Report 199

Behaviour and physiology of day-old chicks during the CO_2 killing method in Dutch hatcheries

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Uitgever
Animal Sciences Group van Wageningen UR
Postbus 65, 8200 AB Lelystad
Telefoon 0320 - 238238
Fax 0320 - 238050
E-mail Info.veehouderij.ASG@wur.nl
Internet http://www.asg.wur.nl

Redactie
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Abstract
Results of an inventory in Dutch hatcheries are described about killing methods. Also an experiment has been conducted to determine behavioural and physiological responses during exposure of day-old chicks to 60 or 80% CO_2. Recommendations are made about the application of CO_2 killing methods with the least possible negative effects on animal welfare.

Keywords
Killing, day-old chicks, methods, CO_2, practice, behaviour, physiology.

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Auteur(s)
A. Lourens
M.A. Gerritzen

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Samenvatting
De resultaten van een inventarisatie worden gepresenteerd over dodingmethoden voor eendagskuikens in Nederlandse kuikenbroederijen. Een experiment is uitgevoerd om het gedrag en fysiologie tijdens blootsteling aan 60% of 80% CO_2 te bepalen. Aanbevelingen worden vervolgens gedaan over het gebruik van CO_2 dodingmethoden waarbij het negatieve effect op dierenwelzijn zo klein mogelijk zal zijn.

Trefwoorden
Doden eendagskuikens, methoden, CO_2, praktijk, gedrag, fysiologie

ANIMAL SCIENCES GROUP
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Report 199

Behaviour and physiology of day-old chicks during the CO_2 killing method in Dutch hatcheries

Sander Lourens
Marien Gerritzen

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Preface

Commercial poultry production is strongly specialized throughout the world. There are breeds that are specialized in laying eggs, others in producing meat. Because the males of layer breeds do not produce eggs and are not profitable in producing meat, they are selected and killed (killed) when one day-old. This happens throughout the world. In The Netherlands 30 million chicks are killed each year. In general they are utilized as feed for zoo animals, reptiles, birds of prey, cats etc.

Killing of day-old chicks is controversial. Alternatives are not yet available. The Dutch Ministry of Agriculture, Nature and Food quality therefore initiated research towards alternatives. Within this project societal acceptability of possible alternatives was examined by a public enquiry. Not all alternatives were ranked higher than accepting the current practice of killing chicks. For instance, killing of embryo's ranked lower that the current practice, probably because the chicks can be utilized as animal feed. If chicks are killed, this has to be done with minimal discomfort for the animals involved. As the current practice of killing day-old male chicks will be in use for several years, the Dutch ministry decided to have an inventory of the methods applied in Dutch hatcheries and to examine their effectiveness. This as part of a large project to examine discomfort among farm animals during slaughter and large scale killing in case of outbreaks of contagious diseases. This report describes how killing of day-old chicks is carried out in Dutch hatcheries. Also the results of laboratory experiments on behaviour and physiology during the process of killing are given, in order to underpin effectiveness of methods used in practice.

Summary

An inventory was made in Dutch hatcheries to describe the methods to kill undesired day-old chicks. For methods using CO_2 gas, duration of exposure and CO_2 concentration during the process were measured. Additionally, behavioural and physiological responses of day-old chicks to different CO_2 concentrations during killing were measured in a controlled experimental setting. Aim of this report is to determine the duration and extent of discomfort caused by killing of day-old chicks in practice, and to determine the minimal demands in terms of exposure time and CO_2 concentration to ensure a minimal reduction in animal welfare.

Common practice of killing day-old chicks
All Dutch layer hatcheries kill day-old male chicks using CO_2 methods. Since 2009, all layer hatcheries apply automatic machines to kill day-old male chicks. These chicks are a valuable by-product to feed carnivorous animals in captivity. Compared to the number of killed day-old chicks in layer hatcheries, the numbers of killed day-old chicks in layer great grand parents stock (GPS) and parent stock (PS) hatcheries are low. Broiler hatcheries kill second grade day-old chicks using either CO_2 methods or a shredder, depending on retailer demands. The undesired female or male chicks in GPS and PS hatcheries related to the broiler industry are not killed, but sold alive as broilers. Despite the variety in methods, exposure time and CO_2 concentrations, all methods applied in the Dutch hatcheries were equally effective: no day-old chicks recovered afterwards. Proper handling of the killing equipment by hatchery personnel appears to be more important for animal welfare than the method used.

Behaviour and physiology
When chicks are exposed to CO_2 gas, a certain discomfort can be observed as gasping or heavy breathing, followed by neck stretching or head shaking and jumping movements. There were no differences in the timing that chicks lost posture, when they were placed in either 60% or 80% CO_2. After loss of posture, heavy breathing continued although on a lower frequency. After loss of posture, convulsive movements with wings and legs were observed longer in day-old chicks exposed to 60% than to 80% CO_2. When day-old chicks were exposed to 80% compared to 60% CO_2, an ECG silence occurred sooner, EEG suppression occurred sooner and faster, and minimal brain activity occurred sooner. Chicks are able to recover after exposure to 60% CO_2 for 10 minutes. Based on literature, it is likely that the welfare of day-old chicks is reduced during the phase of head shaking and jumping. The duration of reduced welfare however is only short (13-16 seconds) and the probability on recovery is very low after exposure of day-old chicks to 80% CO_2 for at least 3 minutes.

It can be concluded that the CO_2 method is an acceptable device to kill day-old chicks. The equipment used should be prefilled with CO_2 gas to induce unconsciousness as soon as possible, and the minimal CO_2 concentration should reach at least 80%. The minimal exposure duration at 80% CO_2 should be at least 3 minutes to suppress EEG signals. With some modifications, all Dutch hatcheries should be able to fulfil these requirements.

Samenvatting

Een inventarisatie is gehouden bij Nederlands kuikenbroederijen om de methoden te beschrijven waarbij de niet bruikbare haankuikens en tweede soort eendagskuikens worden gedood. Van de methoden waarbij CO_2 gas werd gebruikt, werd de blootstellingduur en gasconcentratie bepaald. Aanvullend werden de gedragingen en fysiologische reacties van eendagskuikens op verschillende CO_2 concentraties vastgesteld in een gecontroleerde experimentele proefopzet. Het doel van dit onderzoek is om de tijdsduur vast te stellen waarbij eendagskuikens een verminderd welzijn ondervinden van de gasdodingsmethode, en om de minimale voorwaarden vast te stellen in termen van blootstellingduur en gasconcentratie waarbij dit verminderde welzijn zo veel mogelijk wordt beperkt.

Doden van eendagskuikens in de praktijk
In alle Nederlandse legbroederijen worden de ongewenste haankuikens gedood door middel van een CO_2 gasdodingsmethode. Deze dode eendagshaantjes zijn een waardevol bijproduct dat aftrek vindt als voer voor vleesetende dieren in onder andere dierentuinen en vogelcentra. Vergeleken met deze legbroederijen, is het aantal ongewenste kuikens dat geboren wordt in de (groot) ouderdierbroederijen voor de legsector laag. Vleeskuikenbroederijen doden alleen de tweede soort kuikens die niet als vleeskuiken kunnen worden geplaatst bij een mester door middel van een CO_2 gasdodingsmethode of een shredder (afhankelijk van de eisen van retailorganisaties). De ongewenste kuikens in de (groot) ouderdierbroederijen in de vleessector worden niet gedood, maar als vleeskuikens opgezet bij mesters. Ondanks de grote variatie in gebruikte methoden, blootstellingduur en CO_2 concentraties, waren alle methoden even effectief: er kwamen na afloop van de procedure geen kuikens meer bij bewustzijn.

