations  $\text{CO}_2$  is judged as aversive (Raj et al 1991, Raj et al 1996) and painful to inhale (Hari et al. 1997, Danneman et al. 1997). Signs of asphyxia and behavioural excitation are observed due to hypercapnia and hypoxia (Forslid et al. 1986, Forslid 1987, Lambooj et al. 1999, Gerritzen et al 2000). Introducing birds in containers pre-filled

with 60% CO<sub>2</sub> can therefore be judged as stressful. However, the induction of unconsciousness is not instantaneous; the duration of this stressful period is approximately 30 to 60 seconds. When using low concentrations of CO<sub>2</sub> or adding O<sub>2</sub> this stress is reduced (Hoederken et al. 1994, Coenen et al. 1995) although the induction period is prolonged. For different animal species, like rhesus monkeys (Mattson et al. 1972) and rats (Danneman et al. 1997) it is described that unconsciousness is induced at approximately 17% CO<sub>2</sub>. Behavioural observations and EEG measurements in broiler chickens (Gerritzen et al. 2004), ducks and turkeys (Gerritzen et al. 2006<sup>a</sup>, 2006<sup>b</sup>) demonstrated that also for poultry unconsciousness is induced at CO<sub>2</sub> concentrations around this concentration. The total period of compromised animal welfare should, however, be judged from the moment that birds are caught in the shed until they lose their consciousness due to the killing procedure. The total duration of this period can, when using mobile killing devices (catching, walk to the killing device, induction faze), be easily more than 5 minutes.

Whole house gassing does not involve handling of animals, the birds remaining in their shed with a minimal of disturbance when preparing the process. As described in Chapter 4 CO<sub>2</sub> is the privileged gas for whole house gassing. Point of concern is what the birds experience between the times from starting the gas flow until birds will be unconscious. During whole house gassing the CO<sub>2</sub> concentration will increase gradually and thus it will take some time to reach anaesthetic concentrations. As described in Chapter 6 the period between first notice and the moment of loss of consciousness can be reduced to 3 minutes, depending on the chosen method of gas distribution. However, it can be discussed if bird welfare is compromised during the total 3 minutes or that bird welfare is affected from the moment that birds show the first signs of distress, i.e. when heavy breathing starts. Heavy breathing followed by gasping is presumed to be an expression of breathlessness which can be judged as suffering or at least be classified as unpleasant. The duration of the period of breathlessness as described in chapter 6 can be reduced to approximately 2 minutes. It is therefore obvious that, compared to mobile killing devices, whole house gassing can reduce the negative effects on bird welfare. The distribution of the gas and the temperature development in the barn however are points of concern. As described in Chapter 4 this depends on the chosen method to inject the gas into the shed and upon the control measures during the procedure.

### **Human safety**

Large scale disease control measures conducted on farm and thus on locations that are not designed for these procedures are a potential hazard for people involved. Working with electrocution devices or with gas killing procedures that are developed to kill animals can also be dangerous for man. High safety regulations and the presence of qualified personnel is therefore a precondition.

It became clear that HPAI can be transferred from birds to man (Koopmans et al. 2005, Tam, 2002). A considerable number of cases confirmed that intensive contact with infected birds can result in infection of humans (RIVM, 2004) and can even lead to mortality in man.

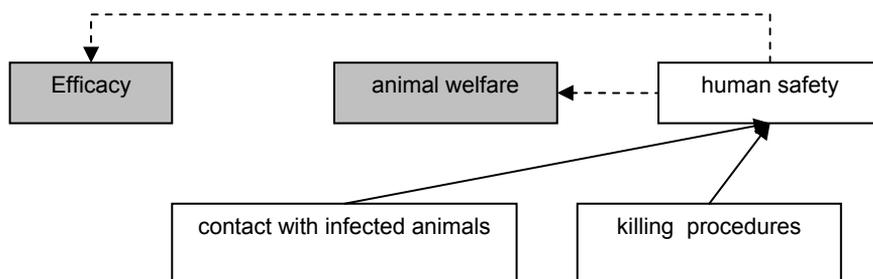


Figure 5: hazards for human safety

In case of HPAI high concentrations of virus are located in faeces and in the respiratory tract of birds. When animals are stressed, as due to catching and handling, respiration increases and most animals will defecate resulting in high virus excretion when handled by man. Preventing intensive contact with live animals is therefore an important measure to protect humans from infection. From this point of view whole house gassing should be preferred above mobile killing devices, because the latter implies handling of live animals. Besides this, efficacy of killing procedures and the speed at which the animals are killed are important to reduce the exposure time.

### Societal and moral aspects

In societal and moral discussions the effective large scale depopulation of poultry farms is an important driving force behind opinion forming. The extensive scale of mass destruction of apparent healthy animals is suddenly visible for the public and raises their suspicion and indignation followed by moral concerns. The confrontation with the massive destruction of living animals elicits societal reactions. Especially when alternatives like vaccination are available, the necessity of the followed strategy is questioned. However, killing large numbers of animals for meat production or even when they are no longer of economical value is widely accepted in our community at least when it is not visible. Animal products including meat are seen as an important source of food which is a justification for killing them. This implies that the reason why animals are killed seems to be crucial for our moral stances. However, killing and destroying large numbers of animals for disease control is often seen as a waste of animal products and judged as

senseless. On the other hand even when no risk is involved many people refuse to eat products from such animals. For farmers and others involved in the production of animal products economical aspects as profit and remaining their rival position is an important interest and at the same time part of their justification to support the non-vaccination policy. Farmers are used to the idea that their animals are killed for the production of food or when they are no longer of economical value. Nevertheless, the killing of productive animals and the destruction of animal products at their own farm is also by them experienced as stressing and as a waste.

