Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Applied Animal Behaviour Science 88 (2004) 275–287
APPLIED ANIMAL BEHAVIOUR SCIENCE
www.elsevier.com/locate/applanim

Assessment of the aversion of hens to different gas atmospheres using an approach-avoidance test

A. Bruce Webster*, Daniel L. Fletcher

Department of Poultry Science, The University of Georgia, Athens, GA 30602, USA

Received 6 October 2003; received in revised form 21 January 2004; accepted 6 April 2004

Abstract

Approach-avoidance tests were conducted to evaluate the responses of laying hens to different gas atmospheres. Twelve hens were trained in a test apparatus consisting of a raised entry chamber connected by a descending chute to a lower chamber into which gas could be injected. Feed was removed from hens overnight before training sessions, and layer feed was presented in the lower chamber as the stimulus to motivate hens to proceed through the apparatus. Once hens were trained to a stable minimum time taken to eat after being placed in the apparatus, approach-avoidance tests were initiated. These resembled training sessions except that they were conducted in mid-afternoon, with feed having been removed from the hens in the morning, and one of six gas treatments was injected into the lower chamber. The gas atmospheres evaluated were: air, 30% CO_2-in-air, 45% CO_2-in-air, 60% CO_2-in-air, 70% Ar/30% CO_2, and Ar. Each hen was tested once in random order with each atmosphere. Days of testing were alternated with days of retraining to minimize extinction of the learned response and the chance that exposure to a given atmosphere might affect a hen in its next test. Exposure to 45% CO_2-in-air and Ar appeared to elevate the time taken by hens to leave the upper chamber of the test apparatus in the subsequent retraining session, but the retraining appears to have extinguished any carryover effect because latencies to leave the upper chamber were small and not different between treatments during tests. Hens were evidently able to detect all the atmospheres enriched with CO_2, and the number of stops and retreats made during the approach to the lower chamber did not differ between these atmospheres. The control and Ar treatments had the lowest incidences of stops and retreats. Every hen but one was stunned on its first encounter with a CO_2-enriched atmosphere. Similarly, every hen but one was stunned in Ar. Overall, there were no significant differences among the stunning gas treatments in the percentages of hens stunned. Hens in the 30% CO_2-in-air treatment entered the lower chamber at almost the same frequency as in the Ar treatment. Most hens became stunned in the corridor when exposed to 60% CO_2-in-air, while the percentages of hens entering the lower chamber before being stunned were intermediate for 45% CO_2-in-air and 70% Ar/30% CO_2. From a behavioral standpoint, there does not appear to be a welfare

* Corresponding author. Tel.: +1-706-542-9154; fax: +1-706-542-8383.
E-mail address: bwebster@uga.edu (A.B. Webster).

doi:10.1016/j.applanim.2004.04.002

disadvantage to the use of mixtures of up to 60% CO_2-in-air to stun/kill chickens compared to 70% Ar/30% CO_2. The results do suggest a modest welfare advantage for Ar, but not one so substantial that it could not be readily balanced by other welfare related considerations.

Keywords: Gas stunning; Laying hen; Carbon dioxide; Argon; Aversion

1. Introduction

Argon (Ar), carbon dioxide (CO_2), nitrogen (N_2), and oxygen (O_2) have been evaluated by various research groups for gas stunning/killing of poultry. Ar and N_2 are essentially inert gases that displace O_2. The resulting anoxia quickly reduces a bird to unconsciousness. CO_2 and O_2 are not inert gases in that they are involved in gas exchange in the lungs and have direct effects on the physiology of animals. When used in sufficient concentration, CO_2 causes unconsciousness at a rate similar to that induced by Ar or N_2 anoxia (Mohan Raj et al., 1992b; Poole and Fletcher, 1995). Stunning by anoxia tends to cause convulsions (Blackshaw et al., 1988; Mohan Raj and Gregory, 1990a; Poole and Fletcher, 1995), which are unsightly and can cause a bird to physically damage itself.

CO_2 suppresses afferent transmission of sensory stimuli to the cortex of the brain, producing anesthesia (Forslid et al., 1986; Mohan Raj et al., 1992b; Raj and Gregory, 1993). It also can diminish convulsions associated with gas stunning and minimize the time needed to stun birds in atmospheres intended to achieve anoxia (Mohan Raj and Gregory, 1990a; Mohan Raj et al., 1992b; Raj and Whittington, 1995). It seems desirable, therefore, to include CO_2 in atmospheres to achieve anesthesia and suppression of motor activity during gas stunning. When mixed directly with air to produce a $CO_2/N_2/O_2$ atmosphere, lower concentrations of CO_2 are needed for stunning than is required for other gas mixtures that rely on reduction of O_2 to very low levels to be effective (Mohan Raj and Gregory, 1990b; Mohan Raj et al., 1992a).