Gedrag en fysiologie
Wanneer eendagskuikens worden blootgesteld aan CO_2 gas, dan kan een zekere mate van stress worden waargenomen zoals het happen naar lucht, zwaar ademen, nekstrekken, kopschudden, en springgedrag. Bij een minimale CO_2 concentratie van 20% wordt bewusteloosheid zo snel mogelijk geïnduceerd. Er waren geen verschillen in het moment waarop de eendagskuikens buiten bewustzijn raakten bij verschillende CO_2 concentraties. Zwaar ademen hield hierna aan, maar op een lagere frequentie. Met name stuiptrekkingen van vleugels en poten werden langer waargenomen bij eendagskuikens die werden blootgesteld aan 60% CO_2 dan aan 80% CO_2. Wanneer eendagskuikens werden blootgesteld aan 80% CO_2 in plaats van 60% CO_2, dan werd er eerder een ECG stilte waargenomen, het EEG werd eerder en sterker onderdrukt, en minimale hersenactiviteit trad eerder in. Eendagskuikens kunnen bijkomen nadat ze gedurende 10 minuten zijn blootgesteld aan 60% CO_2. Op basis van de gedragswaarnemingen is het waarschijnlijk dat het welzijn van de eendagskuikens in geringe mate negatief wordt beïnvloed bij het doden met CO_2. De tijdsduur van dit verminderde dierenwelzijn is echter kort (13-16 seconden) en de kans op bijkomen in minimaal wanneer de eendagskuikens worden blootgesteld aan minimaal 80% CO_2 voor ten minste 3 minuten. Wij concluderen dat de CO_2 dodingmethode een geaccepteerde methode is voor eendagskuikens, waarbij het welzijn van de eendagskuikens kort verminderd is. Deze methode moet bij aanvang een minimale CO_2 concentratie bieden van 20% om bewusteloosheid zo snel mogelijk te induceren. Hierna moet de CO_2 concentratie snel worden opgevoerd naar minimaal 80% om de EEG voldoende te onderdrukken, waarin de kuikens minimaal 3 minuten moeten verblijven. Met enkele aanpassingen voor wat betreft tijdsduur en CO_2 concentratie kunnen alle Nederlands broederijen aan deze eisen voldoen.

Contents

1 Introduction

Recently, much attention has been paid to the general discomfort of the Dutch society to the killing of undesired male chicks born in layer hatcheries. The technological perspectives that may bring future solutions for this problem are summarized in Woelders et al. (2007), whereas the opinions of the general public on the killing of day-old chicks and possible alternatives are discussed in Leenstra et al. (2008), using focus groups and an internet questionnaire. The exact methods used in practice in terms of exposure duration and CO_2 concentration were still unknown, and a lack of knowledge exists on the behavioural and physiological responses of day-old chicks to different CO_2 concentrations during killing. Therefore, an inventory was made in Dutch hatcheries on the methods applied. Next, experiments were carried out to determine behavioural and physiological responses of day-old chicks to CO_2 concentrations used in practice. Aim of this paper is to determine the duration of reduced animal welfare during killing of day-old chicks in practice, and to determine the minimal preconditions in terms of exposure time and CO_2 concentration to ensure the lowest duration of reduced animal welfare.

2 Observations in practice

2.1 Measurements

CO_2 concentrations were measured using an OXYBABY V as shown in Picture 1. Data were retrieved using Witt-logger software and stored in Microsoft Excel. The OXYBABY V was always placed between the chicks, to measure the CO_2 concentrations that chicks were exposed to during the killing process.

Picture 1. The OXYBABY V CO2 measuring device.

2.2 Methods used by hatcheries

The different methods used in Dutch hatcheries are summarized in Table 1. Only one layer hatchery used a self made device at the time of the visit. This hatchery already purchased an automatic machine that was to be installed in January 2009. At the time of publishing this report, all Dutch layer hatcheries apply a automatic machine to euthanize their day-old male chicks. Broiler hatcheries apply different methods to euthanize second grade chicks that are selected by hand from the first grade chicks. One hatchery used plastic bags, 2 hatcheries used containers, 1 hatchery had a automatic machine, and 5 hatcheries used a shredder. All broiler hatcheries preferred a CO_2 killing method, but were forced to use a shredder when they supply to English retail organizations. The GPS and PS hatcheries that hatch the grandparents and parents of the broilers are often able to sell their undesired chicks or by-products to broiler hatcheries. This is obviously not the case for layer by-products.

Table 1. Methods for killing of day-old chicks in Dutch layer, broiler, and (great) grand parent stock hatcheries.

	CO_2 method				Other method	By-products	
	Plastic bags	Container	Automatic Machine	Self made device	Shredder	Dead	Alive
Layer hatcheries							
1			X			X	
2			X			X	
3				X		X	
4			X			X	
5			X			X	
Broiler hatcheries							
1		X					
2					X		
3			X		X		
4					X		
5					X		
6	X				X		
7		X					
(great) grand parent stock hatcheries							
1					X		X
2	X						X
3					X		X
4	X				X		X
5	X						X
6				X			
7		X					
Total	**4**	**3**	**5**	**2**	**8**	**5**	**5**

2.3 CO_2 concentrations and exposure time

2.3.1 Plastic bags

In picture 2, a general device for killing of day-old chicks using plastic bags and CO_2 is shown. In Figure 1, the CO_2 and O_2 concentrations during killing of day-old chicks in plastic bags are shown. The bag was first filled with CO_2 and the chicks were placed in the bag, 30 seconds after filling the bag when CO_2 concentration reached 47% After 70 seconds, CO_2 concentration reached 60% and gradually increased until a maximum value of 74%. The procedure lasted for 440 seconds, and CO_2 concentration remained close to 70%. No chicks recovered within 30 minutes afterwards.

Picture 2. Plastic bag and CO_2.

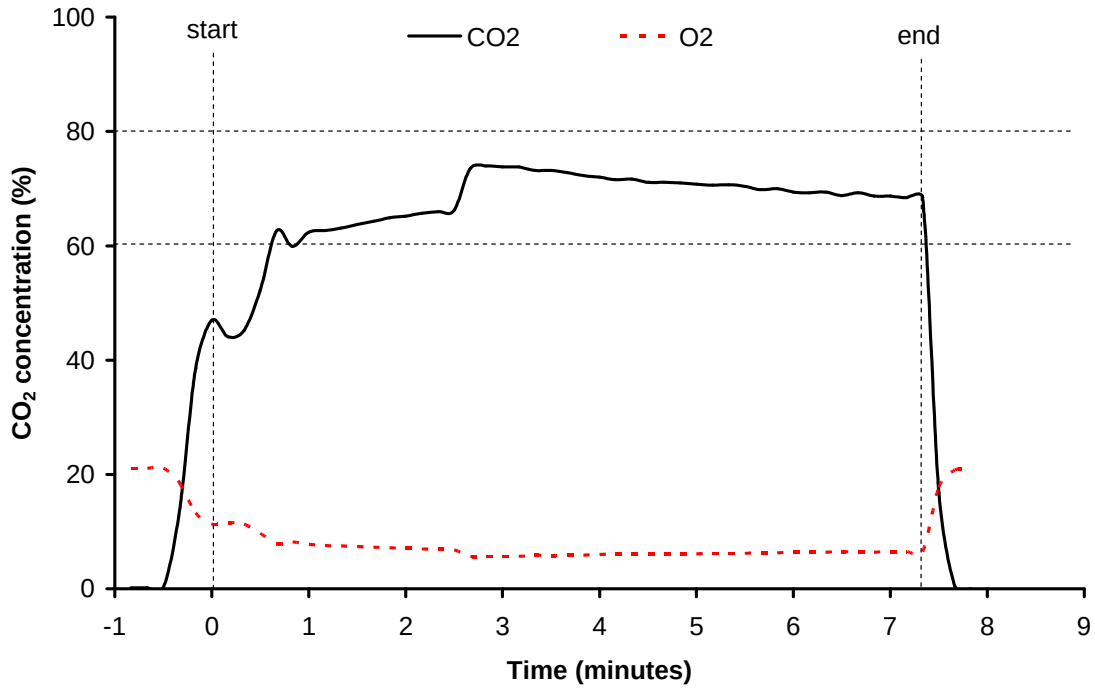

Figure 1. CO_2 and O_2 concentrations during killing of day-old chicks in plastic bags.

2.3.2 Containers

In picture 3, a general device for killing of day-old chicks using a container and CO_2 is shown. In Figures 2 and 3, the CO_2 and O_2 concentrations during killing of day-old second grade chicks in containers are shown. In Figure 2, the container was empty (not prefilled with CO_2) when the chicks were placed, in Figure 3, the container was prefilled with CO_2 before placement of the chicks.

Figure 2 shows that it takes about 11 minutes before CO_2 concentration reached over 60%, when the container was not prefilled with CO_2. chicks were held five more minutes at CO_2 concentrations between 75 and 80%, with a maximum concentration of 83% (Figure 2). When the container was prefilled with CO_2, the initial CO_2 concentration was 20% (Figure 3). 60% was reached after 5 minutes, which was substantially earlier as compared to the non-prefilled container. The chicks were held at CO_2 concentrations between 70 and 75% for 4.5 minutes, with a maximum CO_2 concentration of 76%. In both situations, no chicks recovered within 30 minutes afterwards.