According to the moral viewpoints of many people animals have the right to for live the lives we had in mind for them (Rutgers et al. 2003). This is one reason why the killing of young animals meets a lot of resistance. That most animals raised for meat production are slaughtered before they reach adult age or even before reaching pubic age anyhow, is not realised. However, that also farmed animal have the right to live the live of food producing animals implies that we accept certain autonomy of farmed animals and, therefore, admit their right to exist and that they also have a value of their own. However, animals kept for a different purpose and different animal species are given a different social status (Waelbers et al. 2004) and consequently valued differently. At least in our culture cows are given a higher status than pigs, pigs higher than poultry. Cows are larger, more visible provided that they are kept outdoors for grazing, and live longer than chickens and are kept in relatively small numbers. Subsequently cows are more perceived as individuals than chickens that are usually kept in large numbers and transformed to standardised objects for food production (Swabe et al. 2005). It seems that a greater value is assigned to cows than to poultry. However, it is doubtful whether this distinction is justifiable for reason mentioned above. In legislation it is recognized that all (farmed) animals have a value of their own and that they all can suffer. Because we admit that animals can suffer, we agreed that we shall keep them from unnecessary pain, fear or injury and thus to protect their well-being (FAWC 2005), which is from an animal point of view their most important benefit. That we admit that animals have a value of their own (Brom 1999), a so called *intrinsic value*, is a justification for their right to exist and to live their lives (even if the duration of this life-time is their productive life, planned by human). That this right stops when the moment of slaughter arrives shows that the acceptance of intrinsic value is not consistent and in fact we have adopted a more utilitarian approach. The use of intrinsic value is more a way to express our feelings of concern in a given situation than a general principle applied to husbandry animals. However, our societal and moral attitude reflects species- and culturally dependent values assigned to different animals, which is not in line with the concept of intrinsic value being a qualitative rather than a quantitative property.

## Ethical view

Unambiguously, there are a lot of aspects in the societal and moral opinion forming of humans with regard to animal welfare and in particular, when it concerns killing animals. What matters in a principal utilitarian approach are the interests of who are being affected by what we do independent of whether it concerns the interests of humans or (sentient) animals (Singer 1989). When following this approach in its pure form this would imply that all animals and human have equal rights. In our western community, when discussing animal welfare and animal rights, a more hybrid view (Sandøe et al. 1997), or a pluralist-utilitarian approach is followed. After all, the interest of humans is considered of more importance than that of animals (“*speciesm*”). From a human point of view this form of speciesm is a legitimated and a generally accepted view. However, this speciesm-view is not only adopted when it concerns human interest, but also when we assign different values to different animal species and is even affected by the relation that we have with individual or groups of animals. Animals kept as pets are valued as more important than farmed animals. Not only we value cows above pigs and pigs above chickens but, even poultry in backyard flocks is perceived differently from farmed poultry species, which became obvious during the HPAI outbreak of 2003 in The Netherlands. Our moral concept thus, is strongly influenced by the emotional bond that we have with individual or groups of animals. Besides this speciesm-view, our moral opinion depends on the justification of our actions or in other words on the benefit of our actions; killing poultry for food production is widely accepted, while killing the same number of animals to prevent the spreading of avian influenza in poultry flocks is considered questionable (“*moral agent view*”). When human safety is at risk the large scale killing for disease control, however, meets far less resistance in society, and concerns regarding animal welfare will lose an important part of their relevance.

Accepting the pluralist utilitarian view, a weighing of costs and benefits of animals and man and of different groups of man should meet as many moral concerns as possible. The benefit of all groups involved should be respected as much as possible, however, it is clear that the weight of benefits is not equal for the different groups. The animals pay the costs with their lives to stop the disease from spreading. When it concerns sick or infected animals this can be justified as ‘mercy killing’ or to prevent them from suffering from a devastating disease. From a human point of view this can probably be defended as the ultimate form of protecting the well-being of animals. During large-scale animal disease control, however, most animals killed are healthy, non-infected animals that are killed to create buffer zones to stop the spreading of viruses. For these groups it is difficult to defend that their well-being is protected. Although, it can be defended that the well-being of the total species in a certain area is protected by killing a “small” defined group of the species. Thus, the right to exist, or the autonomy of animals, and the intrinsic value of individual animals is limited to benefit the species. When weighing the costs and benefits of animals involved in disease control it can be

argued that animals that are sick or infected are valued differently as compared to healthy non-infected animals.

Animal producers grow animals from an economical perspective. Due to an outbreak of infectious diseases the survival of their business, their income and the continuity of their farms is at stake. The choice as made in the past for the non-vaccination policy and, thus, for the depopulation strategy of the governments is a choice made on economical grounds to protect the farmers profit and to keep their rival position in exporting animal products. The benefit of the producers and their autonomy (*freedom of choice*) is, therefore, well protected and is justified in national and international (EU) legislation.

