Behavioral Responses of Broilers to Different Gaseous Atmospheres

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

The six test groups were divided into four experimental groups that were exposed to gas mixtures used for stunning and two control groups that were exposed to atmospheric air. The different gas mixtures and their concentrations were a) air, no flow (control–); b) circulating air, flowing (control+); c) >90% Ar in air; d) 60% CO_2 in air; e) 40% CO_2 and 30% O_2 in air; and f) 70% Ar and 30% CO_2 in air. The behavior of the broilers before entering the gas tunnel, the number of birds that moved into the gas mixture, and the behavior in the gas mixture were recorded on video and analyzed afterward.

No differences among the groups were observed in the number of broilers that walked into the gas tunnel or in the number of birds that tried to return to the cage. Exposure of broilers to the 70% Ar and 30% CO_2 mixture resulted in the fastest loss of posture. The number of broilers exhibiting headshaking and gasping was least in the >90% Ar in air mixture. Convulsions were rarely seen in the 40% CO_2 and 30% O_2 mixture; the other gas mixtures resulted in severe convulsions.

The experiment did not indicate that broilers could detect or avoid increased CO_2 or decreased O_2 levels when they come into contact with such atmospheres for the first time.

(*Key words*: behavior, broilers, argon, carbon dioxide, gas stunning)

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INTRODUCTION

The EU Council Directive (1993) on the protection of animals at the time of slaughter states that animals should be stunned, rendered unconscious and insensible, prior to slaughter by exsanguination. Unconsciousness should be induced as soon as possible without any detrimental effect to the welfare of the animal or to the quality of the meat. The state of unconsciousness should be long enough to ensure that the animals do not recover before death occurs by exsanguination. The traditional method of poultry slaughtering in the EU involves shackling of live birds followed by a stun to kill procedure using a waterbath.

In a waterbath stunner, a minimum current of 120 mA per bird is required to irreversibly abolish the brain function and induce cardiac arrest in at least 90% of the birds (Gregory and Wotton, 1990). This minimum current for stunning broilers, proposed by the Council of Europe, increases the quality defects such as hemorrhages and broken bones of carcasses and broiler meat (Veerkamp and de Vries, 1983; Gregory and Wilkins, 1989). The increase in quality defects results in substantial economic losses to the poultry industry (Alisch and Obdam, 1992). It is apparent that the two objectives (animal welfare and meat quality) are in conflict when using the electrical waterbath stunning system for broilers.

An alternative method for broiler chickens is gas stunning, which can eliminate the stress associated with uncrating, or shackling, or both, of live birds before electrical stunning (Lambooij et al., 1999). Because gas stunning or killing does not immediately render the birds unconscious (Raj et al., 1998), the induction of anesthesia should be very smooth, capable of inducing a rapid loss of consciousness, or the best compromise of these criteria.

Gases used for stunning slaughter animals can be divided into gases that displace oxygen from the breathing air, such as Ar and N, and gases that directly affect the central nervous system, such as CO_2 (van den Bogaard et al., 1985). Signs of asphyxia and behavioral excitation have been observed because of both hypercapnia and hypoxia (van den Bogaard et al., 1985; Forslid et al., 1986). It is well known that CO_2 is an anesthetic gas that produces rapid unconsciousness when inhaled at high concentrations (Forslid et al., 1986; Barford and Madsen, 1988). However, CO_2 is an acidic gas and has been found to be painful, causing unpleasant sensations on the nasal mucosa, lips, and forehead in humans when administered in concentrations >65% (Hari et al., 1997). Exposure to CO_2 could be stressful

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to pigs (Hoenderken et al., 1983) and poultry (Raj et al., 1992) because it may impart a sense of breathlessness. The addition of O_2 and humidification of the gas (Coenen et al., 1995) has been shown to ameliorate the negative effects of CO_2 on rats. Similar results have been reported for poultry (Hoenderken et al., 1994, Lambooij et al., 1999).

Anoxia induced by Ar is an alternative to CO_2 gas stunning. Argon can be easily administered in a gas stunning system because it is heavier than air (as is CO_2). Moreover, Ar is tasteless and odorless and may not impart any sense of unpleasantness during the induction phase (Raj and Gregory, 1991). Another option is a low concentration of CO_2 in Ar. Research has shown that both of the last-mentioned gas mixtures caused a rapid loss of brain function in chicken (Raj and Gregory, 1991; Raj et al., 1992), turkeys (Raj and Gregory, 1993, 1994; Raj, 1996), and pigs (Raj and Gregory, 1995). When either of these methods is applied, birds experience convulsions (Raj et al., 1991; Lambooij et al., 1999).