Picture 3. Killing with container and CO_2.

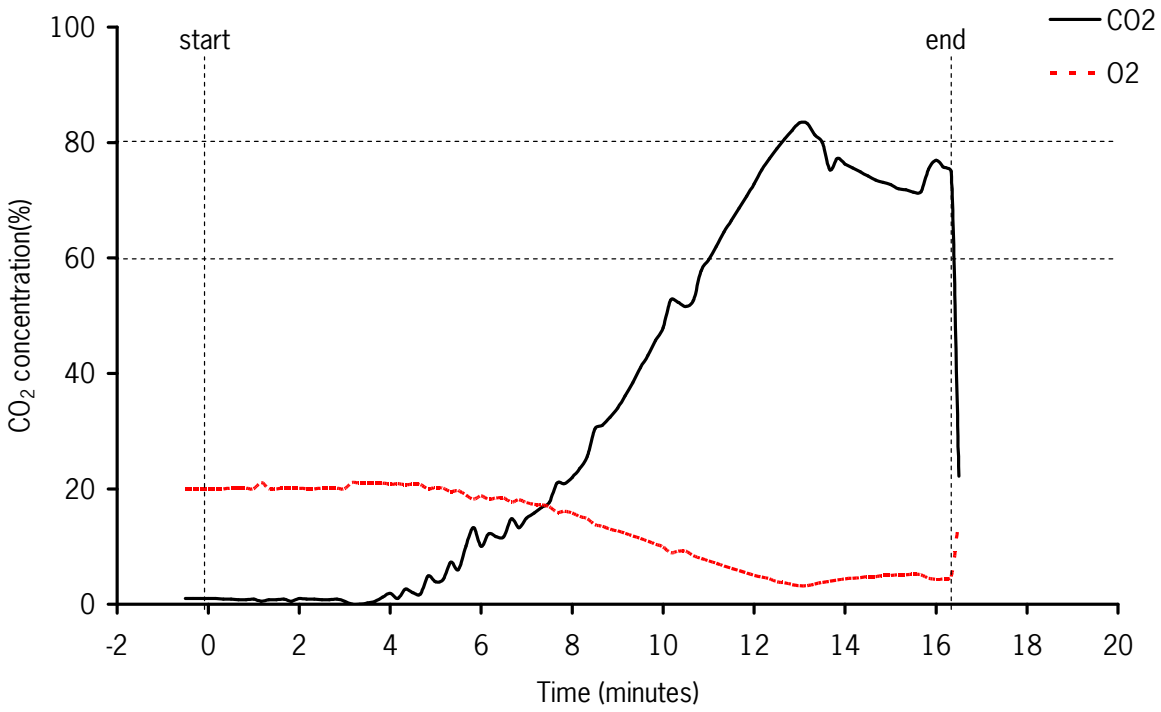

Figure 2. CO_2 and O_2 concentrations during killing of day-old chicks in a container not prefilled with CO_2.

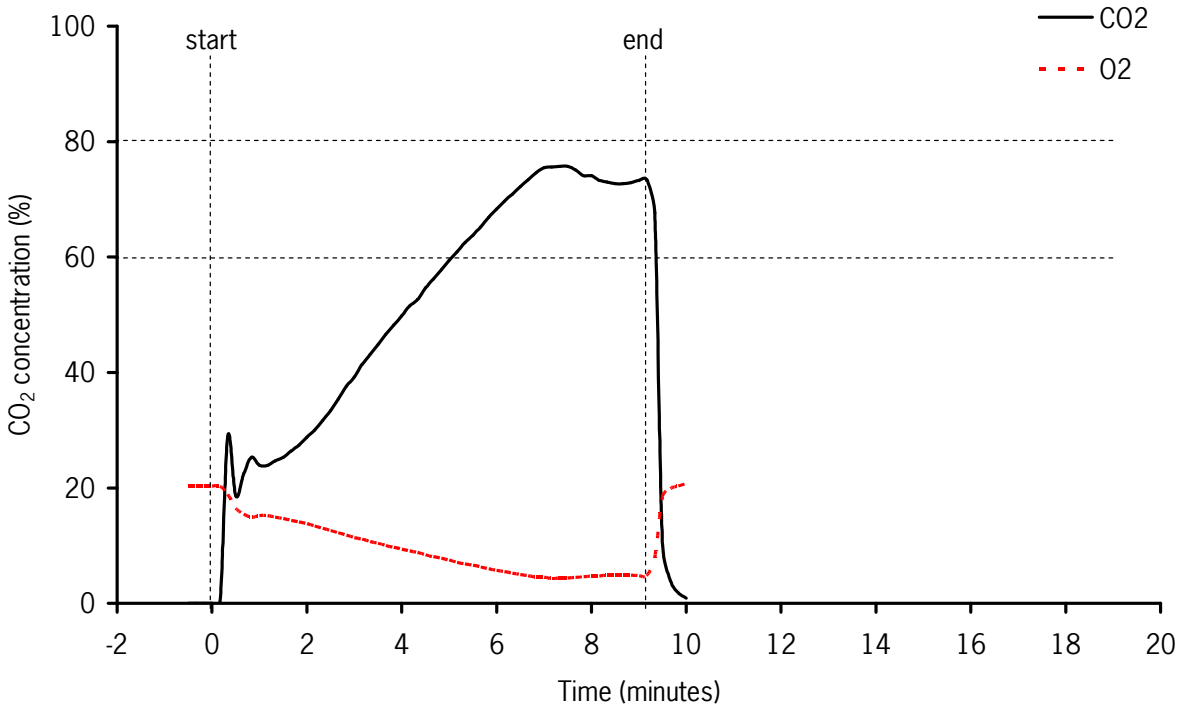

Figure 3. CO_2 and O_2 concentrations during killing of day-old chicks in a container prefilled with CO_2.

2.3.3 Automatic machines (1)

In picture 4, an automatic machine for killing of day-old chicks using CO_2 is shown. Chicks are transported to the machine using a conveyor belt, and fall into the machine on the first belt that transport the chicks into the machine. At the end of the first belt, they fall on the second belt that transport the chicks backwards, where they fall on the third belt. At the end of the third belt, the chicks are transported upwards and outside the machine. Figure 4 shows the different CO_2 concentrations the chicks are exposed to during the 4 minutes the chicks are on the belts.

Picture 4. Automatic machine and CO_2 (1)

In Figure 4 the stepwise development of CO_2 concentration is shown for the consecutive belts. At the first belt, CO_2 levels of up to 30% are measured; at the second belt 76%; at the third belt the CO_2 concentration is above 80% and increased to almost 98%. Chicks remained at CO_2 concentrations of above 60% for more than 3 minutes and no chicks recovered within 30 minutes afterwards.

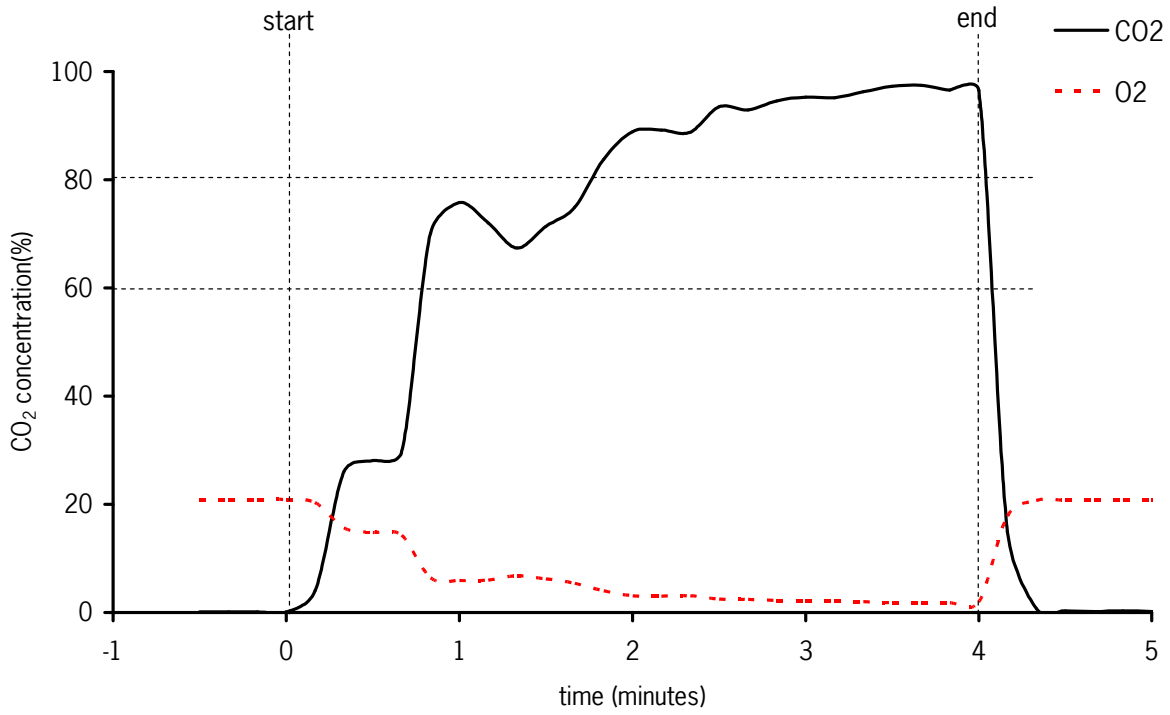

Figure 4. CO_2 and O_2 concentrations during killing of day-old chicks in automatic machines with CO_2 (1)