Consumers demand safe (animal) products for consumption for an acceptable price. It is for their benefit to keep animals under industrialized circumstances to guarantee low prices and in this perspective the non-vaccination policy is also to their benefit. On the other hand consumers or in a broader sense the society, is asking for respect for animal welfare. Societal acceptable treatment of animals with respect for animal lives and welfare is an important issue. An important societal justification to kill animals for disease control is human safety which is an important reason for legislation of disease control measures. When people can get infected and when these infections are a threat for human health or even for human lives the pre-emptive killing of healthy animals meets broad acceptance. This makes it clear that the reason why animals are killed, also within disease control, is important for the level of societal acceptance. Our morality is thus influenced by costs and benefits.

To carefully weight human and animal interest it is important to value the costs and benefits. The value that we give to the benefits of the different groups and the price we accept to pay depend on our own interests and will be different for producers, consumers and for non-consuming society (like vegetarians). Important factors that must be highly valued, however, are animal welfare and human safety. As it seems now no rational framework is available to weigh costs and benefits. It is rather an emotional reaction of society versus the interests of those involved in animal husbandry which determines the political strategy to be followed.

### **To put societal attitude in perspective**

The killing of poultry to stop infectious diseases like HPAI meets a lot of resistance in our western society, especially when alternatives like vaccination are in sight. Furthermore, the visibility of the depopulation of poultry farms makes people aware that large numbers of birds are killed in our society. In 2003 in The Netherlands 30 million birds were killed on-farm to stop the HPAI outbreak. In contrast; in The Netherlands every year approximately 300 million birds are raised and killed for food production (Den Hartog et al. 2003, CBS / LEI 2005). The killing of 30 million birds for disease control was reason for wide indignation whereas the killing of 300 million birds for food production is widely accepted.

From an animal welfare point of view it can be argued that, when using acceptable, well controlled methods, on-farm killing causes less suffering than the normal slaughter procedures that include catching, transportation, stunning and killing in the slaughterhouses. The period of compromised animal welfare can for on-farm killing be reduced to minutes and in worst case takes up to 30 minutes, whereas in the normal production chain the period between catching and killing take at least 4 hours and in periods up to 20 hours are no exception (Nijdam et al 2004). It is obvious that with on-farm killing animal welfare is less compromised than in the normal procedures for slaughter.

From an animal point of view it is not important why they are killed, it even can be questioned if the feelings or expectations of birds (and other sentient animals) are harmed by ending their lives (Singer 1979, Warren 1997). Since animals have no expectations of their live span ( no time awareness ) it is not wrong killing them but the way we kill them is what matters (Bracke 1990). What matters for individual animals (benefit their well-being) is that they are kept from redundant suffering. In regard to on-farm killing for disease control this implies that we should kill animals with a minimum of suffering as is possible. Our obligation in this is to put maximum effort in the development and use of acceptable killing methods.

To do right to societal and moral objections of humans to kill animals without any use, or when they are wasted, the implementation of alternatives like vaccination needs major attentions. However, it is important to realize that vaccination is not applicable in all cases. For instance; for sick and infected animals vaccination comes to late and killing, or in this case euthanasia, is the only opportunity. More important when it concerns broiler chickens ( > 50% of all farmed birds! ) vaccination against HPAI is not relevant because they live to short to be effectively protected by vaccination. Consequently, killing animals for disease control will remain a major intervention measure.

### **Concluding remarks and recommendations**

This thesis aimed to identify and in concept develop acceptable methods to kill poultry on-farm and preferable in their housings for the control of contagious poultry diseases. Acceptable at that moment meant, efficient killing procedures with respect for animal welfare, that are save to proceed and, with a minimal risk for spreading the disease. Outbreaks of HPAI world wide made it clear that there is a need for high capacity killing procedures for different situations. During the AI epidemic of 2003 in The Netherlands, whole house gassing techniques with CO<sub>2</sub> proved to be an efficient method with high capacity.

The results of this thesis support that whole house gassing with CO<sub>2</sub> is suited for all farmed poultry species with respect for birds' welfare. The duration of compromised animal welfare can be reduced to approximately 3 minutes for the individual animals when using optimal gassing techniques.

Also in respect of efficacy and human safety whole house gassing is the most preferred method for large scale on farm killing of poultry. Point of concern with whole house gassing however, is the level of control during procedure. It is evident that CO<sub>2</sub> levels and distribution in the barns must be monitored and controlled to guarantee optimal distribution of the gas.

There are situations in which whole house gassing is not possible. In these situations mobile gassing equipment and mobile electrocution devices appeared to be very useful. The handling of birds prior to killing, however, has a negative effect on birds' welfare, enlarges the risk of virus spreading and enlarges the risk of virus transmission to humans. Therefore, effort should be made to further develop mobile killing devices that can operate with minimal human – animal contact and that can be used in the poultry houses.

It is clear that animal welfare is compromised when killing them. However, when using well controlled on farm killing methods animal welfare is less affected than by catching and transporting them to the slaughterhouse.

To respect animal lives and in respect of the moral attitude of man, the non-vaccination policy should be reconsidered. However, we must realize that there will be situations that farms must be depopulated and therefore, even when it concerns a small number of farms or animals, we should provide maximum effort in further developing acceptable killing methods.