Some researchers have expressed concern that a high concentration of CO_2 is undesirable for gas stunning of poultry because it may be unpleasant or distressing to the birds (Mohan Raj et al., 1992a,b; Raj and Gregory, 1993). They have suggested that Ar or a mixture of Ar and CO_2 may be more humane. Given sufficient exposure, hens learned to avoid areas where the CO_2 concentration was 7.5%, or the O_2 concentration was made 10% or less by dilution with Ar (Mohan Raj and Gregory, 1991). Aversion to the altered atmosphere was outweighed by aversion to a dominant hen, making it apparently less strong than aversive experiences a chicken might normally encounter. It is not clear whether the perceptual mechanisms involved in this learned response to CO_2-supplemented or O_2-depleted atmospheres would govern a hen's perceptions during acute exposure to high levels of CO_2 or very low levels of O_2, as would occur in gas stunning.

Recent studies comparing the behavior of chickens in different gas atmospheres noted that all atmospheres containing 30–60% CO_2 caused the same array of behavioral actions while the birds were still conscious (Lambooij et al., 1999; Webster and Fletcher, 2001), providing little reason to conclude on a behavioral basis that these atmospheres are greatly different in their impact on poultry welfare. Only Ar appeared to cause little reaction before the birds became impaired by anoxia. Both these studies immersed chickens in gas atmospheres, leaving the researchers only to speculate about aversion to the atmospheres based on the birds' reactions once in them.

A better assessment of aversion would be to record the responses of chickens encountering a gas stunning atmosphere in a situation where they are free to avoid the atmosphere. Gerritzen et al. (2000) found no evidence that broilers could detect or avoid gas stunning atmospheres with increased levels of CO_2 or Ar. It is possible that the social stimulus used by Gerritzen et al. (2000) caused approach motivations toward their gas tunnel strong enough to overwhelm aversion to any of the atmospheres tested, giving a false impression of the birds' ability to detect them. Other attractive stimuli might produce more moderate approach motivations that would allow expression of avoidance to disturbing stimuli. Since hunger can be varied according to length of time without eating, moderate food deprivation might induce food approach motivations that do not totally override any aversion that chickens might have to stunning gases. Therefore, approach-avoidance tests were conducted to evaluate the responses of laying hens to different stunning gas atmospheres using feed deprivation to motivate them to approach food placed in the atmospheres.

2. Materials and methods

2.1. Animals and housing

The hens were a commercial White Leghorn strain drawn from a flock kept at the University of Georgia. They were placed in battery cages at a rate of two birds per cage in a room adjacent to the room where the behavioral tests were conducted. Both rooms were environmentally controlled, and negative-pressure fan-ventilated. During all training sessions and behavioral tests, the test room was ventilated continuously to create a unidirectional air movement from inlets on the interior wall to the exhaust fans on the outside wall. Management of the hens was in accordance with the standard University of Georgia protocol, except as dictated by the requirements of behavioral training and testing, which are described below.

2.2. Test apparatus

The test apparatus consisted of two plywood chambers having interior dimensions of 46 cm $L \times$ 46 cm $W \times$ 46 cm H. One chamber was elevated 30 cm above the other and connected to it by a 122 cm corridor that sloped for the first 91 cm to the floor level of the second chamber, with the final 30 cm section serving as a platform from which a hen could enter the lower chamber. The corridor was constructed of plywood and was 20 cm wide with walls 46 cm high. The chamber openings were 20 cm $W \times$ 38 cm H. The floors of the upper chamber and corridor were given a coat of gray, skid-resistant paint to provide secure footing for the hens. The wall and floor junctions of the lower chamber were sealed with silicon caulk. The upper chamber and the corridor were covered with 0.6 cm mesh metal screening during training and testing. The lower chamber had a removable clear plexiglass cover that could be clamped down onto rubber edging to seal the top of the chamber during tests. Metal screening was placed over this plexiglass top to give a similar appearance as the rest of the apparatus from inside. Just inside the entrance to the lower chamber, a 1.9 cm PVC pipe penetrated the side wall 8 cm from the floor. This pipe functioned as the gas inlet. A 90° elbow on the end of the pipe directed incoming gas toward the floor. The entrance to the lower chamber was covered by two overlapping clear plastic sheets stapled to the inside wall on opposite sides of the entrance and along the top of the entrance to the midline. These sheets were intended to hinder gas escape and help keep the lower chamber filled with the appropriate gas during tests. They were not intended to prevent gas from infiltrating the lower portion of the corridor. Hens were able to see through the plastic sheets and could easily push through them to enter the chamber. To aid filling of the chamber with gas by allowing air to be forced out of the chamber as gas was injected, an exhaust pipe made of 1.9 cm PVC penetrated the back wall of the lower chamber at the top corner opposite the gas inlet. Gas flow into the lower chamber was controlled by adjustment of regulators placed on the source gas cylinders.