2.3.4 Automatic machines (2)

In Picture 5, another automatic machine to kill day-old chicks is shown, using the technique similar as described for machine 1 (see 2.2.3).

Picture 5. Automatic machine and CO_2 (2)

Figure 5 shows the CO_2 concentration differs from that in machine 1 (see 2.2.3). CO_2 concentration reached values above 60% after 20 seconds, above 80% after 40 seconds with a maximum value of 93%. The procedure lasted only 110 seconds (1.8 minutes), and no chicks recovered within 30 minutes afterwards.

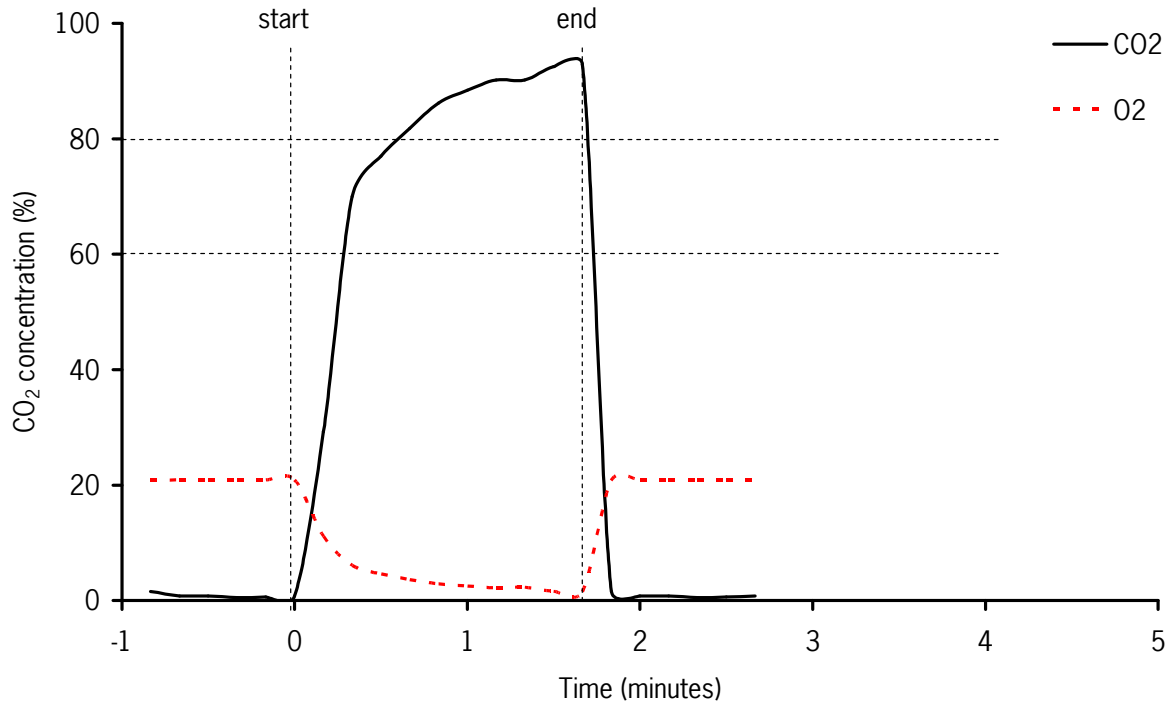

Figure 5. CO_2 and O_2 concentrations during killing of day-old chicks in automatic machines with CO_2 (2)

2.3.5 Self developed device

In one layer hatchery, a self developed device was used to kill day-old chicks with CO_2 (Picture 6) In this device, CO_2 gas enters through the roof panel, sinks down due to gravity and the concentration builds up from the bottom to above. A hole in the roof provides a smooth outlet of the fresh air.

Picture 6. Killing with an own developed device and CO_2

The CO_2 measurements were done between the two stacks of baskets, at the height of 3 (bottom) and 10 (top) baskets from the ground, see Picture 5. At the bottom (3 baskets high), the CO_2 concentration remained low for 70 seconds, then increased and reached 60% after 110 seconds. Next, the CO_2 concentration gradually increased to 80% after 30 minutes. At the top (10 baskets high) it took 16 minutes before CO_2 levels reached the chicks. The CO_2 concentration at that point was 49%, and increased to 58% after 30 minutes (14 minutes later). The gradient in CO_2 concentration may be the result of leakages in de side walls. At all levels in the device, no chicks recovered within 30 minutes afterwards.

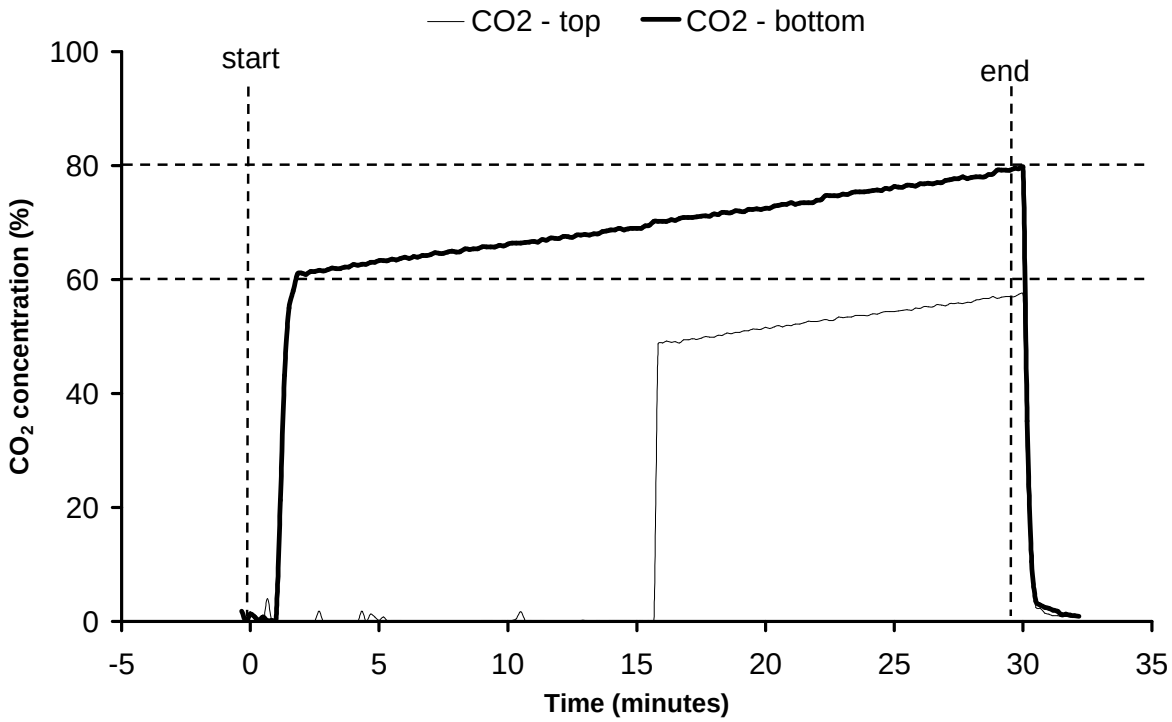

Figure 5. CO_2 concentrations during killing of day-old chicks measured at the top and at the bottom in an own developed device.

