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Behavioural responses during exposure of broiler chickens to different gas mixtures

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Abstract

An alternative stunning system to the generally applied electrical water bath stunner for broiler chickens is gas stunning. This method of stunning can eliminate the stress associated with uncrating and/or shackling of live birds prior to electrical stunning. Behavioural responses to inhalation of gas were examined during exposure of broilers to different gas mixtures. In total, 137 six-week-old broiler chickens were individually immersed in a gas chamber containing one of the following gas mixtures: (a) 90% Ar/air, (b) 30% CO_2/60% Ar/air or (c) 40% CO_2/30% O_2/30% N_2. The birds moved freely or were restrained. Behavioural parameters were recorded on video and analysed for gasping, headshaking, wing flapping and loss of posture. The number of gasps before loss of posture declined progressively on exposure to gas mixtures Ar/air or Ar/CO_2/air or CO_2/O_2/N_2, respectively, and remained high after loss of posture in the latter gas mixture. Gasping occurred rarely in broilers during exposure to gas mixture Ar/air. When gas mixture CO_2/O_2/N_2 was used the loss of posture was significantly delayed compared to the gas mixture Ar/air and Ar/CO_2/air. The number of headshakes and wing flapping was significantly higher in the restrained groups of birds compared to the free moving group. Wing flapping was low before and after loss of posture in gas mixture CO_2/O_2/N_2. It is concluded that during the immersion in gas mixtures broilers show gasps, head shakes and wing flapping which start before loss of posture, which may cause some distress. However, it can be argued that gas stunning compared to water bath stunning is preferred in practical applications, because the live broilers do not need to be uncrated and/or shackled. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

A basic requirement for humane slaughter of animals including poultry is that they should be stunned, rendered unconscious and insensible, prior to slaughter by exsanguination. Under commercial conditions, although red meat animals (horses, ruminants and pigs) are stunned using either a penetrating captive bolt, non-penetrating concussion bolt, electric current or carbon dioxide gas (pigs only), poultry species are stunned using electrified water bath stunners only (Council Directive, 1993). There are two main reasons for this common practice in poultry. Firstly, it may be difficult to apply captive or concussion bolt owing to a very small target area (head) for shooting in poultry without compromising bird welfare under commercial conditions. Secondly, the throughput rates required in medium to large chicken processing plants (killing five to nine thousand birds per hour) can be easily achieved with water bath stunners rather than with captive bolt or concussion stunners.

An alternative stunning system to the generally applied electrical water bath stunner for broiler chickens is gas stunning. This method of stunning can eliminate the stress associated with uncrating and/or shackling of live birds prior to electrical stunning. A lot of gas mixtures can be used to stun or kill animals, however, for slaughter animals the use is restricted. At present, the gas mixtures 90% Ar (argon) in air, 30% CO_2 (carbon dioxide) and 60% Ar in air and 40% CO_2 and 30% O_2 (oxygen) and 30% N_2 (nitrogen) are used for poultry in a few plants under commercial conditions.

The used gasses can be divided in gasses, such as N_2 or Ar, that displace O_2 from the air to be breathed and gasses, such as CO_2 (in concentrations higher than 12%), that affect directly the central nervous system (Boogaard et al., 1985). It is well recognised that CO_2 is an anaesthetic gas which produces rapid unconsciousness when inhaled at high concentrations (Forslid, 1987); however, signs of asphyxia and behavioural excitation are observed due to occurrence of both hypercapnia and hypoxia (Boogaard et al., 1985; Forslid, 1987). Moreover, it is an acidic gas and has been found to be painful, causing unpleasant sensations on the nasal mucosa, lips and forehead in human subjects, when gas puffed stimuli are administered in concentrations over 65%. The right hemisphere of the human SII cortex is dominantly involved in this response, which may suggest emotional/motivational aspects of trigeminal pain, and is in agreement with the role of the trigeminal pathways as a general warning system (Kobal and Hummel, 1985; Huttunen et al., 1986; Hari et al., 1997). Exposure could be stressful to animals as it may impart a sense of breathlessness (Raj et al., 1992; Raj and Gregory, 1993, 1995) and stimulate a warning system. In rats, a low concentration of CO_2 and addition of O_2 and use of humidified gases could ameliorate the negative effects. In that case, almost no signs of asphyxia and excitation were observed. The main action of CO_2 is not its suffocating activity, but its anaesthetic activity (Coenen et al., 1995). A problem with O_2-replacers is its lower efficacy in younger animals (Glass et al., 1944; Wooley and Gentle, 1988). However, Ar can be easily administered in gas stunning, because it is heavier than air (as is CO_2), tasteless and odourless. Another option is a low concentration of CO_2 in Ar. Research has shown that both last mentioned gas mixtures caused a rapid loss of brain function in chickens (Raj and Gregory, 1991; Raj et al., 1992), turkeys (Raj and Gregory, 1993, 1994; Raj, 1996) and pigs (Raj and Gregory, 1995).

