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Short communication

A note on behaviour of poultry exposed to increasing carbon dioxide concentrations

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Abstract

Killing poultry by means of whole house gassing with carbon dioxide (CO_2) is an important tool in disease control. The behaviour of ducks, broilers, laying hens and turkeys was observed to assess differences in susceptibility between species and to assess animal welfare implications following exposure to CO_2 treatment. All birds were individually exposed to CO_2 concentrations, which increased from 0% to 45% at a rate of 14 l/min. The results discussed in this paper suggest that there are slight to occasionally significant differences, which might also be age related between different poultry species exposed to whole house gassing with CO_2. However, these differences are not so substantial so as to make it necessary to set different welfare criteria in relation to whole house gassing for the examined species.

Keywords: Disease control; Carbon dioxide; Killing; Broilers; Laying hens; Ducks; Turkeys

1. Introduction

The regulation for the control of HPAI viruses is governed by EU Council Directive 92/40/EEC within the European Union (EU). Killing and removal (culling) of infected poultry flocks (and other possibly uninfected flocks in the immediate surrounding geographical area) is the most important control measure currently employed to stop the spreading of such disease. For disease control purposes, carbon dioxide (CO_2) is often used in on farm killing of large groups of poultry, in both mobile gas container units and whole house gassing exercises. An epidemic of Avian

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Influenza occurred in the Netherlands during 2003 and the great majority of the birds that were eliminated, were killed by whole house gassing with carbon dioxide (Gerritzen et al., 2006a). Compared to mobile killing devices, whole house gassing techniques increases the killing capacity enormously, which facilitates the clearance of infected farms more rapidly. Observations in practice during whole house gassing of broilers and laying hens have indicated that 40% carbon dioxide in inhaled air is sufficient to kill all chickens within 30 min.

Inducing unconsciousness with CO_2 is associated with breathlessness, hyperventilation (Raj and Gregory, 1995) and irritation of the nasal mucosa (Iwarsson and Rehbinder, 1993). The suitability of CO_2 for killing ducks has been questioned based on the assumption that diving birds, including ducks, are less sensitive to asphyxia and hypoxia and that they possess physiological mechanisms enabling them to withstand hypercapnia (Hawkins, 2001). However, electro-physiological measurements and blood-gas values confirmed that white Peking ducks and turkeys loose consciousness and die at comparable CO_2 concentrations as broilers and laying hens (Gerritzen et al., 2006b). Despite the physiological comparability, it is not clear if effects on animal welfare between different species can be easily compared.

The objectives of the study reported here was to: (a) examine differences in behavioural reactions of different poultry species, i.e. chickens, ducks and turkeys, and (b) of different ages, i.e. broilers versus laying hens, to slowly increasing CO_2 concentrations in an attempt to assess issues of animal welfare.

2. Materials and methods

2.1. Animals

At the poultry facilities of the Animal Sciences Group (ASG), Lelystad, The Netherlands 25 2-week-old White Peking ducks (Anas platyrhynchos), 25 3-week-old turkeys (Meleagris gallopavo), 25 1-week-old broilers (Gallus Domesticus) and 25 40-week-old laying hens (Gallus Domesticus) were raised under similar conditions to those routinely used in the poultry industry. All of the animals were housed on litter and had ad libitum access to a commercial feed and water. Experimental trials started when the animals were 6–7 weeks of age and at 42 weeks of age for the laying hens. Ethical aspects of the experiment were considered and approved by the animal ethical committee of the ASG.

2.2. Experimental design

Forty subjects were selected randomly for the experimental treatments comprised of 10 animals from each of the four groups of poultry. Individual animals were placed in a cuboidal Perspex test box measuring 0.8 m × 0.8 m × 0.8 m and subsequently exposed to slowly increasing CO_2 concentrations. The test box was fitted with a 1-cm∅ CO_2 inlet 5 cm above the base of the box, a CO_2 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. Carbon dioxide from a 100% CO_2 source was released at a rate of 14 l/min into the test box until the air at the measuring point consisted of a 40% CO_2 concentration and the birds remained in this CO_2 environment until they died. The moment animals died 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 followed by complete relaxation.

2.3. Measuring behaviour

The behavioural profile of the birds was recorded continuously during the procedures using a digital video camera (Sony camcorder, IP7E). The video recordings were analyzed afterwards (Table 1) using

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 backwards
Sit	Sagging trough their 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

behavioural observation analysis software (Etholog, version 2.2). The total number of behavioural events, the CO_2 concentration level where these events started and the number of replications within the various behavioural events (while the birds were still conscious) were used to assess the impact on animal welfare.

2.4. 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).

3. Results and discussion

In this study, we investigated the effects of slowly increasing CO_2 concentration (from 0% to 45%) on the behaviour of broilers, laying hens, ducks and turkeys. The purpose of the trials was to determine the effects on animal welfare and to determine if there were differences between ducks, turkeys, broilers and layers with regard to exposure to increasing CO_2 concentration. If there are differences in sensitivity between age (broilers versus layers) and/or species (ducks, chickens, turkeys) in this regard, this could infer the need to set different criteria for different groups of poultry during whole house gassing.