2.3.6 Summary and discussion

In Table 2, a summary is given of the exposure time and CO_2 concentrations measured in practice.

Table 2. Summary of methods, duration, concentration and CO_2 exposure time and recovery.

Methods	Total duration (mm:ss)	CO_2 reached after (mm:ss)			Exposure time (mm:ss)		Max CO_2 (%)	Recovery
		>20%	>60%	>80%	>60%	>80%		
Plastic bags	07:20	00:00	01:10	-	06:10	-	74.0	No
Container empty	16:20	07:40	11:00	12:40	05:20	02:40	83.4	No
Container prefilled	09:20	00:40	05:10	-	04:10	-	75.7	No
Automatic Machine 1	04:00	00:10	00:50	01:50	03:10	02:10	97.5	No
Automatic Machine 2	01:40	00:10	00:20	00:40	01:20	01:00	92.5	No
Own Device bottom	30:00	01:10	01:50	-	28:10	-	79.7	No
Own Device top	30:00	15:50	-	-	-	-	57.5	No

Total duration of the killing, exposure time and CO_2 concentrations differed between the different methods used. However, the effect of the different methods was the same since no chicks recovered within 30 minutes afterwards.

The duration that day-old chicks are exposed to CO_2 concentrations under 20% may be critical for animal welfare. This can be concluded from Figure 6 (after Gerritzen et al., 2007), where CO_2 concentration increased in time. The first vertical dotted line indicates the moment of loss of consciousness; the second vertical dotted line marks the moment all animals are dead. Lines parallel with the x-axis indicate the duration of the behaviour characteristics. At CO_2 concentration of 20%, animals lost posture and from that moment onwards it was determined that animals were unconscious and EEG suppression occurred as well.

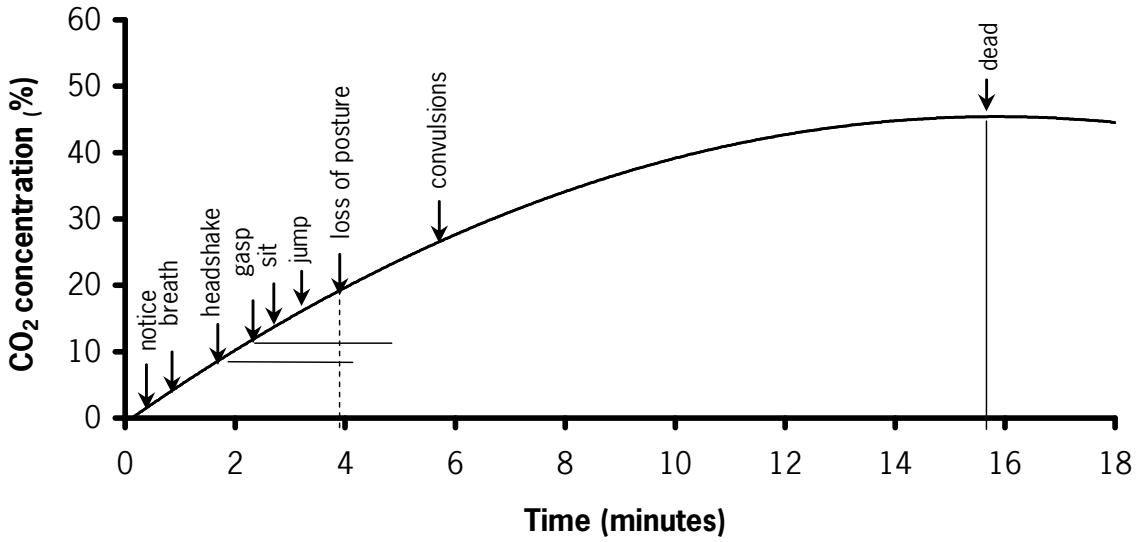

Figure 6. Schematic overview of the increase of the CO_2 concentration in time and the onset of the behavioural parameters.

Using the plastic bag method, day-old chicks are immediately exposed to high CO_2 concentrations and lost posture within seconds. In an empty container, CO_2 concentration slowly increased and it took over 7 minutes before CO_2 concentration reached values more than 20%. In a container prefilled with CO_2, this time period was only 40 seconds. The automatic machines can only be started after CO_2 concentrations were over 80%, ensuring a sufficiently high CO_2 concentration at the start of the killing process. In the own developed device, it took a long time (16 minutes) before CO_2 concentration in the baskets at the top increased, but at that time the chicks were instantly exposed to 50% CO_2. Using the automatic methods, CO_2 concentrations increased to more than 90%, which may decrease the time to induce death. Therefore the 1 minute and 40 seconds duration time in one of these automatic methods may be sufficient to avoid recovery. However, any failure in gas delivery may cause recovery of the chicks. The method using the own developed device increased CO_2 concentration in the upper baskets to the maximum of only 57.5%, but due to the long duration this was apparently sufficient to avoid recovery of the chicks (see also Figure 6).

3 Behavioural and physiological aspects

3.1 Indication of death

Until now judgment of impact on animal welfare when killing day-old chicks is based on behavioural observations. More specific, measuring the time it takes until animals are motionless, used as indicator for unconsciousness, is the most important animal welfare indicator. The time it takes before day-old chickens reach unconsciousness when using exposure to different carbon dioxide concentrations has never been confirmed by physiological data. When a method is applied for the killing of individual or (large) groups of animals it is of major importance that the initial method is effective, or in other words the methods applied should inevitably lead to death. Measurements of electro-cardiogram (ECG) together with general electro encephalogram (EEG) will give a good indication for the induction of death. Measuring ECG and EEG induce a certain level of discomfort to the animals. Therefore, the experiments are judged and approved by the ethical committee of the Animal Sciences Group.

3.2 Experimental set-up

To measure physiological and behavioural responses of day-old chicks during the gas killing process we received 103 day-old male chicks from a commercial layer hatchery. All animals used in these experiments were meant to be killed at the hatchery and thus no additional animals were killed. To measure behavioural responses, 73 day-old male chicks were exposed to 60 or 80 % carbon dioxide. Individual chicks were placed into a prefilled gas box (0.8x0.8x0.8 m) for a period of 2 or 5 minutes. During this exposure to CO_2, the duration of loss of posture, heavy breathing and uncontrolled movements where recorded. The animals could move freely in the gas box. After exposure, it was registrated if animals recovered.

Brain activity by means of a general electro-encephalogram (EEG) and heart activity (beats per minute; BPM) were recorded to analyze the duration until animals lost consciousness and died. All physiological data were recorded using a biomedical amplifier (BMA 931)[1], a DI-720 data converter[2] and windaq software[3]. To measure electro physiological parameters in total 30 day-old chickens were equipped with electrodes just before exposure to 60% or 80% CO_2. To register general brain activity, two needle electrodes ((55% silver, 21%copper, 24% zinc) of 5 mm in length and 1 mm in diameter were punctured trough the skin just under the skull approximately 3 mm left and right from the sagital line on the imaginal line ear to eye. Electrodes and wires were fixed on the animals head using medical bandage tape. To measure heartbeats, two needle electrodes of 2 cm in length and 1 mm in diameter were placed subcutaneously at the breastbone and at the back, between the wing base of the animals. To minimize noise on the signal an earth connecting electrode was placed subcutaneous at the back of the animals. All electrodes were pre-connected with coaxial shielded wires that were connected with the recording device.

The electrodes were placed without the use of any anaesthesia. Anaesthesia will conflict with the measurement of consciousness and will need time (days) to be out of the animals system and was therefore not applicable. Day old chicks are too small in size and the micro voltage signal is too low to use available surface electrodes. The method used here, (with needle electrodes) is chosen because it had minimal impact on animal welfare and we still had the possibility of measuring a clean EEG and ECG signal. After placing the electrodes, a baseline signal was recorded for 30 seconds before the chicks were placed in the gas mixture. It has to be noticed that the recorded baseline represents a baseline of a stressed animal. However, in practice animals will also be in a stressful situation just before they are killed. All chicks were exposed to the gas mixtures (60% or 80% CO_2) until an iso-electric ECG occurred.

[1] MODEL BMA-931, CWE, Inc., Ardmore, USA
[2] DATAQ instruments, Akron Ohio, USA.
[3] DATAQ instruments, Akron Ohio, USA.