The aim of this study was to determine differences in the spontaneous behaviour in broiler chickens when immersed in the different gas mixtures. In this study, the behavioural responses of free (non-restrained), restrained, or equipped with brain electrodes and restrained birds, during exposure to gas mixtures are presented.

2. Materials and methods

2.1. Experimental set up

The required number of birds was delivered at the institute and lairaged with water and fed 2 or 3 days before the experiment. The day before the experiment, a number of 28 birds were separated and 9 of them were equipped with electrodes for measuring the brain activity as part of another experiment. Feed was withdrawn 4 h before surgery and 4 h before the experiment, while water remained available. The birds were randomly distributed over the experimental groups, where all groups were equally used each day of experiments. In total, 137 six-week-old broiler chickens were individually immersed for 2 min in a gas chamber containing one of the following gas mixtures: (a) 90% Ar in air, (b) 30% CO_2 and 60% Ar in air and (c) 40% CO_2 and 30% O_2 and 30% N_2. BOC Gases (Belgium) delivered the used gases in bottles. The different gas mixtures were humidified before entering the gas chamber. The gasses CO_2 and O_2 (Servomex analyser, Series 1400, Zoetermeer, Netherlands) and humidity (Testoterm, type 4510, Almere, Netherlands) were recorded just before immersion, at 40 s and at 90 s during immersion. (Gas was pumped to the analyser for approximately 30 s, and after that period the display showed the value.) The size of the Perspex gas chamber was $70 \times 70 \times 70$ cm where the opening to allow the birds in the chamber was 35×35 cm. The valve was just opened to let the bird in.

The number of birds immersed in gas mixtures a, b and c was 44, 45 and 48, respectively. The birds (a) moved freely ($n = 47$) in the gas chamber, as a control for restrained, or (b) were restrained by hand on both sides of the breast and legs ($n = 39$), as a control for equipped with brain electrodes, or (c) were equipped with brain electrodes and restrained by hand ($n = 51$). The results of the brain measurements are presented in a different paper by Raj et al. (1998).

Behavioural parameters were recorded on video and analysed for gasping, head shaking, wing flapping and loss of posture. The behavioural parameters scored in this study were defined as follows:

- Headshake: broiler shakes its head
- Gasp: broiler takes a deep breath through the wide-open mouth, which may involve stretching of the neck and vocalisation
- Wing flapping: broiler shows severe wing flapping
- Loss of posture: broiler is unable to maintain sitting position and neck tension and may fall on its side or back; a behavioural indicator of the onset of unconsciousness (Raj et al., 1992; Raj and Gregory, 1995)

2.2. Statistical analysis

Observations are either count data or time intervals. In addition, count data will be reduced to binary data, to study the proportions of animals showing the different types of behaviour. For each type of data the statistical model and statistical techniques employed will be discussed below.