We must realize that the killing of large groups of animals and the non-vaccination policy is an economical policy to protect our export position and acceptable product prices, something that benefits both producers and consumers. From this viewpoint it can be stated that being against the killing of animals for disease control is being against the intensive way of producing animal products.

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## CHAPTER 7

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## Summary

## SUMMARY

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In our western communities the production of poultry products for consumption is an economical activity. For the production of eggs and poultry meat only in The Netherlands over 300 million birds are slaughtered every year. The production of poultry products is concentrated on large farms with high animal densities that are situated close together, resulting in areas with high poultry densities. Due to the high concentration of birds the introduction of contagious diseases, like for instance high pathogenicity avian influenza (HPAI), have devastating consequences for the producers, society and for the animals. For the protection of the industry the control of these contagious diseases is regulated in international guidelines and legislations. To protect the rival position of farmers and to protect the European export position, vaccination to control HPAI was excluded in these legislations. Therefore, the most important control measure to stop an outbreak of HPAI is to kill all animals of susceptible species on infected and suspected farms. This implies that large numbers of infected and probably healthy animals are killed on the farms. At the start of this project however, killing methods that had been tested for their suitability for large scale on farm killing were unavailable.

The aim of this thesis was to assess the possibilities for large scale on-farm killing of poultry during an outbreak of a contagious disease. Pre-limiting conditions were that methods must be efficient, safe to proceed, and acceptable from an animal welfare point of view. Moreover, a starting point from the point of animal welfare and epidemiology view, were that animals are preferably killed in their housings to avoid handling and transportation of possibly infected animals. Second condition is that animals can not escape or evade the procedure. Considering this, the use of gas mixtures for whole house gassing and in mobile (outdoor) devices was investigated.

In Chapter 2 we aimed to determine if broiler chickens would freely enter an atmosphere containing high levels of CO<sub>2</sub>, CO<sub>2</sub> mixed with O<sub>2</sub>, Argon, or a mixture of Argon with CO<sub>2</sub> when contacting these gas mixtures for the first time. Furthermore, the response of broilers inhaling these gas mixtures was assessed to determine animal welfare implications. Broilers were individually placed in a box that was connected to a descending tunnel, pre-filled with a) air, no flow (control-); b) circulating air, flowing (control+); c) >90% Ar in air; d) 60% CO<sub>2</sub> in air; e) 40% CO<sub>2</sub> and 30% O<sub>2</sub> in air and f) 70% Ar and 30% CO<sub>2</sub>. The tunnel with a perspex side was placed adjacent to the pen so birds could hear and see their companion animals that were used as the stimulus to enter the gas-filled tunnel. Since no difference between the groups was observed in the number of broilers that walked into the gas tunnel or in the number of birds that tried to return to the cage we concluded that there was no difference in aversion to the used gas mixtures. Continuous inhalation of 70% Ar and 30% CO<sub>2</sub> mixture resulted in the fastest loss of posture. The number of broilers exhibiting headshaking and gasping was least in

the >90% Ar in air mixture but convulsions were rarely seen in the 40% CO<sub>2</sub> and 30% O<sub>2</sub> mixture, whereas the other gas mixtures resulted in severe convulsions. The experiment did not indicate that broilers would avoid increased CO<sub>2</sub> or decreased O<sub>2</sub> levels when they come into contact with such atmospheres for the first time.

The gas mixtures and conditions described in Chapter 2 have initially developed for stunning poultry and to be used in pre-filled systems. In Chapter 3 we aimed to investigate the suitability of gas mixtures for the euthanasia of broilers in their housings by gradually increasing the percentage of CO<sub>2</sub> in the breathing air. Three gas mixtures, a) 100% CO<sub>2</sub>, b) 50% N<sub>2</sub>+50% CO<sub>2</sub> and c) 30% O<sub>2</sub>+40% CO<sub>2</sub>+30% N<sub>2</sub> followed by 100% CO<sub>2</sub> were injected in the test situation for 15 minutes and the achieved CO<sub>2</sub> concentration was maintained for an other 15 minutes. To assess the level of discomfort before loss of consciousness and the killing rate, groups of 20 broiler chickens per trial were exposed to the increasing CO<sub>2</sub> concentration. At the same time 2 broilers per trial equipped with brain electrodes were observed for behaviour and brain activity. The highest level of CO<sub>2</sub> in the breathing air was reached with the 100% CO<sub>2</sub>-mixture, 42% against 25% for the other two mixtures. In all three-gas mixtures head shaking, gasping and convulsions were observed before loss of posture. Suppression of electrical activity of the brain and loss of posture occurred almost simultaneously at approximately 17% CO<sub>2</sub> in the breathing air, indicating that loss of posture is a useful parameter for unconsciousness. Ten percent of the 2-week-old broilers survived the 30% O<sub>2</sub>+40% CO<sub>2</sub>+30% N<sub>2</sub>-mixture and therefore this mixture was excluded for further testing at six weeks of age. At 6 weeks of age 30% of the broilers survived the mixture of 50% N<sub>2</sub> with 50% CO<sub>2</sub>. The results of this experiment indicate that euthanasia of groups of broilers in a condition where the normal atmosphere is gradually displaced by CO<sub>2</sub> is well possible. However, gasping and head shaking are observed before loss of posture and also the occurrence of convulsions before loss of consciousness can occur. To prevent possible discomfort a gradual increase to an anaesthetic level of 17% CO<sub>2</sub> in the breathing air, using 100% CO<sub>2</sub> can be recommended. To kill practically all animals levels of at least 40% CO<sub>2</sub> in the breathing air, maintained for 30 minutes are necessary.

