CO_2 CULLING WITH INFLUENZA CONTAINMENT SYSTEM (I.C.S.): PHYSIOLOGICAL AND ETHICAL CONSIDERATIONS

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Summary

This study evaluates the use of the I.C.S.-bag compared with current killing methods for poultry. The ICS culling method was evaluated from several ethical standpoints with the 'Animal Disease Intervention Matrix' (ADIM, Aerts 2006). This system provides governments with a tool to take more ethically justified decisions about animal diseases. In a series of gassing experiments on laboratory scale, the changes in the physiological mechanisms and the behavioural changes of birds after exposure to rising CO_2 were investigated. Finally, it was determined if the I.C.S.-bag was bio-secure for Highly Pathogenic Avian Influenza (HPAI) over a period of 48 hours. This study was performed by the Instituto Zooprofilattico delle Venezie (IZSVe, Italy).
Finally, this system was introduced at the University of Lomé, Togo. There the method was used during one of the latest out-brakes. Also, based on the ability of the system to contain HPAI and no leakage when gases are injected into the system, storages tests were conducted on poultry feed and hatching eggs. The results showed that the I.C.S.-bag has (1) a higher ADIM-score, (2) death within 40 seconds and (3) no dispersion of the virus in the environment within 48 hours. The other experiments indicated (4) a higher Feed Conversion Ratio when stored in the I.C.S.-bag, as well as (5) higher hatching rate of day old chickens.

Introduction

Avian influenza Directive 2005/94/EC of the Council of 20 December 2005, which was incorporated into national legislation at the end of 2006, differentiates between high pathogenic (HPAI) and low pathogenic (LPAI) avian influenza. Low pathogenic avian influenza does not make the animals ill, but according to scientists, it may mutate to high pathogenic avian influenza, which can make chickens seriously ill and may infect humans. In both cases the poultry at the infected site and its surroundings must be evacuated. As a pest control measure, all infected animals and those within a certain radius of the infected location are killed. This applies to poultry kept by both amateurs and professionals.

The most commonly used procedures for large-scale emergency depopulation of birds, e.g. in the case of avian influenza, consist of exposing poultry to gasses. Many different gas types and mixtures are used for stunning and killing poultry. With the exception of CO and HCN killing, gas mixtures contain three important components: CO_2, N_2 and Ar. Gas killing can be done without removing the animals from their housing ('whole house gassing'), in an environment containing at least 45% CO_2 for killing all animals (Gerritzen et al., 2006; Raj et al., 2006; OIE guidelines, 2005).

From the point of view of both human and animal welfare, the killing of animals in the shed with a minimum of man-animal interaction is preferred. Hitherto, the killing of poultry in the shed has only been possible by means of shed gassing with CO_2. It is not possible to use this method in every shed because - sheds with excessive leakage cannot be effectively responsibly filled with CO_2 gas.

Recently, a new gassing system was developed in which gassing is performed in an I.C.S.-bag filled with CO_2 gas or dry ice. However, it was not clear how the birds would react and how blood acid-base related parameters change when birds are exposed, immediately or gradually, to an environment with very high CO_2 / very low O_2 concentrations. Moreover, information was lacking on spatial and time distribution of CO2 in an I.C.S.-bag and in the close surroundings of the bag, since safety of the workers has to be guaranteed. These issues were clarified in several of our experiments.

Materials and Methods

A group of 6-week old broilers was divided into three groups and the broilers were individually killed by direct exposure to high CO2 (approx. 57% CO2) in the I.C.S.-bag (exp 1), by means of gradual and rapid build-up of CO2 in a plexibox (exp 2) and by means of gradual and slow build-up of CO2 in a plexibox (exp 3). Venous blood samples were taken from the animals' wing vein before and after gassing and immediately analysed using a blood gas analyser. These samples were used to measure the parameters relating to the acid-base control in the blood (blood pH, partial pressure of CO_2, partial pressure of O_2, HCO_3^-, base excess, haematocrit, acid saturation, Na^+, K^+, Ca^{2+}) by means of a blood gas analyser (pGEM3000, Instrumentation Laboratories). In addition, glucose and lactate were measured.