Count data, e.g., number of gasps before loss of posture, were analysed with a log–linear model (McCullagh and Nelder, 1989). Main effects and interactions for the three gasses, for electrodes fitted or not and for animals restrained or not, were introduced on the log-scale (McCullagh and Nelder, 1989). This implies that multiplicative effects were assumed, rather than additive effects. The variances of the counts were taken to be proportional to the corresponding means μ: $\mathrm{Var}(y) = \phi * \mu$, i.e., data were analysed as overdispersed Poisson counts. Effects on the log-scale were estimated by the method of quasi-likelihood (McCullagh and Nelder, 1989). The dispersion parameter ϕ was estimated from Pearson's generalised chi-square statistic: $\phi = X^2/d$, where $X^2 = \Sigma(y-\hat{y})^2/\hat{y}$ is Pearson's chi-square statistic with associated number of degrees of freedom d (McCullagh and Nelder, 1989).

$\hat{y}$ time intervals, e.g., time-to-loss of posture, were also analysed with a log–linear model. Now, variances were assumed to be proportional to the square of the mean: $\mathrm{Var}(y) = \phi * \mu^2$, i.e., a constant coefficient of variation ϕ (McCullagh and Nelder, 1989). In this instance, the quasi-likelihood method is equivalent to the maximum likelihood method under assumption of a gamma distribution. Time-to-loss of posture was mildly censored on the right-hand side. This variable was also analysed assuming a truncated log–normal distribution, employing an EM-algorithm (Taylor, 1973) for maximum likelihood estimation under normality, as implemented in Genstat 5 procedure CENSOR (Lane, 1994). Obviously, when the type of behaviour studied did not occur, there was no corresponding interval in the data set. To study the proportions of animals showing the type of behaviour, separate analyses were performed where counts were reduced to binary data: 0, when the behaviour was not observed or 1, when the behaviour was observed (i.e., when the count was larger than 0).

For binary data, a logistic regression model was assumed (McCullagh and Nelder, 1989). Now, we focus on probabilities under the various experimental conditions, e.g., the probability that an unrestrained animal without electrodes will show severe wing flapping for Ar. Probabilities are between 0 and 1, which impedes straightforward use of main effects and interactions. Therefore, the probabilities are 'stretched' before effects are introduced: $\mathrm{logit}(p) = \log(p/(1-p))$ = combination of main effects and interactions, for a probability p. Here, $\mathrm{Var}(y) = p(1-p)$, i.e., small variation at the extremes and large variation around 0.5. Effects were estimated by the maximum likelihood method. Maximum likelihood is a large sample technique. For gasping and head shaking, Fisher's exact test (Yates, 1984), employing Genstat 5 procedure FEXACT2X2 (Ridout and Patefield, 1995) was performed as an additional back up for a simplified model.

Finally, some technical remarks. For counts obtained after loss of posture, the analysis is affected by the large number of observations equal to 0. Reduced models are apparently more stable. For example, for gasping after loss of posture, most observations

Table 1
Number (mean ± s.d.) of gasps, headshakes and wing flapping of broiler chickens during exposure to gas mixtures in a box when free moving or restrained by hand and equipped with brain electrodes