The avian influenza outbreak of 2003 in The Netherlands made the experiments as described in Chapter 2 and 3 to become real. During this outbreak, the disease was controlled by destroying all the poultry in large areas. In total 30 million birds were killed on 1242 farms and in more than 17000 hobby flocks, by using mobile containers filled with carbon dioxide, mobile electrocution lines and whole house gassing with CO and with CO<sub>2</sub>. Observations of these methods as described in Chapter 4 were used to compare their effectiveness, and capacity, and their effects on the welfare of the birds. Culling of flocks of chickens in different types of housings by a gradually increase of CO<sub>2</sub> was frequently applied during this epidemic. It appeared to be well possible to increase CO<sub>2</sub> concentrations in the

breathing air in large barns up to 40% air in approximately 30 minutes. Gassing whole poultry houses had a much greater capacity than mobile equipment. Furthermore, catching live birds to bring them to a mobile killing device caused extra stress and could cause pain due to injuries inflicted when catching and handling them. However, whole-house gassing is not suited for all types of housing, and under such circumstances mobile killing devices were a useful alternative. The characteristics and availability of the different methods enables to choose the most suitable method for each poultry farm. The choice of the method should be based on the size of the farm, for example, high capacity equipment for large farms, and on the type of housing. Although whole-house gassing appeared to be effective there are points for concern. During the epidemic there were few controls on the procedure. Little information was generated about the gas flows and build-up of its concentration. To reduce the birds' suffering to a minimum it is essential that the killing procedure is carefully controlled. This control should be based on physiological and behavioural data derived from birds' at the most critical places in the system or animal house. Furthermore, due to sometimes large numbers of survivors there were questions about the suitability of whole house gassing for ducks and turkeys. However, it is not clear if this was because of different conditions or due to that they are less susceptible to CO<sub>2</sub>.

In Chapter 5 physiological responses of White Pekin ducks and turkeys to increasing CO<sub>2</sub> concentrations are described. Blood gas values showed similar reaction patterns for both species: a strong increase in pCO<sub>2</sub> from approximately 40 mmHg to 200 mmHg, decreasing pO<sub>2</sub> and O<sub>2</sub> saturation, a decline in pH from 7.4 to 6.7 and a strong shift in acid base equilibrium (averaging 0 to -23). On the EEG,  $\theta$  and  $\delta$  waves occurred at 21-23% CO<sub>2</sub> and suppression to a near iso-electric EEG between 41.8 and 43.4% CO<sub>2</sub> in inhaled air. Heartbeat declined from approximately 300 bpm at the start to 225 bpm at loss of posture to 150 bpm one min. before heartbeat ceased. During the last phase of heart activity an irregular rhythm and fibrillation were observed in addition to a decline in beats per min. Blood gas values and electrophysiological data confirmed that ducks and turkeys lose consciousness before a level of 23% CO<sub>2</sub> in inhaled air is reached and that both ducks and turkeys die within 13 min in 45% CO<sub>2</sub> in inhaled air. The conditions, 40% CO<sub>2</sub> maintained for 30 min., as stated earlier for chickens may be critical for ducks and turkeys. Therefore, to kill all farmed poultry species it is recommended that a minimal concentration of 45% CO<sub>2</sub> in inhalation air be maintained for 30 minutes.

Aim of Chapter 6 was to assess the effects of slowly increasing CO<sub>2</sub> concentrations on animal welfare. Behaviour of broilers, ducks, laying hens and turkeys was observed to identify differences in susceptibility between species and to assess animal welfare implications. All birds were individually exposed to CO<sub>2</sub> concentrations which increased from 0 to 45% with a speed of 14l/min (comparable

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with whole house gassing in practice). Heavier breathing, gasping and headshaking were observed in all species and started 2-3 minutes before loss of posture. Convulsions were observed in broilers, ducks, and laying hens but not in turkeys. The convulsions occurred mainly after loss of posture, only 1 broiler and 2 ducks showed convulsions before loss of posture. At approximately 24% CO<sub>2</sub> loss of posture occurred in ducks and at approximately 19.5% CO<sub>2</sub> in the other groups. It is obvious that killing animals always conflicts with animal welfare. To make a decision in acceptability of a killing method all aspects have to be considered. Advantages of whole house gassing are the large capacity and the fact that animals don't have to be handled alive. These advantages are important enough to make whole house gassing, if executed properly, an acceptable method in emergency disease control for all farmed poultry species.

In Chapter 7 the results of this thesis are discussed in regard of efficacy, animal welfare and humane safety aspects of the different killing procedures. Societal and moral aspects are discussed from an ethical view point and considered in perspective with the general accepted production of animal products.