The aim of this study was to determine the different responses of broilers on inhalation of gas mixtures containing CO_2 or CO_2 with O_2, Ar, or a mixture of Ar and CO_2 when they come into contact with these gas mixtures for the first time.

MATERIALS AND METHODS

Experimental Protocol

On the first day of this experiment, 209 5-wk-old broiler chickens were randomly distributed over six test groups. The broilers were housed in a commercial rearing house (floor pen) where they were accustomed to finding their feed during the light period of the light regimen (1.5 h light, 1.5 h dark). The gas mixtures were randomly distributed in four or five test periods (during a light period) per day over 5 d of 1 wk. During these test periods, broilers were tested individually. The animals were the experimental units, and the observations on the animals were independent.

The six test groups were divided into two control groups and four experimental groups. The test gases and their concentrations were a) atmospheric air, no flow (control –); b) circulating air, flow similar to the flow used for the gas mixtures (control +); c) >90% Ar in air; d) 60% CO_2 in air; e) 40% CO_2 and 30% O_2 in air (humidified mixture); and f) 70% Ar and 30% CO_2. The number of birds used in test group a, b, c, d, e, and f were 38, 43, 28, 36, 35, and 29, respectively.

On experimental days, the broilers were separated from the rest of the flock and individually placed in a 0.7-m × 0.7-m cage that was connected with a descending tunnel at an angle of 7° and was 1 m high, 0.7 m wide, and 5 m long. Location of the tunnel at a lower level was to allow the retention of the dense gases, carbon dioxide and argon. The cage and tunnel were placed adjacent to the floor pen. The left sides of the cage and tunnel were placed against a wall. The right side of the tunnel had a plexiglass wall. Broilers in the cage could hear and see their companions outside the descending tunnel, which was the stimulus for the broilers in the cage to enter the gas tunnel. All of the activity in the broilerhouse (other broilers, people, and equipment) was to the right of the cage. The various gas mixtures were supplied to the tunnel until a desired level was established inside the tunnel. Carbon dioxide and O_2 concentrations were recorded (Servomex analyzer, Series 1400[2]) 30 s before and 1 and 2 min after the start of each trial. When incorrect gas conditions were measured, data of these tests were excluded from the analysis (one broiler excluded in the >90% Ar group, two broilers excluded in the 60% CO_2 group, two broilers excluded in the 70% Ar and 30% CO_2 group). The relative humidity in the tunnel was monitored directly before and after a broiler was exposed to the gas tunnel (Testoterm, type 4510[3]).

Behavioral Parameters

The behavior of the broilers was recorded on videotape and analyzed afterward using behavioral observation analysis software (The Observer, version 3.0).[4] The behavioral parameters scored in the cage before entering the tunnel were body positions, such as sitting or standing, and the facing direction, such as to the wall, forward, or to the open side. Time until entering the tunnel, the distance that birds moved into the tunnel, and turning back from the tunnel to the cage were the parameters used to determine whether broilers could detect and avoid the different gas concentrations. The behavioral parameters scored in the gas tunnel were headshaking, gasping, sagging, loss of posture, and convulsions. These parameters can be defined as follows: headshake, broiler shakes head; light gasp, broiler takes a deep breath; heavy gasp, broiler takes a very deep breath together with stretching of the neck and, sometimes, vocalization; sagging, broiler is unable to stand on its legs and sits down; loss of posture, broiler is unable to maintain sitting position and neck tension and, in most cases, falls on its side or back; convulsions, severe wing flapping.

Because it was not possible to score the headshaking and gasping of broilers with convulsions, headshaking and gasping were scored until the convulsions started. When no convulsions were observed, headshaking and gasping were scored for 60 s.

Approval for this experiment was obtained from the committee on animal care in research of the Institute for Animal Science and Health.[5]

Statistical Analysis

To analyze the number of broilers that entered the tunnel, data were transformed into binary data. The binary

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variable Y denotes whether an animal enters the tunnel (Y = 1) or not (Y = 0). Suppose that P is the probability that an animal enters the tunnel, i.e., P is the probability that Y equals 1. A probability is difficult to model because it is limited to the range 0 to 1. Therefore, it is customary in the model to transform the probabilities, to escape from the boundaries 0 and 1. This transformation is carried out with the Probit link function, which is the inverse of the standard normal probability function. Effects are included for experimental day, group, light regime, and gas mixture. The model is an example of a generalized linear model (McCullagh and Neder, 1989). The likelihood ratio statistic is applied to test for interactions and main effects (McCullagh and Neder, 1989). Additional pairwise comparisons are based on approximate normality of the estimators on the probit scale.