	Gas	Animals (n)	Before loss of posture			After loss of posture		
			Gasps (n)	Head-shakes (n)	Wing flapping (n)	Gasps (n)	Head-shakes (n)	Wing flapping (n)
Free	Ar	16	0.1 ± 0.3^{a}	1.6 ± 1.0^{a}	0.1 ± 0.5^{a}	0 ± 0^{a}	1.1 ± 1.1^{a}	2.9 ± 1.3^{a}
	$Ar + CO_2$	15	3.1 ± 1.9^{b}	2.8 ± 1.7^{ab}	0.9 ± 1.0^{b}	0.1 ± 0.2^{a}	0.2 ± 0.7^{b}	2.4 ± 1.5^{a}
	$CO_2 + O_2$	16	8.8 ± 3.0^{c}	4.4 ± 2.1^{b}	0.1 ± 0.3^{a}	6.8 ± 5.0^{b}	0.2 ± 0.5^{b}	0.4 ± 1.0^{b}
Restrained	Ar	9	0.1 ± 0.3^{a}	3.6 ± 1.7^{a}	1.1 ± 0.9^{a}	0.3 ± 0.7^{a}	2.0 ± 1.9^{a}	1.8 ± 1.7^{a}
	$Ar + CO_2$	9	4.1 ± 1.4^{b}	2.0 ± 1.2^{b}	2.0 ± 1.7^{b}	0 ± 0^{a}	0.4 ± 0.7^{b}	1.4 ± 1.3^{a}
	$CO_2 + O_2$	14	11.1 ± 3.8^{c}	2.6 ± 2.8^{ab}	0.4 ± 0.6^{c}	7.4 ± 4.9^{b}	0.4 ± 1.1^{b}	0.1 ± 0.3^{b}
Restrained + electrodes	Ar	14	0.5 ± 1.0^{a}	3.4 ± 1.9^{a}	1.4 ± 0.9^{a}	0.5 ± 0.8^{a}	0.8 ± 1.3^{a}	0.8 ± 1.0
	$Ar + CO_2$	13	4.8 ± 1.0^{b}	3.5 ± 2.6^{ab}	1.8 ± 1.2^{b}	0 ± 0^{a}	0.3 ± 0.5^{b}	0.1 ± 0.4
	$CO_2 + O_2$	16	7.5 ± 1.8^{c}	1.5 ± 1.1^{b}	0.2 ± 0.4^{c}	8.4 ± 7.7^{b}	0.3 ± 0.5^{b}	0.4 ± 0.7

Means with a different superscript differ significantly $p < 0.05$, according the analysis of variance model.

Table 2
Start of the behavioural parameter gasps, headshakes, wing flapping before loss of posture of broiler chickens in seconds (mean ± s.d.) after exposure to gas mixtures and the percentage of animals that show the behaviour

	Gas	Animals (n)	Start of behaviour after immersion in the gas			Loss of posture (s)	Percentage of animals that show the behaviour		
			Gasps (s)	Head-shakes (s)	Wing flapping (s)		Gasps (%)	Head-shakes (%)	Wing flapping (%)
Free	Ar	16	9 ± 0^{a}	8 ± 4^{a}	7 ± 8^{a}	15.6 ± 3.6^{a}	6.3^{a}	87.5	18.8^{a}
	$Ar + CO_2$	15	4 ± 1^{b}	6 ± 6^{ab}	10 ± 5^{a}	16.7 ± 5.2^{a}	73.3^{b}	93.3	53.3^{b}
	$CO_2 + O_2$	16	3 ± 1^{b}	5 ± 2^{b}	32 ± 5^{c}	31.6 ± 9.3^{b}	100.0^{b}	100.0	12.5^{c}
Restrained	Ar	9	19 ± 0^{a}	7 ± 4^{ab}	12 ± 6^{a}	24.6 ± 12.1^{a}	11.1^{a}	100.0	55.6^{a}
	$Ar + CO_2$	9	4 ± 2^{b}	4 ± 3^{a}	9 ± 6^{b}	22.8 ± 10.8^{a}	100.0^{b}	88.9	88.9^{b}
	$CO_2 + O_2$	14	4 ± 1^{b}	10 ± 7^{b}	10 ± 7^{c}	42.3 ± 12.8^{b}	100.0^{b}	71.4	28.6^{c}
Restrained + electrodes	Ar	14	16 ± 9^{a}	13 ± 9	12 ± 8^{a}	29.5 ± 10.9^{a}	21.4^{a}	100.0	85.7^{a}
	$Ar + CO_2$	13	5 ± 3^{b}	10 ± 9	10 ± 6^{b}	26.8 ± 5.5^{a}	100.0^{b}	84.6	92.3^{b}
	$CO_2 + O_2$	16	4 ± 1^{b}	9 ± 6	11 ± 9^{c}	34.2 ± 11.5^{b}	100.0^{b}	75.0	25.0^{c}

Means with a different superscript differ significantly $p < 0.05$, according the analysis of variance model.

for Ar and Ar/CO_2 are equal to zero. This leads to very large effects and standard errors on the log-scale. Test results (tentatively assuming that these are not seriously affected) suggest that neither effect of electrodes or restrain is significant. The same conclusion follows when the analysis is restricted to data corresponding to gas mixture $CO_2/O_2/N_2$ only. Consequently, the model was reduced to main-effects for gas mixtures only. The dispersion factor in the reduced model is 2.4 (0.32). The reduced model is far less affected by the zero values, although the dispersion factor appears to be deflated: a separate estimate for gas mixture $CO_2/O_2/N_2$ is 4.5 (0.98). Differences between gas mixtures are marked and significant for this larger estimate as well.