The time of death was diagnosed by measurement of heartbeat, respiration and corneal enlargement. The behaviour of the animals was observed and the time of behaviour-related change, as defined by Gerritzen et al. (2004), was observed and noted. The time and occurrence of the different behaviours during the CO_2 stunning were recorded with a PC-based data-acquisition system (National Instruments).

Post-mortem muscular rigidity was checked using the PUFF system (Bamelis et al., 2006) as an indicator of post-mortem meat quality. The PUFF control animals were four additional broilers that were killed by cervical dislocation.

An O_2-sensor (Vaisala OMT355) was chosen to measure the changes of CO_2, instead of a CO_2-sensor. The reason was that the O_2-sensor was more accurate and reliable than a CO_2-sensor. Since all experiments were performed under normal atmospheric conditions, it may be assumed that a constant ratio subsists between the % of O_2 measured and the % of CO_2. The ratio was calculated as follows: Assuming a standard air mixture consisting of 20.94 % O_2, 0,03 % CO_2 and residual gases (N_2, Ar, …): "the % CO_2 after the addition of CO_2" = 100(1-('%O_2'/20.94)).

Results

Experiment 1. Immediately after being placed in the I.C.S.-Bag, all the animals began wing flapping and showed uncontrolled behaviour. Death of all the animals was diagnosed within 40 seconds (Table 1). Venous blood values pointed to significant acidification of the blood (fall of pH – Table 2). Nevertheless, no other blood parameters changed significantly through direct exposure to high CO_2 concentrations, except Na^+ and Ca^+. Most probably, the time of exposure was too short to produce any significant changes in the venous blood.

Table 1. Time of reaching well-defined behaviour on exposure to a rapidly rising concentration of CO_2. The times are shown as an average ± standard deviation. Likewise given are the O_2 and CO_2 concentrations at which a well-defined behaviour-related change occurs.

	Deep respiration with gasping and neck stretching	**Loss of posture**	**Wing flapping and uncontrolled muscular movement ('convulsions')**	**Loss of movement ('motionless')**
Experiment 1 Very rapid gassing in I.C.S.-Bag (n=6)	/	/	/	<= 40 seconds
Experiment 2 Rapid gassing in a plexibox (n=7)	12 ± 3 seconds	49 ± 12 seconds	59 ± 11 seconds	106 ± 15 seconds
	O_2: 19.6 ± 0.1 %	12.5 ± 0.2	11.1 ± 0.1	
	CO_2: 6.2 ± 0.4 %	40.1 ± 0.8	46.8 ± 0.7	6.9 ± 0.2
				66.7 ± 0.9
Experiment 3 Slow gassing in a plexibox (n=3)	227 ± 100 seconds	420 ± 176 seconds	900 ± 101 seconds	1209 ± 345 seconds
	O_2: 18.8 ± 0.5%	17.7 ±0.4 %	16.0 ± 0.7 %	14.2 ± 0.2 %
	CO_2: 10.1 ± 2.5%	15.7 ± 2.1 %	23.6 ± 3.2 %	32.1 ± 0.9 %
	Gerritzen (2004): 46 seconds CO_2: 6 %	Gerritzen (2004): 172 seconds CO_2: 15.7 %	Gerritzen (2004): 177 seconds CO_2: 16 %	Gerritzen (2004): 700 seconds CO_2: 31.5 %