In subsequent analyses, data were restricted to those animals that entered the tunnel. Continuous variables, such as time to start of a behavioral parameter and the duration of a certain behavior, were analyzed with an analysis of variance model. Count data, such as number of broilers that show certain behavior, were analyzed using a log-linear model (McCullagh and Neder, 1989). In this model, effects were introduced on the logarithmic scale. An overdispersion factor was introduced, which was estimated from Pearson's chi-square statistic (McCullagh and Neder, 1989). Estimated effects on the logarithmic scale were transformed back to multiplicative effects on the original scale. All statistical calculations were done with Genstat 5 (1993).

RESULTS

During the experiment the average temperature in the broilerhouse was 25 C (ranging from 23 to 27 C). The average relative humidity was 72% (ranging from 68 to 75%). Relative humidity, CO_2, and O_2 concentrations in the tunnel are presented in Table 1.

Initially, interaction terms were included in the model, but because they were not significant or hardly ever sizeable, they were dropped from the model in later analyses. The results of the analysis of body position and facing direction in the cage before entering the gas tunnel and the percentage of birds that entered the gas tunnel are presented in Table 2. There were no differences ($P > 0.05$) found between the test groups in behavior of the broilers in the cage or the number of birds that entered the gas tunnel. The time taken for the broilers to move out of the cage and into the tunnel and the distances the birds moved into the tunnel (measured 10 s before and 10 s after loss of posture) are presented in Table 3. There were no significant differences among the groups in entering time or in the distance broilers walked into the gas tunnel.

Behavior of the broilers after they had entered the gas tunnel is presented in the Tables 4 and 5. The incidence of headshaking in the broilers was significantly higher in the control+ group compared with the control– group. The lowest number of broilers exhibiting headshaking was observed in the Ar group, followed by the 60% CO_2 and 70% Ar and 30% CO_2 groups and was highest in the 40% CO_2 and 30% O_2 group. Exposure to >90% Ar caused no gasping (including no light gasps). The number of broilers exhibiting heavy and light gasping was higher ($P < 0.05$) in the three gas mixtures containing CO_2. Convulsions did not occur in either control groups and was only observed for one bird in the 40% CO_2 and 30% O_2 mixture. All of the broilers in the >90% Ar, 60% CO_2, and 70% Ar and 30% CO_2 groups had convulsions. In the >90% Ar and 40% CO_2 and 30% O_2 groups, the onset of convulsions was later ($P < 0.05$) later than that in the 60% CO_2 and the 70% Ar and 30% CO_2 groups. Sagging occurred significantly earlier in the experimental groups than in the control groups. Loss of posture occurred first in the 70% Ar and 30% CO_2 group followed by both the >90% Ar and the 60% CO_2 groups and occurred last in the 40% CO_2 and 30% O_2 group.

DISCUSSION

Our study with the six different gas mixtures revealed no differences in the number of birds entering the gas-filled tunnel. Moreover, the percentage of broilers of the control groups entering the tunnel did not differ from those of the experimental gas mixtures. Raj and Gregory (1991) concluded that hens can detect an atmosphere containing increased concentrations of CO_2 or decreased concentrations of O_2, and, given a free choice, they learned to avoid such atmospheres. We found no indication that the broilers avoided or could detect increased CO_2 or decreased O_2 levels when they come into contact with such atmospheres for the first time. Raj and Gregory (1991) suggested that the unpleasantness of the stunning gases

TABLE 1. Conditions during the experiments: relative humidity (%) and O_2 and CO_2 concentrations (%) of the gas mixtures in the gas tunnel

			Gas concentrations					
			Before		After 1 min		After 2 min	
Gas mixture	n	Humidity	O_2	CO_2	O_2	CO_2	O_2	CO_2
Control–	38	71 ± 3	. . .	. . .	. . .	. . .	. . .	. . .
Control+	43	73 ± 2	. . .	. . .	. . .	. . .	. . .	. . .
>90% Ar	28	38 ± 5	1.1 ± 0.2	0.0 ± 0.0	3.6 ± 1.3	0.0 ± 0.0	2.8 ± 0.8	0.0 ± 0.0
60% CO_2	36	40 ± 7	6.3 ± 0.7	67.6 ± 3.3	7.7 ± 1.3	61.6 ± 6.7	8.1 ± 0.5	58.7 ± 2.7
40% CO_2 and 30% O_2	35	51 ± 8	33.6 ± 2,5	41.3 ± 1.5	31.8 ± 2.4	38.0 ± 4.9	31.3 ± 2.7	42.9 ± 3.4
70% Ar and 30% CO_2	29	31 ± 6	1.5 ± 0.4	30.6 ± 1.9	3.6 ± 1.2	30.0 ± 1.9	2.5 ± 0.7	30.9 ± 1.5