3.3 Results

3.3.1 Behaviour

Within 5 seconds after placement of individual animals into the 60% or 80% CO_2 they started gasping or heavy breathing. In all animals heavy breathing was immediately followed by neck stretching or head shaking. There were no differences in induction time of these behaviours between both CO_2 concentrations. After loss of posture, heavy breathing continued although on a lower frequency. Loss of posture was in approximately 50% of the cases, in both CO_2 concentrations, preceded by a strong jumping movement. Convulsive movements with wings and legs were seen in the 60% CO_2 group between 30-90 seconds after loss of posture and in the 80% CO_2 between 15-35 seconds after loss of posture.

The number of animals that recovered after different exposure times to 60% or 80% CO_2 is presented in Table 3. The delay time from removal from the gas mixture until recovery varied from 2 up to 15 minutes. There was more variation in delay time to recovery between animals than between exposure times.

Table 3. Recovery and ECG silence after different exposure times in 60% or 80% CO_2.

Gas concentration	Exposure time (min)	Recovery (n/total)	ECG Silence ± SEM (sec) [1)]
60% CO_2	2	3/3	800±114
	5	3/4	
	10	6/8	
80% CO_2	2	1/24	311±24
	3	0/25	
	5	0/4	

[1)] ECG silence is measured for animals that stayed in the CO_2 concentration until all heart activity ceased.

3.3.2 Electro physiological responses

There was a large variation in induction of ECG silence between animals in both CO_2 concentrations. In the 60% CO_2 group ECG silence never occurred before 380 seconds (±6 min) and in one occasion occurred after 1670 seconds (±28 min). The induction of ECG silence in the 80% CO_2 group varied between 120 seconds (±2 min) and 500 seconds (±8 min).

At the time of, or immediately after loss of posture a small change in the EEG signal was observed. This change could not be used to describe an EEG suppression that would indicate loss of consciousness. It was therefore not clear if animals were less conscious at or immediately after loss of posture. Suppression of the EEG signal which indicated unconsciousness occurred in the 60% CO_2 after 52 seconds and in the 80% CO_2 group after 24 seconds, see Figure 2. A near iso-electric EEG or the induction of a phase of minimal brain activity occurred in the 60% CO_2 group after 269 seconds and in the 80% CO_2 group after 111 seconds (Figure 7).

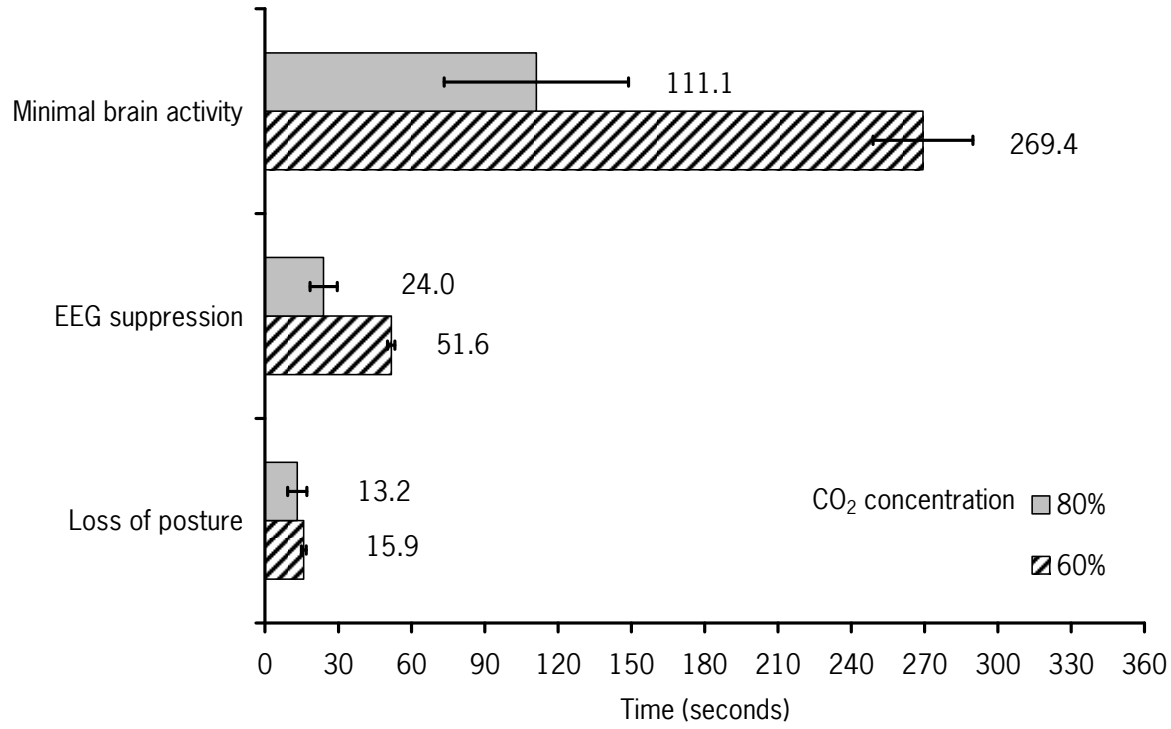

Figure 7. Time in seconds (mean ± SEM) to loss of posture, to EEG suppression, and to a near iso-electric point (minimal brain activity).

Immediately after exposure of the chicks to both the 60% and the 80% CO_2, the heartbeat declined with approximately 50%. Conspicuous in both CO_2 concentrations is the sharp decrease in heart rate shortly after the chicks are introduced to the CO2 box (Figure 8). Before 30 seconds after immersion the heart rate increased to a more or less normal level. After this recovery of the heart rate in both CO_2 concentrations the heart rate of the 80% CO_2 started to decrease almost immediately whereas the decrease in heart rate in the 60% CO_2 group started not before 120 seconds after being immersed into the CO_2.

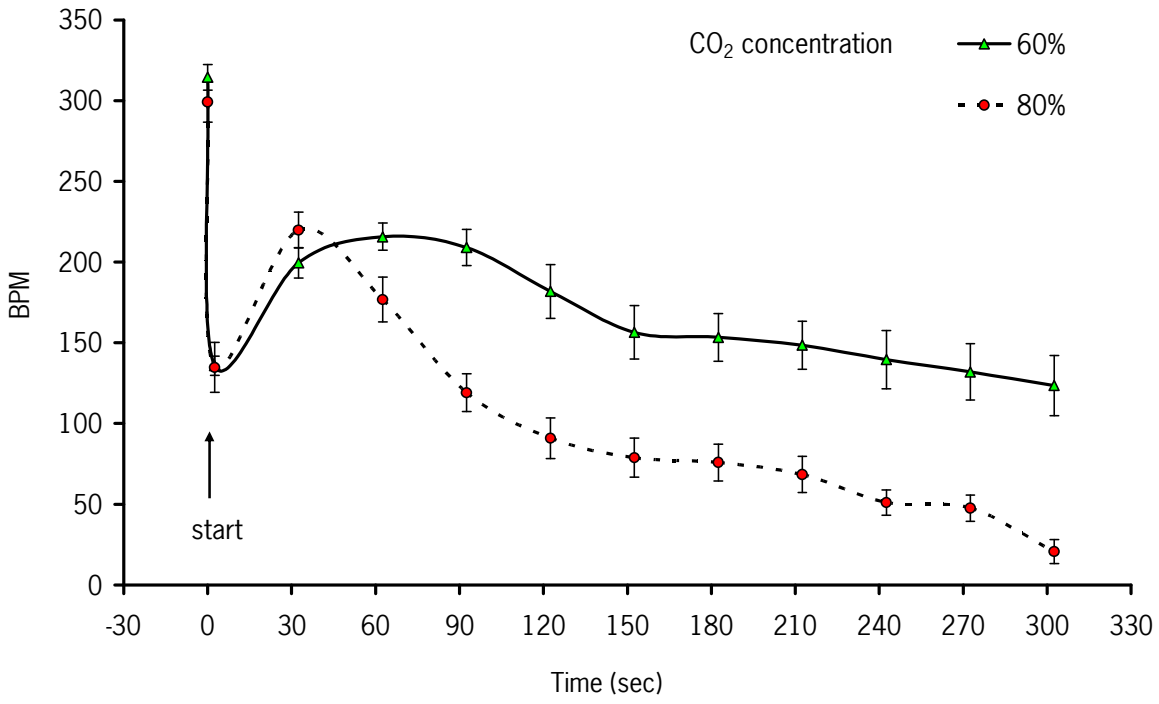

Figure 8. Development of heart rate (BPM) of day-old chicks after placement in 60 or 80% CO_2.