All calculations were performed with the statistical programming language (Genstat 5 Committee, 1993).

3. Results

The measured (mean $\pm$ s.e.) percentages in the different gas mixtures were in (a) Ar/air an O_2 percentage of $1.4 \pm 0.2\%$, (b) a CO_2 and O_2 percentage of $29.8 \pm 0.2\%$ and $1.3 \pm 0.1\%$, respectively, and (c) a CO_2 and O_2 percentage of $40.3 \pm 0.1\%$ and $31.8 \pm 0.2\%$, respectively. The humidity during exposure of the different gas mixtures in the gas chamber was $16 \pm 1.3\%$, $15 \pm 1.5\%$ and $76 \pm 2.3\%$, respectively. When the birds were not killed by the gas mixtures, i.e., mixture c, they were exsanguinated as soon as possible after leaving the gas chamber.

The results of the analysis of behaviour are summarised in the Tables 1 and 2. In restrained birds, the position of the animal in relation to the video picture sometimes made it difficult or impossible to score the time to loss of posture. Free moving birds were easy to score. It should be noted that loss of posture in particular was delayed ($P = 0.00$) in the restrained birds compared with free moving birds. When gas mixture c was used, the loss of posture was significantly ($P = 0.05$; $P = 0.00$) delayed compared to the gas mixtures a and b (Table 2). Gasping occurred rarely in broilers during exposure to gas mixture a and the chance to show the behaviour is lower ($P = 0.00$). There was a significant ($P = 0.00$) increase in the number of gasps before loss of posture in gas mixtures a, b and c, respectively, and remained significantly ($P = 0.00$) higher in gas mixture c after loss of posture (Table 1). This difference was consistent in broilers irrespective of whether they were free moving or restrained, however, there was a significance ($P = 0.00$) between restrained and restrained and equipped with brain electrodes groups of birds. These last birds showed more ($P = 0.00$) gasps, too. The number of head shakes and wing flapping was higher ($P = 0.01$; $P = 0.00$) in the restrained groups of birds compared to free moving birds. Moreover, the start of both behaviours was earlier ($P = 0.03$; $P = 0.02$). Wing flapping was significantly ($P < 0.05$) lower before and after loss of posture in gas mixture c compared to a and b.

4. Discussion

Exposure of birds to 90% Ar in air or 60% Ar/30% CO_2 in air results in an anoxic condition. Under these conditions depression of activity in the brain extends progressively from the telencephalon to the diencephalon and then to the mesencephalon. Anoxia results in suppression of the rostral reticular formation and therefore loss of consciousness and in suppression of the caudal reticular formation and therefore onset of convulsions (Ernsting, 1965). Wing flapping is observed before the onset of loss of posture when exposed to Ar or Ar/CO_2 gas mixtures (Tables 1 and 2). This pointed to the suggestion that wing flapping during immersion in the gas might be a response to an anoxic condition. This may be supported by a study of physiological and behavioural responses in adult hens and chicks. When subjected to decreasing oxygen concentration adult birds slowly became unconscious, without showing any signs of distress, until respiratory failure supervened. Chicks showed similar results but loss of motor control was observed while still conscious, which might cause distress (Wooley and Gentle, 1988).