Table 2. Blood parameters of broilers gassed with CO2 in different circumstances

	Experiment 1 Very rapid gassing in I.C.S.-Bag (n=6)		**Experiment 2 Rapid gassing in a plexibox (n=7) ***		**Experiment 3 Slow gassing in a plexibox (n=3) ****	
	before	**after**	**before**	**after**	**before**	**After**
pH	7.34 ± 0.02[a]	7.24 ± 0.03[b]	7.39 ± 0.04[a]	7.24 ± 0.03[b]	7.35 ± 0 .01	6.86 ± 0.06
pCO_2 (mm Hg)	37.5 ± 3.0	37.5 ± 2.2	51.8 ± 7.0	62.2 ± 6.3	42.5 ± 4.5	99.5 ± 5.5
pO_2 (mm Hg)	53.8 ± 3.7	47.7 ± 4.8	47.0 ± 1.6[a]	30.2 ± 2.8[b]	50.5 ± 1.5	31 ± 2.0
SO_2 (%)	83.7 ± 3.8	65.2 ± 9.4	81.5 ± 2.6[a]	45.2 ± 7.0[b]	83 ± 1.0	22.0 ± 0.0
Glucose (mg/dl)	207.3 ± 10.5	186.8 ± 14.4	202.3 ± 7.5	181.0 ± 8.0	211.5 ± 5.5	181.5 ± 30.5
Lactate (mmol/l)	4.4 ± 0.5	5.7 ± 0.5	4.3 ± 0.8	7.0 ± 0.6	4.2 ± 0.3	11.4 ± 2.6
Haemato-crit	25.5 ± 0.7	23.8 ± 1.8	30.2 ± 1.3	33.8 ± 0.7	26.5 ± 2.5	25.0 ± 4.0
HCO_3^- (mmol/l)	20.3 ± 1.6	16.5 ± 1.6	30.7 ± 2.3	25.5 ± 1.6	23.7± 1.7	17.8 ± 1.5
Base excess (blood) (mmol/l)	-4.9 ± 1.5	-9.9 ± 1.8	5.66 ± 1.8	-5.3 ± 3.9	-1.7 ± 1.3	-15.3 ± 2.9
Na^+ (mmol/l)	132.5 ± 2.9[a]	118.5 ± 4.1[b]	147.8 ± 1.8	151 ± 1.0	133.5 ± 0.5	129.5 ± 2.5
K^+ (mmol/l)	5.23 ± 0.1	5.73 ± 0.17	/	/	5.3 ± 0.3	5.7 ± 0.2
Ca^{2+} (mmol/l)	1.22 ± 0.05[a]	0.99 ± 0.07[b]	1.39 ± 0.03	1.36 ± 0.03	1.23 ± 0.02	1.29 ± 0.01

* Statistical analysis is carried out by means of repeated measurements (SAS); various letters point to significant differences of a particular parameter before and after gassing.

** in view of the small number of observations, no statistical analysis of experiment 3 data was carried out

Experiment 2. A typical progress of the O_2 concentration and related CO_2 concentration, during the gradual build-up (rapid) is shown in Figure 1. This figure shows also the changes in the heartbeat rhythm. The duration of behaviour related modification is shown in Table 1. The variation between animals was not great, pointing to the same behaviour pattern of the animals' reaction to high CO_2 concentrations. The sequence of the occurrence of these behaviour-related changes was the same in all the animals. Following the diagnosis of death, all animals were lying on their backs. Table 2 shows the blood parameters of the animals before and after gassing. Blood pH fell significantly, coupled with an insignificant rise of partial pressure of CO_2 and a fall in HCO_3^-. Base excess fell significantly as a result of exposure to CO_2. The partial pressure of O_2 and oxygen saturation of venous blood, were significantly lower after gassing. There was a tendency towards a high lactate and a lower glucose concentration after gassing, electrolytes were not changed. The pattern of progress of *rigor mortis* observed using the PUFF system did not differ between CO_2-gassed animals and animals killed by cervical dislocation (results not shown).