3.3.3 Heart rate analysis

The characteristic development of the heart rate during exposure to CO_2 is presented in the figures 3 to 7. Each vertical line represents 0.48 seconds. In Figures 9.1 – 9.5, an example of the development of heart rate of 1 representative animal is shown in time after exposure to 60% CO_2. The pattern is characteristic for all animals but the time frame can differ strongly between CO_2 concentrations and between individual animals.

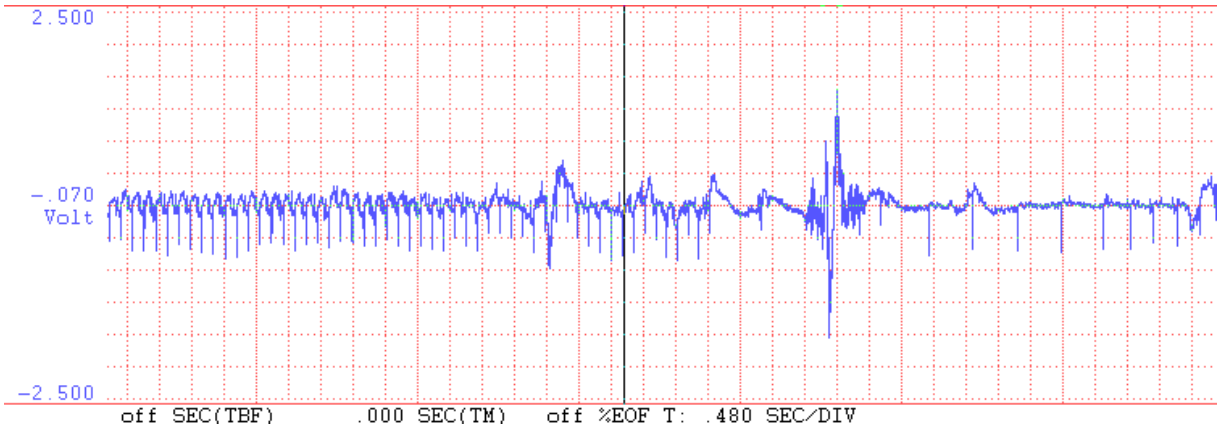

Figure 9.1. Heart rate before and immediately after exposure to 60% CO_2

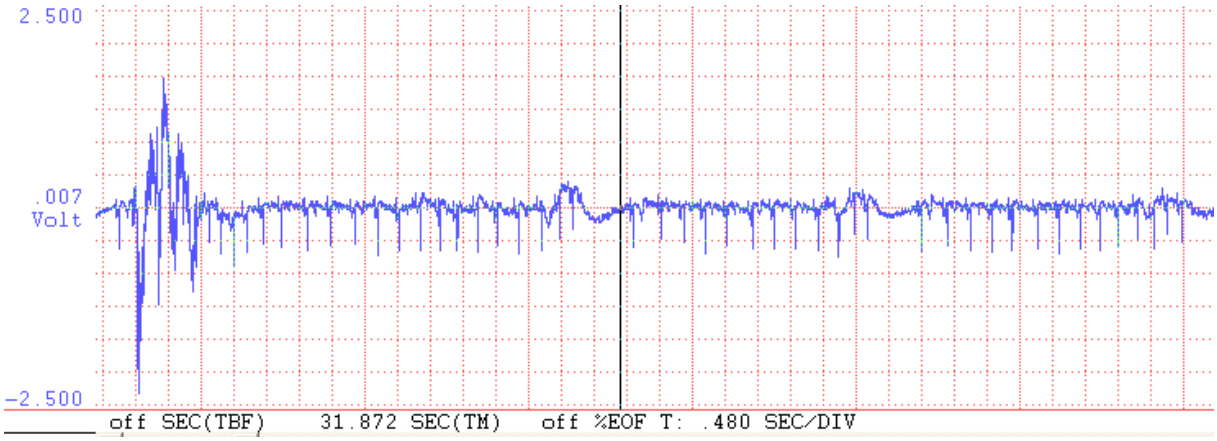

Figure 9.2. Heart rate 32 seconds after exposure to 60% CO_2

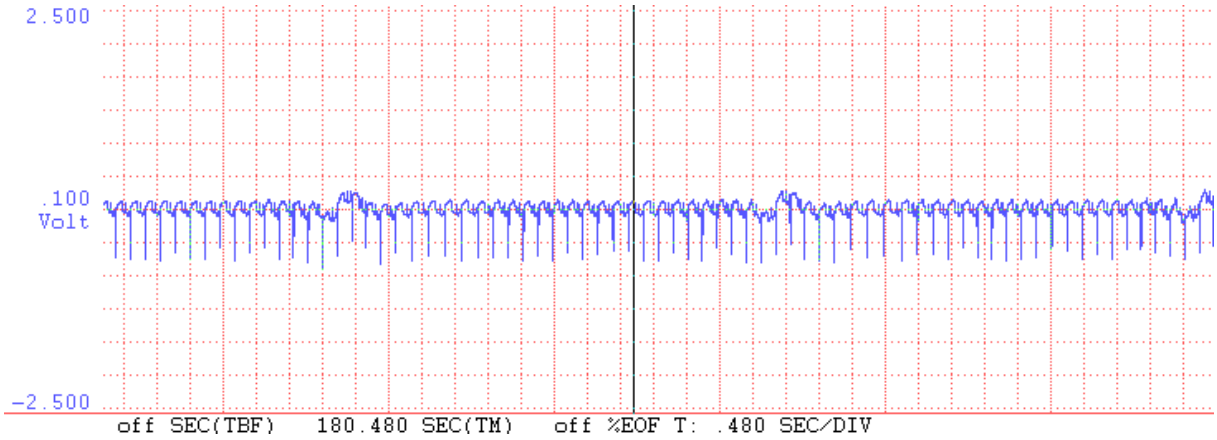

Figure 9.3. Heart rate 180 seconds after exposure to 60% CO_2

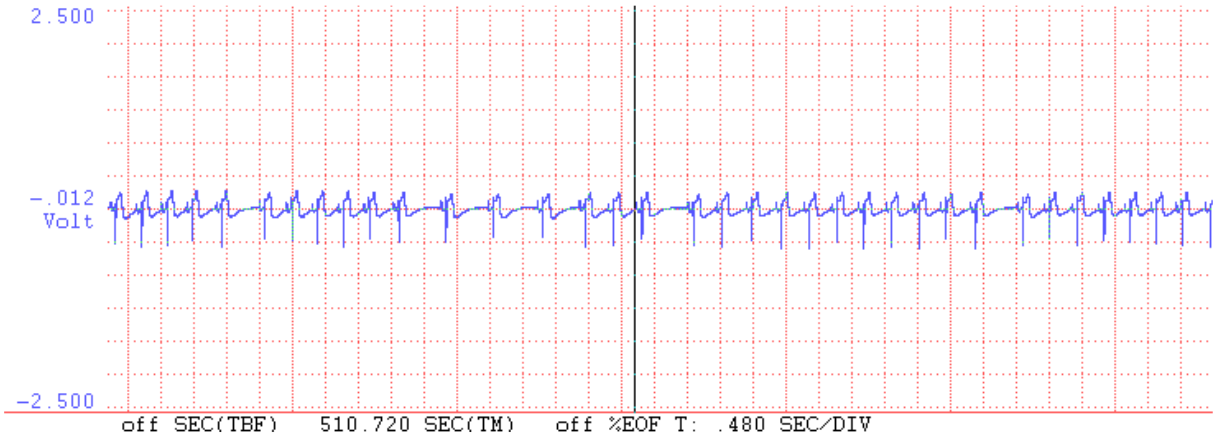

Figure 9.4. Heart rate 510 seconds after exposure to 60% CO_2

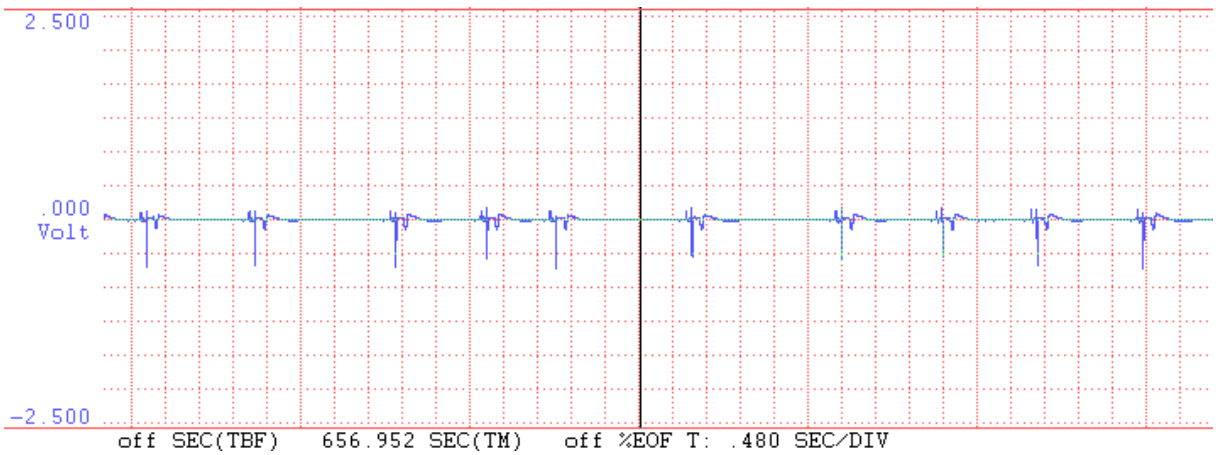

Figure 9.5. Heart rate 660 seconds after exposure to 60% CO_2.