For effective disease control in a densely populated area large killing capacity is required to stop the disease. Whole house gassing has a large capacity, is used in the barns and there is minimal contact of human with possibly infected animals. It is unmistakable that during slowly increasing CO<sub>2</sub> concentrations, unavoidable with whole house gassing, breathlessness and agitation occur during a period of approximately 2-3 minutes. When killing animals in mobile equipment however, the duration of compromised animal welfare is not seldom over 5 minutes due to catching, handling, and the killing procedure. Therefore, considering the duration and level of compromised birds' welfare, whole house gassing is especially on larger farms preferable above methods that involve handling of living animals. Distribution of the gas and temperature development in the barn however is point of concern and should be well controlled.

Killing large numbers of animals for other reasons than for human consumption meets a lot of societal resistance especially when alternatives like vaccination are probably available. That the non-vaccination policy is based on economical grounds, to protect our export position is not fully recognized. Also the fact that on farm killing is causing less harm for the birds than when they are handled and transported to slaughterhouses is not recognized in our society. Our moral attitude depends strongly on our own benefits and not on that of the animals involved. Therefore it can be stated that; being against large scale killing for disease control is being against the intensive way we are producing animal products.

## Samenvatting

De productie van pluimvee producten is in westerse landen een belangrijke economische activiteit. Alleen al in Nederland worden voor de productie van eieren en vlees jaarlijks 300 miljoen kippen, gefokt en geslacht. De productie van deze pluimveeproducten is voornamelijk geconcentreerd op grote bedrijven die dicht bij elkaar liggen, met veel dieren per m<sup>2</sup> met als gevolg gebieden met een hoge pluimvee dichtheid. Door deze hoge concentratie dieren in een beperkt gebied hebben uitbraken van besmettelijke dierziekten, zoals hoog pathogeen Aviaire influenza (HPAI), ernstige consequenties voor pluimvee, de pluimveehouders en de gemeenschap. Om de pluimvee-industrie te beschermen tegen de gevolgen van een uitbraak, is de bestrijding van besmettelijke dierziekte geregeld in nationale en internationale wetgeving. In de huidige Europese wetgeving is, om voornamelijk economische redenen, vaccinatie tegen HPAI niet of beperkt toegestaan. De belangrijkste bestrijdingsmaatregel bij een uitbraak van HPAI is daarom het doden van alle AI gevoelige dieren op besmette en verdachte pluimveebedrijven. Dit heeft tot gevolg dat er tijdens een uitbraak van HPAI grote aantallen besmet, van besmetting verdacht en gezond pluimvee wordt gedood. Voor de start van dit onderzoek was er geen onderzoek bekend naar de geschiktheid van methoden voor het op grote schaal doden van pluimvee op het pluimveebedrijf

Het doel van deze studie was om de mogelijkheden te onderzoeken voor het doden van pluimvee op het bedrijf. Belangrijke voorwaarde was dat de methoden efficiënt zijn, veilig zijn uit te voeren, en acceptabel zijn vanuit het oogpunt van dierenwelzijn. Uitgangspunt vanuit zowel dierenwelzijn als vanuit epidemiologisch oogpunt was dat de dieren bij voorkeur in de stal worden gedood waardoor vangen, hanteren en transporteren van mogelijk besmet pluimvee wordt vermeden. Een volgende belangrijke voorwaarde was dat dieren niet kunnen ontsnappen of zich niet kunnen onttrekken aan de methode. Vanuit deze voorwaarden is gekozen om de mogelijkheden van stalvergassen en het doden met gas in mobiele apparatuur te onderzoeken.

In hoofdstuk 2 is onderzocht of vleeskuikens vrijwillig een ruimte betreden die is gevuld met een hoge concentratie CO<sub>2</sub>, CO<sub>2</sub> met O<sub>2</sub>, of met een mengsel van CO<sub>2</sub> en Argon. Daarnaast is, om de gevolgen op hun welzijn te beoordelen, de reactie van de vleeskuikens op het inademen van deze gasmengsels bestudeerd. Vleeskuikens werden individueel in een box geplaatst die was verbonden met een tunnel gevuld met a) stilstaande lucht (negatieve controle), b) circulerende lucht (positieve controle), c) >90% Ar, d) 60% CO<sub>2</sub>, e) 40% CO<sub>2</sub> + 30% O<sub>2</sub>, of f) 70% Ar + 30% CO<sub>2</sub>. Door een perspex wand aan het eind van de tunnel konden de dieren hun soortgenoten zien en horen waardoor de dieren werden gestimuleerd om de tunnel te betreden. Het feit dat er geen verschil was in het aantal dieren dat de gas tunnel in ging en ook niet in het aantal dieren dat probeerde terug te keren naar de box leidde tot de conclusie dat er geen verschil in aversie is tussen de verschillende gasmengsels.

De inhalatie van 70% CO<sub>2</sub> met 30% O<sub>2</sub> leidde het snelst tot bewusteloosheid. In het mengsel met 90% argon kwamen kopschudden en zwaar ademen het minst voor. Spierkrampen kwamen bijna niet voor bij dieren in het 40% CO<sub>2</sub> + 30% O<sub>2</sub> mengsel terwijl in de andere gasmengsels (uitgezonderd de controle groepen) dieren ernstige spierkrampen vertoonden.

Belangrijkste conclusie uit dit hoofdstuk is dat er geen reden is om aan te nemen dat vleeskuikens een atmosfeer met een hoog CO<sub>2</sub>% of een laag O<sub>2</sub>% vermijden wanneer ze voor de eerste keer in contact komen met deze omstandigheden. Het is echter duidelijk dat bewusteloosheid niet direct intreedt en dat het welzijn van de dieren wordt beïnvloed. Impact op het dierenwelzijn kan op basis van dit experiment niet worden geclassificeerd.