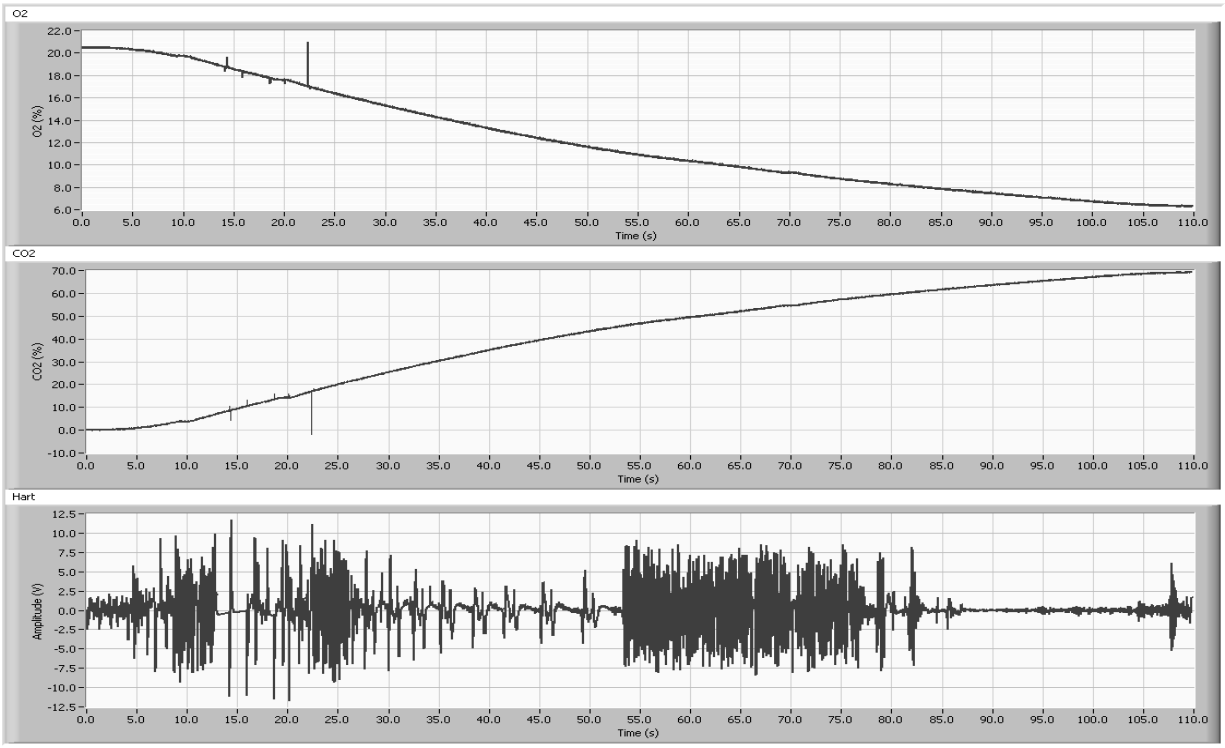

Figure 1. Progress of O_2 and CO concentrations and of heartbeat during rapid gassing.

Experiment 3. A typical progress of the O_2 concentration during slow gassing with CO_2 is shown in Figure 2. Since the build-up of CO_2 occurred very slowly, the timing of the sequence of the different behaviour patterns was also appreciably delayed (Table 2), with occurrence of death after only 20 minutes. Likewise note the great similarity in CO_2 concentrations to the results of Gerritzen et al. (2004), although the timing in our experiment was different. Marked differences can also be noted in O_2 and CO_2 concentrations, respectively in experiments 2 (rapid) and 3 (slow). All animals died lying on their stomachs. The blood parameters of the animals are shown in Table 2. There was a marked fall of blood pH occurred coupled with a markedly higher partial pressure of CO_2 and lactate concentration. Base excess and bicarbonate concentrations fell strongly. The

partial pressure of O_2 underwent a fall and oxygen saturation was strongly reduced after gassing. Haematocrit and electrolytes remained almost unchanged.