3.4 Discussion

Aim of this experiment was to provide us with information necessary to judge the acceptability of different CO_2 killing methods with respect to animal welfare. The two CO_2 concentrations (60% and 80%) as well as the minimal exposure times were chosen because they are regularly applied in commercial hatcheries in The Netherlands.

Effects on animal welfare due to exposure to high CO_2 concentrations is subject of international discussion (Coenen 2000; Gerritzen, 2000, 2004; Lambooij 2000; Leach 2002; Raj 1998) and is judged from mildly aversive to highly aversive. The heavy breathing and headshaking observed in this experiment indicate a period of reduced animal welfare (Gerritzen et al., 2006). The duration of period of reduced animal welfare from immersion into the gas mixture to the moment animals lost consciousness. In general loss of posture is seen as the behavioural parameter for the induction of unconsciousness (Gerritzen 2006) and is followed by a suppression of alpha and beta waves on the EEG. In this experiment we observed a delay between loss of posture and EEG suppression which we can not explain. The suppression of alpha and beta waves on the EEG is generally accepted as the moment animals are unconscious. Based on this, it is assumed that the maximum duration of reduced animal welfare in the 60% CO_2 group is on average 52 seconds and in the 80% CO_2 group on average 24 seconds.

Another aspect of effective killing is that animals should not recover. In the 60% CO_2 group, 3 out of 4 animals recovered after a 10 minute exposure time and based on ECG measurements, animals still had minor heart activity (Figure 6) after exposure durations of up to 20 minutes. After exposure of day-old chicks to 80% CO_2 for 2 minutes, 1 out of 24 chicks recovered. After exposure to 80% CO_2 for 3 minutes, no chicks recovered.

Based on the behavioural aspects we can not exclude a certain impact on animal welfare while day-old chicks are killed with CO_2. However, based on the physiological aspects we can conclude that the duration of reduced welfare is less than one minute. Recovery is minimal after exposure to 80% CO_2 for at least 3 minutes.

4 Conclusions

4.1 Measurements in practice

- All Dutch layer hatcheries kill day-old male chicks using CO_2 methods
- From January 2009 onwards, all Dutch layer hatcheries apply automatic machines to kill day-old male chicks
- Killed male day-old chicks using CO_2 methods are valuable by-products to feed carnivorous animals in captivity.
- Broiler hatcheries kill second grade day-old chicks using either CO_2 methods or shredder, depending on retailer demands.
- Undesired female or male chicks in GPS and PS hatcheries related to the broiler industry are not killed and can be sold alive as broilers.
- Compared to the number of killed day-old chicks in layer hatcheries, the numbers of killed day-old chicks in layer GPS and PS hatcheries are only low.
- Exposure time to low CO_2 concentrations differs between different methods
- Despite the variety in methods, exposure time and CO_2 concentrations, all methods applied in the Dutch hatcheries were equally effective: no day-old chicks recovered within 30 minutes afterwards.
- Killing devices prefilled with CO_2 offer sufficiently high concentrations (higher than 20%) to induce loss of posture within seconds.

4.2 Experiments

- When chicks are exposed to CO_2 gas, behavioural indicators of discomfort can be observed, as gasping or heavy breathing, followed by neck stretching or head shaking and jumping movements.
- There were no differences in the moment that chicks lost posture, when they were placed in either 60% or 80% CO_2.
- After loss of posture, heavy breathing continued although on a lower frequency.
- After loss of posture, convulsive movements with wings and legs were observed longer in day-old chicks exposed to 60% than to 80% CO_2
- An ECG silence occurred sooner in day-old chicks exposed to 80% than in 60% CO_2.
- EEG suppression occurred sooner and faster in chicks exposed to 80% than in 60% CO_2.
- Minimal brain activity occurred sooner in chicks exposed to 80% than in 60% CO_2.
- Chicks are able to recover after exposure to 60% CO_2 for 10 minutes.
- Based on behavioural observations, it is likely that animal welfare is reduced for a short period when chicks are killed with CO_2.
- Day old chicks lost posture after 16 seconds when placed in 80% CO_2, and after 13 seconds when placed into 80% CO2.
- Loss of posture is generally the moment that animal are unconscious, which occurs with suppression of the EEG signal.
- However, in day-old chicks the suppression of EEG signals occurs later: after 52 sec in 60% CO_2 and after 24 sec in 80% CO_2.
- Therefore, the estimated duration of reduced welfare during killing of day-old chicks in CO_2 is 52 sec in 60% CO_2 and 24 sec in 80% CO_2.
- The probability to recover is minimal after exposure of day-old chicks to 80% CO_2 for at least 3 minutes.

4.3 Recommendations

- The CO_2 method is an acceptable method to kill day-old chicks when the initial CO_2 concentration is higher than 20%.
- The method should provide CO_2 concentrations higher 80% to suppress EEG as soon as possible.
- The minimal exposure duration should be 3 minutes at 80% CO_2.

5 References

Coenen, A.M.L., Li Zhonghua, A. S., Luijtelaar van, Gilles, (2000). Gas mixtures for anaesthesia and euthanasia in broiler chickens. World's Poultry Science 56, 225-234.

Gerritzen, M. A., Lambooij, E., Hillebrand, S. J. W., Lankhaar, J. A. C., Pieterse, C., (2000). Behavioural responses of broilers to different gaseous atmospheres. Poultry Sci. 79, 928-933.

Gerritzen, M.A., Lambooij, E, Reimert, H., Stegeman, A.,Spruijt, B.M., (2004). On Farm Euthanasia of Broiler Chicken: effects of Different gas Mixtures On Behaviour and Brain Activity. Poultry Science 83, 1294-1301.

Gerritzen, M., B. Lambooij, H. Reimert, A. Stegeman, B. Spruijt (2007) A note on behaviour of poultry exposed to increasing carbon dioxide concentrations. Applied Animal Behaviour Science, Volume 108, Issue 1-2, Pages 179-185 M.

Lambooij, E., Gerritzen, M.A. , Engel, B. , Hillebrand, S.J.W. , Lankhaar, J. , Pieterse, C., (1999). Behavioural responses during exposure of broilers to different gas mixtures. Applied Animal Behaviour Science 62, 255-265.

Leach, M.C., Bowell, V.A:, Allan, T.F., Morton, D.B., (2002). Measurement of aversion to determine humane methods of anaesthesia and euthanasia. Animal Welfare 13,S77-86.

Leenstra, F., Munnichs, G. Beekman, V., Van den Heuvel-Vromans, E., Aramyan, L and Woelders, H. 2008. Alternatives for killing one day-old male chicks. Report 142, Animal Sciences Group of Wageningen UR, ISSN 1570-8616.

Raj, Mohan. A.B., Wotton, S.B., McKinstry, J.L.,Hillebrand, S.J.W., Pieterse, C., (1998). Changes in somatosensory evoked potentials and spontaneous electroencephalogram of broiler chicken during exposure to gas mixtures. Br. Poultry Sci. 39, 686-695.

Woelders, H., Brom, F.W.A., and Hopster, H. 2007. Alternatieven voor doding van eendagskuikens; technologische presperctieven en ethische concequences. Rapport 44, Animal Sciences Group of Wageningen UR, ISSN 1570-8616.