De gasmengsels zoals gebruikt in hoofdstuk 2 zijn in eerste instantie ontwikkeld voor het verdoven van pluimvee in het reguliere slachtproces en worden gebruikt in voorgevulde systemen. In hoofdstuk 3 is onderzocht of deze of hiervan afgeleide gasmengsels ook geschikt zijn voor het doden van dieren in de stal, en dus bij een geleidelijke toename van de CO<sub>2</sub> concentratie in de inademinglucht. Om de mate van ongerief te onderzoeken zijn in een experimentele opzet groepen vleeskuikens, bij een bezettingsgraad die vergelijkbaar is met de praktijk (20 dieren per m<sup>2</sup>), blootgesteld aan geleidelijk toenemende CO<sub>2</sub> concentraties. Gelijktijdig is bij 2 dieren per experiment de hersenactiviteit (EEG) gemeten. Gedurende 15 minuten zijn drie verschillende gasmengsels, a) 100% CO<sub>2</sub>, b) 50% N<sub>2</sub>+50% CO<sub>2</sub> and c) 30% O<sub>2</sub>+40% CO<sub>2</sub>+30% N<sub>2</sub>, met een snelheid van ca 14 liter per min. geïnjecteerd in de testruimte.

Het hoogste percentage CO<sub>2</sub> werd bereikt in de 100% CO<sub>2</sub> groep, nl. 42% tegen gemiddeld 25% in de andere 2 groepen. Suppressie van het EEG signaal en 'omvallen' trad nagenoeg gelijktijdig op bij gemiddeld 17% CO<sub>2</sub> in de inademinglucht. Dit indiceert dat 'omvallen' een bruikbare gedrags parameter is voor het intreden van bewusteloosheid. Voor 'omvallen' werden in alle groepen kopschudden, zwaar ademen en spierkrampen waargenomen. Bij 40% CO<sub>2</sub> in de inademinglucht werden geen levende dieren gevonden terwijl bij 25% CO<sub>2</sub> na 30 minuten nog 10 tot 30% levende dieren werden aangetroffen.

Uit dit experiment is duidelijk geworden dat het goed mogelijk is vleeskuikens met een langzaam oplopend CO<sub>2</sub>% te doden. Echter, zwaar ademen, kopschudden en spierkrampen treden op voor verlies van bewusteloosheid. Om onnodig lijden te voorkomen is het noodzakelijk geleidelijk, maar voldoende snel, een concentratie van 17% CO<sub>2</sub> te bereiken. Om alle dieren te doden is ten minste 40% CO<sub>2</sub> in de inademinglucht gedurende 30 minuten nodig.

De Nederlandse uitbraak van HPAI in 2003 heeft een belangrijke richting aan dit onderzoek geleverd. Om deze uitbraak te controleren zijn 30 miljoen dieren (pluimvee) gedood, verspreid over 1242 commerciële bedrijven en van meer dan 17.000 hobby dierhouders. Voor het doden van commercieel gehouden pluimvee zijn mobiele gascontainers van verschillende afmetingen, mobiele slachtlijnen en

stalvergassen ingezet. Observaties van verschillende methoden zijn beschreven in hoofdstuk 4 waarbij de aandacht vooral uit is gegaan naar effectiviteit, capaciteit en dierenwelzijn. Verreweg het grootste aantal dieren is gedood door stalvergassing met CO<sub>2</sub> gevolgd door stalvergassing met CO. Het bleek heel goed mogelijk om in grote pluimveestallen in circa 30 minuten een concentratie van 40% CO<sub>2</sub> te realiseren. Voorkeur voor het gebruik van CO<sub>2</sub> boven CO is gebaseerd op vooral de veiligheids risico's bij het gebruik van CO. Groot voordeel van stalvergassen boven het gebruik van mobiele apparatuur is het feit dat dieren niet worden gevangen en gehanteerd waardoor angst, stress en verwondingen door vangers worden voorkomen. Echter, niet in alle gevallen is stalvergassen mogelijk. Stallen met teveel lekkage, en zeer kleine stallen kunnen beter met mobiele apparatuur geruimd worden. Belangrijk aandachtspunt bij stalvergassen blijft de controleerbaarheid van het proces. Tijdens de AI epidemie is te weinig informatie verzameld over het overlevingspercentage van de verschillende pluimveesoorten en van de gas verdeling in de stallen. Om dierenleed tot een minimum te beperken is het noodzakelijk om het proces te controleren op de meest kritische plaatsen in de stallen en mogelijk bij te sturen.