TABLE 2. Body position and facing direction of broiler chickens before entering the tunnel and the percentage of animals that entered the tunnel

Gas mixture	n	Body position		Facing toward			Entering tunnel
		Sitting	Standing	Wall	Forward	Open side	
		(%)					
Control−	38	38	62	0	62	38	45
Control+	43	48	52	0	68	32	42
>90% Ar	28	47	53	16	54	32	32
60% CO_2	36	42	58	8	59	33	33
40% CO_2 and 30% O_2	35	25	75	6	56	38	54
70% Ar and 30% CO_2	29	29	71	7	57	36	52

was less severe than the social pressure of being alone. Broilers in our experiment did not try to return to the cage, the companion birds on the other side of the transparent wall were perhaps seen as a safer situation. Fear-related behavior patterns can be contradictory, such as active avoidance in contrast with immobility or freezing (Boissy, 1995). Freezing can be an explanation of not trying to escape from the experimental gas mixtures. In the control groups, sagging refers to the moment broilers actually sit down. (Sometimes control broilers started walking in the tunnel again after they had sat down.) Sagging occurred much earlier in the experimental gas mixtures than in the control groups. Therefore, sagging in the experimental gas groups refers to the moment broilers can no longer stand on their legs as a result of the anesthetic effect of the gas mixture. When the perception of the presence of a gas as threatening comes too late, the anesthetic effect of the gas makes it impossible for them to turn back to the cage.

The distance traveled by the broilers through the gas tunnel was similar across experimental groups. Once entering the tunnel, the distance covered when loss of posture was observed was the result of various parameters, such as the anesthetic on brain and related muscle function (uncoordinated movement) during the period prior to or at loss of posture. The time between entering the tunnel and loss of posture also affects the distance traveled by the broiler in the tunnel; the longer the time until loss of posture occurred, the longer the time, and, thereby, the distance that the broiler could have entered the tunnel. Unable to separate these effects, it was not possible to draw conclusions on the negative effect of the gas mixtures based on the travel distance of the bird in the gas tunnel.

The negative effects of a stressor on the animal's welfare are dependent on the severity and duration of the stressor. Therefore, a very smooth induction of anesthesia using a gas mixture or a rapid induction of loss of consciousness is essential to ensure minimum levels of distress in live birds. Loss of posture is a behavioral indicator of the onset of unconsciousness (Raj et al., 1992; Raj and Gregory, 1995). An increase in the level of O_2 could considerably lengthen the time until loss of posture (Lambooij et al., 1999). The combination of CO_2 and Ar results in the shortest period between sagging and loss of posture and, thus, the shortest induction period until the onset of unconsciousness.

When humans are subjected to high concentrations of CO_2, breathlessness is induced; it also can be pungent (Gregory et al., 1990). The perception of the stimulus is an abruptly starting pungent pain, which decays slowly over a couple of seconds (Hari et al., 1997). Gasping and headshaking are seen as indicators of breathlessness and the pungency of gas mixtures. Furthermore, headshaking starts earlier, and the number of headshakes is increased, by the flow of air compared with stagnant air. The period between the onset of headshaking and loss of posture was shortest in the 60% CO_2 group and the 70% Ar and 30% CO_2 group.

Gasping in the control groups might have been the result of the high temperature and the high relative humidity in the broiler house. If the number of heavy gasps per broiler were corrected for the time between onset of gasping and loss of posture (in other words, the period of gasping), the number of heavy gasps per second per bird is significantly lower in the 40% CO_2 and 30% O_2 group than in the other gas mixture groups. The period between onset of gasping and loss of posture was shortest in the 70% Ar and 30% CO_2 group and longest in the 40% CO_2 and 30% O_2 group. The addition of O_2 to CO_2 and humidification of the gas mixture reduced the intensity of the negative effects of

TABLE 3. Time (s) that broilers entered the tunnel after opening the tunnel and the average distance (m) the birds moved into the tunnel (mean ± SD) at 10 s before and 10 s after loss of posture