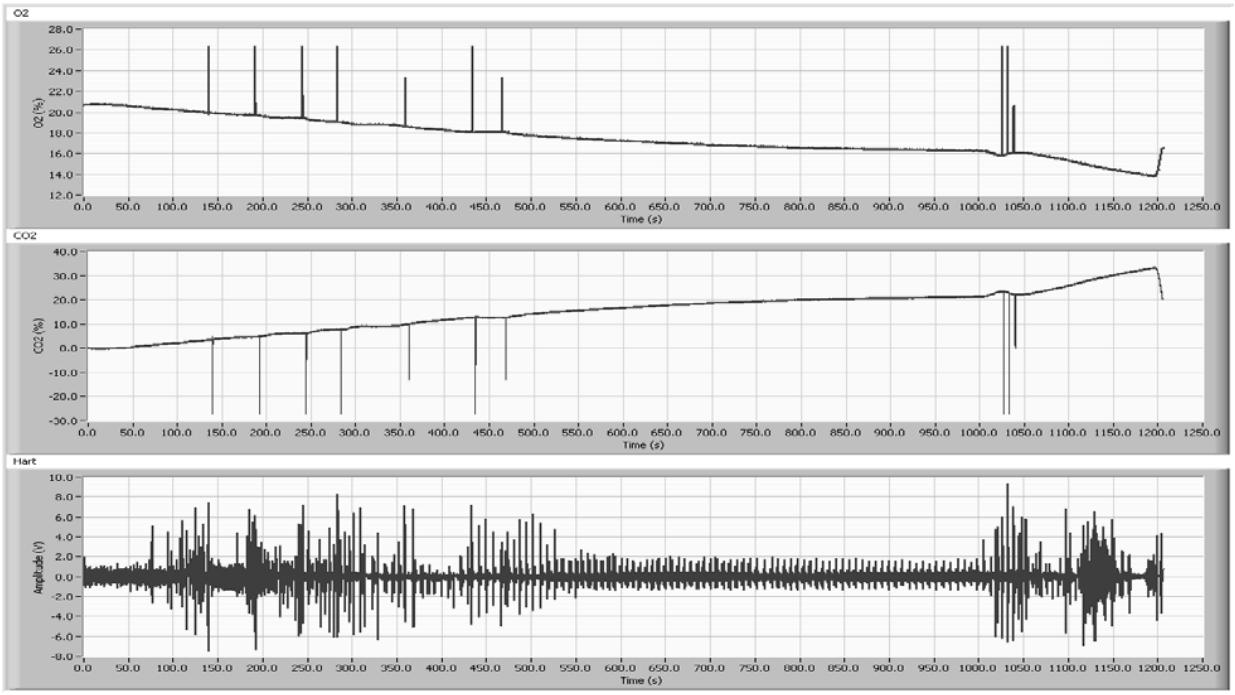

Figure 2. Progress of O_2 and CO_2 concentrations and of heartbeat during slow gassing.

Discussion

It is clear from the observations that ***CO_2 gassing in the I.C.S.-Bag*** (experiment 1) resulted in very rapid death (within 40 seconds). This rapid death was probably attributable to a number of causes. Both an acute exposure to a very low O_2 concentration (9%) and to a very high CO_2 concentration (calculated: 57%) caused specific reactions in the animal.

The results of Zeller et al. (1988) showed that in the case of an acute exposure to a high CO_2 concentration (50% for 30 seconds), an immediate rise of pCO_2 occurred (from 20 mmHg to 120 mmHg) in the arterial blood of cannulated broilers. This was coupled with an immediate fall of blood pH (from 7.5 to 6.9 in 30 seconds), which was also to be observed in our experiment, although the fall was less pronounced. There was no pronounced fall of the partial pressure of O_2 within 30 seconds in arterial blood (Zeller et al., 1988), or in venous blood in our experiment.

Exposure to 40% CO_2 for 60 seconds caused a rapid fall of both systolic and diastolic blood pressure, together with a heart arrhythmia and marked bradycardia (slow heartbeat). These data of Zeller point to the fact that acute exposure to very high CO_2 concentrations; resulted into rapid changes of acid-base parameters, but not of partial pressure of O_2 blood values neither in arterial nor venous blood, at least over a very short space of time. In the case of inhalation of very high CO_2 concentrations, breathing stops immediately (Zeller et al., 1988). In contrast with mammals, birds possess intrapulmonary chemoreceptor's that are acutely sensitive to CO_2, but insensitive to hypoxia or anoxia (Ludders, 2001), stimulation of the said receptors leads to a suppression of

breathing via the vagus nerve. The extent and speed of the inhibition depends on the inhaled CO_2 concentration or the blood partial pressure of CO_2.

Zeller et al. (1988) also described a rapid fall of blood pressure and bradycardia which point to a direct toxic effect of CO_2 on heart function; this can be related to the flapping around of the animals, comparable with the situation of the so-called flip over of broilers (indication of heart failure). These immediate effects of direct exposure to very high CO_2 concentrations explains why no significant rise occurred in venous blood partial pressure of CO_2, because gas exchange stopped almost immediately. There was a trend to a lower degree of oxygen saturation in venous blood; this change was probably more pronounced in arterial blood. Although the trends were present, the duration of time during which the animals were killed was probably too short to observe other significant blood changes in relation to the acid-base balance.