The loss of posture of the subjects (indicating loss of consciousness) occurred in broilers at 19.0% CO_2, which was significantly different than in ducks (23.8% CO_2), while loss of posture occurred in layers and turkeys at 19.9% and 19.3% CO_2 (Table 2), respectively, in the inhaled air in the test box, which occurred within 4 min. The first signs that animals noticed a change in their environment, i.e. an increase in CO_2, was recorded from as little as 2% CO_2 in the air within the test box for broilers, ducks and turkeys which was at a significant ($p < 0.05$) lower concentrations than for laying hens (6.6% CO_2). This behaviour was manifest as a more alert attitude, i.e. looking around and walking to and fro within the test box. However, while this was obviously a sign of detecting changes in the breathing air, it does not prove conclusively that it was specifically a detection of CO_2 by the subjects. In the case of chickens, it has been reported

Table 2
CO_2 concentration (%) in the breathing air at the start of behavioural events

Parameter	Broiler	Duck	Layer	Turkey	$p(F)$[a]	SED
Notice	2.4 a	1.5 a	6.6 b	2.3 a	0.001	1.1
Breath	5.6 ab	2.8 a	8.0 b	4.1 a	0.034	1.7
Headshake	8.3 a	5.1 a	13.0 b	5.5 a	0.001	1.9
Gasp	9.2 a	9.5 a	14.0 b	6.4 a	0.004	1.9
Sit	10.2 a	11.8 ab	16.0 b	10.4 ab	0.143	2.6
Jump	13.2 a	11.5 a	18.6 b	9.2 a	0.002	2.2
Convulsions	30.0 a	24.0 a	28.0 a	26.0 a	0.447	4.7
Loss of posture	19.0 a	23.8 b	19.9 ab	19.3 a	0.023	2.3

Means within a row with no common letters are significantly different at $p < 0.05$ using students *t*-test. Data presented are ANOVA means.

[a] ANOVA significance levels of the *F*-test for the factorial effects; d.f. 3,32.

that they can detect CO_2 at a 10% level (McKeegan et al., 2005). The first behavioural signs of any physiological effects of CO_2, which typically shows as heavier breathing started between approximately 3% and 8% CO_2 depending on the species. Carbon dioxide is the most important chemical stimulator for respiration and an increase in CO_2 concentration will increase pCO_2 in the blood, which is subsequently detected by the ‘‘respiratory centre’’ in the medulla oblongata and ultimately leading to an increased rate of breathing in order to lower the pCO_2—typical homeostasis (Guyton and Hall, 2000). Heavier breathing therefore is not necessarily associated with reduced welfare but may be simply a physiological reaction.

Shortly after the onset of heavy breathing, at 5–8% CO_2 for broilers, ducks and turkeys and at a significant higher ($p < 0.05$) level of 13.0% CO_2 for layers first headshaking behaviour occurred followed by gasping at approximately 6.4–9.5% CO_2 for ducks broilers and turkeys and at 14% CO_2 ($p = 0.004$) in the breathing air for layers. Any interpretation of the effect of headshaking on animal welfare is controversial; it has been described as indicative of an aversive reaction to CO_2 and respiratory distress (Webster and Fletcher, 2001) or as an alerting response or an attempt to regain an alert state (Hughes, 1983). Gasping is in many cases almost directly followed by headshaking, especially when birds are in a sitting posture. It is likely that when birds are feeling dizzy and start fainting they want to regain alertness and headshaking may follow as a result. Furthermore, CO_2 appears to have a stimulating effect on headshaking (Gerritzen et al., 2000) and is dose dependent (McKeegan et al., 2005). Therefore, it cannot be excluded that headshaking is related to reduced welfare. Gasping or very deep breathing has been described as a respiratory distress associated with breathlessness (Raj and Gregory, 1995) and interpreted as a state that can be qualified as atleast very unpleasant and can therefore also be associated with reduced welfare. Furthermore, headshaking and gasping are repetitive up to, or even after, the moment of loss of consciousness and thus is not an instant event, and as such may indicate a period of reduced welfare. Animals changed to a sitting position, which is related to a reduced level of balance, at approximately 11–16% CO_2. In most cases, after a short time this sitting position was followed by a strong jump followed by returning to the sitting position again. Ducks changed more often to a sitting position than the other three species although, this was not significantly different from broilers. It is most likely that the jumps were attempts to keep control over their body position. During this period, the animals were still considered to have been aware of their environment and thus this period could be classified as at least having an impact on animal welfare. At on average 20% CO_2, which was reached within approximately 4 min,