Gas mixture	n	Entering tunnel	Average distance in tunnel at 10 s	
			Before loss of posture	After loss of posture
		(s)	(m)	
Control−	17	18 ± 12	. . .	. . .
Control+	18	17 ± 11	. . .	. . .
>90% Ar	9	14 ± 9	0.4 ± 0.4	0.8 ± 0.6
60% CO_2	12	11 ± 10	0.6 ± 0.8	1.0 ± 0.8
40% CO_2 and 30% O_2	19	14 ± 12	0.7 ± 0.6	0.9 ± 0.5
70% Ar and 30% CO_2	15	13 ± 10	0.5 ± 0.8	1.3 ± 0.7

TABLE 4. Number of broilers showing the behavioral parameters headshaking, heavy and light gasping, and convulsions and the average number (mean ± SD) of the behavioral parameters headshaking and heavy and light gasping

		Headshaking		Gasping				Convulsions
				Heavy		Light		
Gas mixture	Animals	Broilers	Average	Broilers	Average	Broilers	Average	Broilers
	(n)							
Control–	17	7[c]	1 ± 1	0[b]	0	2[b]	0 ± 1	0[b]
Control+	18	18[a]	2 ± 2	0[b]	0	2[b]	1 ± 2	0[b]
>90% Ar	9	4[c]	1 ± 1	0[b]	0	0[b]	0	9[a]
60% CO_2	12	11[b]	2 ± 2	8[a]	1 ± 1	12[a]	4 ± 2	12[a]
40% CO_2 and 30% O_2	19	19[a]	2 ± 1	11[a]	1 ± 1	19[a]	5 ± 3	1[b]
70% Ar and 30% CO_2	15	14[b]	2 ± 2	8[a]	1 ± 2	14[a]	2 ± 1	15[a]

[a–c]Values with a different superscript differ ($P < 0.05$) according to the logistic regression model.

CO_2. This result is in agreement with Hoenderken et al. (1994) who recommended a gas mixture of CO_2 and O_2 and humidification of the gas mixture to prevent breathlessness in hens during exposure to CO_2.

When subjected to decreasing O_2 concentrations, adult birds gradually became unconscious, without showing any signs of distress, until respiratory failure supervened. Chicks showed similar results, but loss of motor control was observed while the chicks were still conscious, which might cause some distress (Woolley and Gentle, 1988). Broilers exposed to >90% Ar were observed to exhibit some headshaking before loss of posture, but no gasping was observed.

Severe convulsions were observed when broilers were exposed to gas mixtures containing high levels of CO_2 or Ar. The convulsions started a few seconds after loss of posture. In the 40% CO_2 and 30% O_2 group, only one broiler showed one short convulsion, which was just before loss of posture. The presence of O_2 in a gas mixture seemed to reduce the number and severity of convulsions significantly.

For the application of most stunning methods, it is necessary to restrain the animals. 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). Animals may be free moving when stunned in a gas chamber.

In this experiment, there were no indications that broilers can detect or avoid increased CO_2 or decreased O_2 levels when they come into contact with such atmospheres for the first time. Gasping by broilers exposed to gaseous atmospheres containing CO_2 can be reduced by the addition of O_2. For broilers under all experimental gas mixtures, headshaking occurred before loss of posture. Broilers exposed to gas mixtures containing Ar or high concentrations of CO_2 were observed to have severe convulsions just after loss of posture. It is likely that these behavioral responses pointed to some distress for the birds. However, it is possible that shackling and hanging upside down causes as much or much more stress, and it may be preferable to use one of the studied gas mixtures for practical applications.

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TABLE 5. Onset of behavioral parameters in seconds (mean ± SD) after entering the gas tunnel

Gas mixture	n	Sagging	Loss of posture	Headshaking	Gasping	Convulsions
		(s)				
Control–	17	50 ± 16[a]	. . .	22 ± 21	17 ± 10[a]	. . .
Control+	18	56 ± 26[a]	. . .	13 ± 17	26 ± 11[a]	. . .
>90% Ar	9	15 ± 6[b]	21 ± 8[b]	9 ± 7	. . .	25 ± 11[a]
60% CO_2	12	10 ± 4[b]	17 ± 5[b]	3 ± 3	6 ± 3[b]	19 ± 7[b]
40% CO_2 and 30% O_2	19	13 ± 3[b]	30 ± 8[a]	3 ± 2	7 ± 3[b]	25*
70% Ar and 30% CO_2	15	9 ± 2[b]	12 ± 2[c]	3 ± 2	5 ± 2[b]	15 ± 2[b]

[a–c]Means with a different superscript differ ($P < 0.05$) according to the analysis of variance model.

*Excluded from analysis; only one observation.

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