Gassing in a plexibox (experiment 2), characterised by exposure to ***gradually rising CO_2 and falling O_2 concentrations***, caused a mild metabolic acidosis in the animals (by definition a primary HCO_3^- reduction coupled with a fall of pH) in venous blood. Under this condition the animal will try to compensate for this metabolic acidosis by an increase in the volume of breath per minute. However, owing to the high concentration of CO_2 in the inhaled air, the partial pressure of CO_2 will rise dramatically, whereby breathing will be suppressed more rapidly. This was reflected in the deep slow breathing of the animals in this experiment.

The rapid fall in the blood pH observed in our experiment caused a fall in pH of the cerebrospinal fluid and intracellular in the brain cells, as previously reported in dogs and pigs (Eisele, 1967; Martoft et al., 2003). Blood pH and the cerebrospinal fluid pH moreover appear to be strongly correlated. The said fall of pH has been shown to cause anaesthetic effect, since CO_2 is capable of suppressing nerve cell function and cerebral electrical activity (Eisele, 1967). Attaining this pH threshold value that causes anaesthetic effect may coincide with the loss of balance and the closing of the animal's eyes (after 50 seconds); this type of behaviour will indicate a loss of consciousness (Raj et al., 1998).

As well as the said acidosis, there was also a clear effect of associated *hypoxia*, which translated into a significant fall in partial pressure of O_2 and oxygen saturation. Hypoxia normally causes a rising stroke volume and heartbeat. In terms of behaviour however, no increase in the speed of breathing could be observed, which may point to interference with the consequences of the high CO_2 concentrations. Presumably, the hypoxia only increased after the loss of consciousness and this occurred only at the end of bradycardia and arrhythmia. This finally led to heart failure and the animals died on their backs in experiments 1 and 2 wich is another indication of heart failure.

Animals that were subjected to gradually increase in CO_2 concentration continued to breathe for a time, causing a significant fall in partial pressure of O_2 due to a prolonged exposure to low O_2 concentrations. Animals that were immediately exposed to very high concentration of CO_2 / low concentration of O_2 presumably suffered immediate breathing arrest and heart failure. Similar blood changes were noted in turkeys and ducks (Gerritzen et al., 2006), although absolute blood values differed strongly from those of broilers as higher CO_2 concentrations were needed to reach a loss of consciousness.

Typical consequence of rapid gassing (experiment 2) was the rapid sequence of behaviour-related changes that can be linked to the attainment of a clearly defined O_2/CO_2 concentration. A similar sequence of behaviour-related changes was described by Webster and Fletcher (2001) and by Gerritzen et al. (2004), although the timing of the occurrence of this behaviour differed appreciably from that of slow CO_2 build-up (Gerritzen et al., 2004). In addition, we were able to establish clearly in our study that the occurrence of the loss of balance, which may be a first indication of loss of consciousness, preceded the phase of convulsions. This could not be established in the large test group used by Gerritzen et al. (2004).

Slow gassing (experiment 3) showed the same sequence of behaviour-related changes over a longer period, but with the difference that the animals died slowly lying on their stomachs. The loss of consciousness occurred after approximately 420 seconds at an 0_2 concentration of 17.7% and a CO_2 concentration of 15.7%, exactly the same CO_2 concentration which Gerritzen et al. (2004) quoted for reaching loss of consciousness. In the case of shed gassing, the attainment of 40-45% CO_2 concentration and its maintenance for 30 minutes has been quoted as adequate for killing animals (Gerritzen et al. 2006). This corresponds to 12.6 – 11.5% O_2. In our experiment the animals died on their stomachs after a gradual build-up of CO_2 to a final concentration of 32.1% (O_2 14.2 %) after approximately 20 minutes.

The slow CO_2 gassing resulted in very pronounced *metabolic acidosis*, which was characterised by a fall of pH and also by strong falls of the buffering systems (bicarbonate and non-bicarbonate buffer bases). In addition, there was a strongly increase in lactate concentration in the blood. The very low level of oxygen saturation indicated a *severe deficit of oxygen.*

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