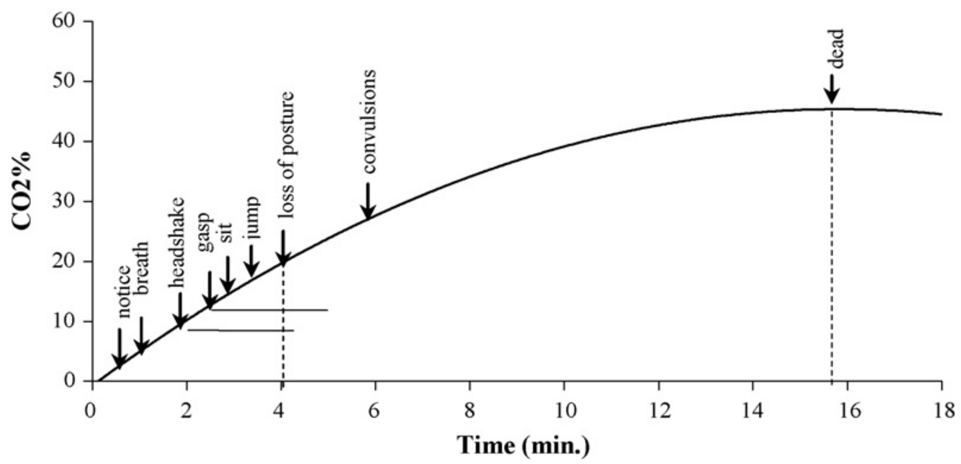

Fig. 1. Schematic overview of the increase of the CO_2 concentration in time and the onset of the behavioural parameters (average of all animals from the four groups). (1) The first vertical dotted line indicates the moment of loss of consciousness. (2) The second vertical dotted line marks the moment all animals are dead. (3) Lines parallel with the x-axis indica.

animals lost posture and thus their consciousness (Gerritzen et al., 2004). Behavioural changes that can be associated with breathlessness like deep breathing and gasping, with aversive or unpleasant experiences like headshaking, and with reduced control like sitting–jumping all occurred in a period of 2.5–3 min before the loss of consciousness.

The order in which behavioural events occurred and the time (min) and CO_2 concentration (%) at which they were observed is schematically presented in Fig. 1. Since the order of occurrence for all eight behavioural parameters was the same for all groups and although there are some significant differences, they are presented as an average of all birds of the different groups. The schedule suggests that birds exhibited alerting reactions (notice) from approximately 3.2% CO_2 in the air within the test box. Deeper breathing (breath) started at CO_2 concentrations of approximately 5% followed by headshaking at 8% and gasping at 9.8%. Shortly after gasping, the birds adopted a sitting position, and from this position they jumped up and returned to a sitting position again. Loss of posture occurred at approximately 20.5% CO_2 in the air within the test box, which was followed by convulsions in a small number of the birds at approximately 27% CO_2 penetration of the air. The duration of the period between first notice CO_2 penetration of the air and loss of posture was approximately 3 min. The duration of the period of headshaking and gasping before loss of posture was approximately 2 min (Fig. 1). Therefore, the timeframe for the occurrence of potentially reduced welfare levels was at maximum 3 min long. After the loss of posture, between approximately 25% and 30% CO_2, a few convulsions occurred in a small number of animals (Table 2). But, these convulsions had no impact on animal welfare as the started only after the loss of posture and thus after the birds had lost consciousness.

The resistance to use CO_2 for stunning or killing is based on experiences of killing animals in an atmosphere of atleast 60% CO_2 concentration. Such high concentrations of CO_2 are irritating to the nasal mucosa and constitute an unpleasant stimulus in humans (Hari et al., 1997) and other animal species (Raj and Gregory, 1994; Leach et al., 2004). During whole house gassings, as simulated in this experiment, CO_2 concentrations increase gradually and animals do not experience such high CO_2 concentrations while conscious. When using low or gradually increasing CO_2 infusions, the time to loss of posture and thus to loss of consciousness, may therefore be lengthened as a result, but the loss of consciousness will be smoother (Lambooij et al., 1999; Gerritzen et al., 2000). From an animal welfare perspective, it is always important to reduce the period in which an animal's welfare might be affected in a negative way. However, during whole house gassings used for emergency killing of chicken flocks, the CO_2 flow may be technically limited due to the maximum available flow from the mobile gaseous source such as the tanker or lorry. This has implications for practical purposes, as it may be difficult to reduce the period between starting the CO_2 inflow and loss of consciousness in the poultry unit. There is an obvious difficulty in that any killing of animals has the propensity to conflict with animal welfare. To make an informed decision regarding the acceptability of any killing method, all aspects regarding the method and welfare issues of the species involved must be considered. The advantages associated with whole house gassings include the large capacity and the fact that animals are not handled alive. These rewards are important enough to make whole house gassing, if executed properly, an acceptable method in emergency disease control.

4. Conclusion

The findings presented here show that there were slight and some significant behavioural differences between the poultry species (and age) examined with regard to killing by means of gassing with carbon dioxide. However, the behavioural differences, as reported in the current study were not of such dimensions that make it necessary to set different criteria for chickens, turkeys or ducks in relation to culling with whole house gassing techniques.

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