Tijdens de AI epidemie van 2003 bleek het niet altijd goed mogelijk om eenden en kalkoenen te doden door middel van stalvergassen met CO<sub>2</sub>. Het was echter niet duidelijk of hieraan fysiologische redenen ten grondslag liggen of dat de uitvoering van de methode of de verschillen in huisvesting hiervoor reden waren. In hoofdstuk 5 is de fysiologische respons van eenden en kalkoenen op verhoogde CO<sub>2</sub> concentraties beschreven. Resultaten uit dit onderzoek laten zien dat er tussen eenden en kalkoenen geen verschillen in fysiologische reacties (bloedgas waarden, EEG's en ECG's) zijn op sterk verhoogde CO<sub>2</sub> concentraties in de inademinglucht. Belangrijke fysiologische verandering is de sterke verzuring in het bloed (dalende pH van 7.4 naar 6.7 en ABE van 0 naar -23) die leidt tot een onomkeerbaar proces. Gevolgen hiervan zijn suppressie van het EEG en ECG en hartritme stoornis gevolgd door hartstilstand. Op basis van deze resultaten kan worden geconcludeerd dat eenden en kalkoenen op een zelfde manier als vleeskuikens kunnen worden gedood doormiddel van stalvergassen met CO<sub>2</sub>.

Het doden van dieren heeft altijd gevolgen voor het dierenwelzijn. In hoofdstuk 6 is het gedrag van vleeskuikens, leghennen, eenden en kalkoenen beschreven met als doel inzicht te krijgen in welke mate hun welzijn tijdens stalvergassen met CO<sub>2</sub> wordt aangetast. Belangrijk onderscheid is of reacties plaats vinden voor of na het verlies van bewusteloosheid. Zwaar ademen en kopschudden waren de eerste reacties die gerelateerd worden met ongerief gevolgd door tekenen van benauwdheid. Deze reacties starten vanaf 3 minuten voordat bewustzijnsverlies intreedt. De duur van het ongerief was in dit experiment dan ook maximaal 3 minuten, immers bij terminale experimenten is er na bewustzijnsverlies geen invloed meer op het welzijn. Spierkrampen werden gezien na bewustzijnsverlies en hebben daarom geen impact op het dierenwelzijn. De in dit experiment

gerealiseerde CO<sub>2</sub> opbouw (concentratie en tijdsduur) is vergelijkbaar met mogelijke CO<sub>2</sub> opbouw in praktijkstallen. Het is dus mogelijk om ook in de praktijk het ongerief voor de dieren te beperken tot een periode van ca 3 minuten. Echter, zeker bij grote en middelgrote stallen is te verwachten dat de duur van het ongerief beduidend minder is dan het ongerief dat gepaard gaat met vangen en hanteren van dieren zoals nodig is bij het gebruik van mobiele apparatuur.

Voor een effectieve bestrijding van besmettelijke dierziekten zoals AI is het van groot belang dat de toegepaste methoden een grote capaciteit hebben. Stalvergassen is een methode met een dergelijk grote capaciteit. Daarnaast heeft stalvergassen als groot voordeel dat het niet nodig is om levende dieren te vangen en te hanteren waardoor risico's voor aantasting van het dierenwelzijn minder worden. Het niet hanteren van levende dieren vermindert het intensieve contact tussen mens en dier waardoor mogelijke risico' voor de humane gezondheid afnemen. Het is echter duidelijk dat ook bij stalvergassen een periode van ongerief voor de dieren ontstaat waarin benauwdheid en angst waarschijnlijk de belangrijkste negatieve effecten zijn. Belangrijke aandachtspunten voor de toekomst bij stalvergassen is de verdeling van het gas in de stalruimte. Een goede controle en bijsturing van het proces door middel van adequate meting van de gasconcentratie tijdens het proces is van groot belang om snel voldoende hoge concentraties op dierniveau te bereiken.

Het doden van dieren voor andere doeleinden dan consumptie zoals bij dierziektebestrijding roept grote maatschappelijke weerstand op, vooral als een alternatief als vaccinatie mogelijk beschikbaar is. Het is echter van belang om ons te realiseren dat het non vaccinatie beleid een beleid is om onze export positie te behouden en te zorgen voor laag geprijsde producten. Het feit dat vangen, hanteren en transporteren van pluimvee naar een slachterij waarschijnlijk meer ongerief veroorzaakt dan het doden van de dieren op het bedrijf lijkt geen grote rol te spelen in onze afweging. Onze morele opvatting in deze is sterk afhankelijk van het nut en of het risico dat het handelen voor ons betekend en niet wat het risico voor het dier is.



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## Curriculum Vitae

Marien Gerritzen werd op 6 augustus 1964 geboren in Heteren en groeide op in Lelystad. In 1980 behaalde hij het MAVO diploma aan de Christelijke scholengemeenschap "De Brug" in Lelystad. Daarna doorliep hij de middelbare agrarische school in Emmeloord, waar hij in 1984 zijn vakdiploma veehouderij behaalde. Op 1 mei 1986 werd hij aangesteld bij het landbouwkundig onderzoek. Tot 1997 was hij werkzaam als biotechnicus bij achtereenvolgens het Instituut voor Veeteeltkundig Onderzoek, het Centraal Diergeneeskundig Instituut en het Instituut voor Veevoedings Onderzoek, allen in Lelystad. Van september 1992 tot januari 1997 werd naast zijn werk de HLO opleiding medische biologie gevolgd aan de Hogeschool Utrecht, die werd afgerond met het behalen van het diploma in de differentiatie proefdierkunde. Vanaf januari 1997 is hij werkzaam als hbo onderzoeker op het gebied verdoven en doden van dieren bij het toenmalige ID-Lelystad, dat nu onderdeel is van de Animal Sciences Group van Wageningen UR. Begin januari 2002 werd begonnen aan het onderzoek dat is beschreven in dit proefschrift.



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