Exposure of birds to concentrations of CO_2 saturates the tissues with CO_2, which causes depression of cell function, including neurones. There is partial blockage of impulses across synaptic junctions (Katz et al., 1963). The direct effects of CO_2 on the neurones are an increase in the cell membrane potential and cessation of spontaneous activity. This results in a desynchronisation and lowered amplitude and frequency of electrocortical activity, finally resulting in an isoelectric trace (Bauer, 1982). As expected, in our study the birds show a delayed loss of posture when O_2 was added to the gas mixture. This phenomenon may point to a main anoxic effect of the gas mixtures Ar/air or Ar/CO_2 /air. The behavioural response to the different gas mixtures was different. The number of gasps before loss of posture declined progressively on exposure to the gas mixtures Ar/air, Ar/CO_2/air or CO_2/O_2/N_2, respectively. In all gas mixtures gasps, headshakes and wing flapping were observed before and after loss of posture, except wing flapping in CO_2/O_2/N_2. It may be that these behavioural parameters are related to the increase of CO_2 and/or decrease in O_2 in the body of the bird.

When humans are subjected to high concentrations of CO_2 it induces breathlessness and it can be pungent (Gregory et al., 1990). The perception of a CO_2 puff ($> 65\%$) stimulus delivered to the nostrils was an abruptly starting pungent pain which decayed slowly during a couple of seconds (Kobal and Hummel, 1985; Huttunen et al., 1986; Hari et al., 1997). After exposure of pigs to high concentrations of 80 to 90% of CO_2 the pigs remain quiet for the first 10 to 20 s and show motoric movements the next 10 s (Forslid, 1987). It is difficult to perceive what the pig experiences during this induction period. At best, it may be mildly unpleasant. At worst, it could be unpleasant. Following the immersion of CO_2 the pigs are well stunned and remain so provided they are stuck within 1 min of stunning. For stunning of pigs, high concentrations of 80 to 90% of CO_2 are recommended, because of rapid induction of anaesthesia (Lambooij, 1990). To prevent breathlessness in hens during exposure Hoenderken et al. (1994) recommended a gas mixture of CO_2 and O_2 and use of a humidified gas mixture. However, Zeller et al. (1988) reported that broilers showed the same response when exposed to a gas mixture of 50% CO_2 and 50% O_2 as compared to high concentrations of CO_2 in air. The motoric activity (wing flapping) and the number of birds that showed the behaviour were low using the gas mixture CO_2/O_2/N_2 which suggest an effect of O_2.

The use of a low concentration CO_2 in Ar combines the anaesthetic and anoxic effects. Different experiments were conducted to examine if there are aversive responses of different species to this combination. Experiments in pigs showed that the majority of the subjects did not show any aversion to the presence of 30% CO_2 in the box (Raj and Gregory, 1995). This mixture induces a more rapid loss of brain function in turkeys compared to 90% Ar in air, and it also reduces the effect of the pungency associated with high concentrations of CO_2 in air (Raj and Gregory, 1993; Raj, 1996). The susceptibility of the turkeys brain to the toxic nature of the CO_2/Ar mixture appeared to be similar to that of hens, as it has been reported that hens lost somatotrope evoked responses in 19 s during exposure (Raj et al., 1992). Our experiments with broilers are also in agreement, because the birds lost posture in the same time schedule, however, it was not more rapid than in 90% Ar in air (Table 2).

For the application of most stunning methods it is necessary to restrain the animal. The effectiveness of any method of preslaughter stunning can be seriously impaired by improper application of the restraining device to the animal (Lambooij, 1992). It is suggested that shackling is potentially painful and the welfare of poultry at shackling may be compromised (Sparrey and Kettlewell, 1994). Under practical conditions broilers may be free moving when stunned in a gas chamber. However, for physiological measurements in our study they had to be restrained. The results showed that restraining the bird might affect the behavioural parameters and speed of stunning when immersed in a gas mixture.

In summary, the results indicated that during the exposure to the different gas mixtures broilers showed gasps, headshakes and wing flapping which were affected by restraining and were started before loss of posture. It is likely that these behavioural responses pointed to some distress of the birds. However, it may be assumed that shackling and hanging upside down cause much more stress and it is preferred to use one of the studied gas mixtures for practical applications.

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