2.3. Training

To create the setting for approach-avoidance conflict, it was necessary to train hens to expect the presence of food in the lower chamber. Thus, if hungry, a hen would be motivated to approach the lower chamber to reach the food. During a behavioral test, this motivation to approach food would weigh against whatever motivation the hen had to avoid entering the lower chamber caused by aversion to the atmosphere in the chamber.

Twelve hens were trained in the apparatus. Feed was available in a small feeder placed at the end wall of the lower chamber during training and testing. Training proceeded in several stages. Cage mates were placed in the apparatus for periods of up to an hour on separate days. This continued until the hens had demonstrated willingness to walk from the upper chamber to the lower chamber and eat after having been left unfed in their cages overnight. In subsequent stages of training, feed was removed from hens the evening before training. On training mornings, the hens were placed individually into the upper chamber of the apparatus. The observer withdrew and monitored the bird's progress by means of video cameras positioned to cover the entire interior of the apparatus. Once hens demonstrated little hesitation to approach the feeder to eat, the plastic sheets were installed at the entrance to the lower chamber. When the hens overcame their hesitation to push through the entrance of the lower chamber, the training environment was brought to its final level of complexity by letting air from a gas cylinder into the lower chamber through tubing attached to the gas inlet. The passage of gas into the lower chamber was adjusted to a constant level for all events of training and testing. Hens were returned to their cages immediately after training and were fed when all training was done for the day. Training was considered complete when the hens achieved a stable, minimum time to travel from upper to lower chamber to eat.

2.4. Behavioral testing

Six gas treatments were evaluated: 1, air (control); 2, 30% CO_2-in-air; 3, 45% CO_2-in-air; 4, 60% CO_2-in-air; 5, 70% Ar/30% CO_2 mixture; 6, Ar. These were obtained pre-mixed from a local gas supply company. Each hen was tested once in random order with each gas atmosphere. All hens were tested once on each test day (two hens per test gas), thus behavioral tests were conducted on 6 days, for a total of 72 tests. To minimize extinction of the learned response and the chance that exposure to a given gas atmosphere might affect a hen's behavior in its next test, days of testing were alternated with days of retraining. Since testing and retraining days occurred in succession, the time of feed withdrawal was reduced to avoid the possibility of hens becoming underfed. For both behavioral tests and retraining sessions, feed was removed from the hens at about 8:00 am in the morning and testing or retraining began about 3:30 pm. Feed was restored to the hens after testing or retraining was completed for the day.

During all tests and retraining sessions, the long axis of the test apparatus was oriented toward the exterior wall of the room with the lower chamber located immediately in front of the room's exhaust fan. Thus, gas emerging from the apparatus would be drawn away from the upper chamber and removed from the room.

The procedure for behavioral tests and retraining sessions was as follows. Individual hens were carried in a closed cardboard box from the room where they were housed to the observation room immediately prior to their test or retraining. The lower chamber was flushed with the appropriate gas atmosphere and then the gas inflow was maintained at a lower level to keep the chamber filled. After a hen was placed into the upper chamber, the observer withdrew out of the hen's sight and watched the test on a television monitor. The hen's behavior was recorded onto videotape using three video cameras which together scanned the interior of the apparatus. The test continued until the hen became unconscious or until 150 s had passed. Unconscious hens were removed immediately after loss of posture. Hens in the Air treatment were removed immediately after reaching the feeder. Between tests, the plexiglass top of the lower chamber was removed and the whole apparatus was purged with air using an electric blower. All hens being retrained were given 120 s after they started eating. The occasional hen being retrained that had not entered the lower chamber within 150 s was placed into the lower chamber. Hens were carried in the cardboard box back to their home cages immediately following a test or retraining.

2.5. Data collection

Behavioral data were recorded from videotapes for both tests and retraining sessions. It was clear that hens came into contact with test gases in the corridor on their approach to the lower chamber, and some hens lost consciousness in the corridor before they could enter the chamber. Therefore, the measures recorded for the tests were: latency (i.e., time from the start of the test) to leave the upper chamber (UCH), the number of occasions the hen stopped for at least 1 s in its approach to the lower chamber (STOP), the number of occasions the hen backed up or turned around and retreated away from the lower chamber (RETREAT), and whether or not the hen was rendered unconscious (STUN), defined by loss of posture (Raj et al., 1998). The location of the hen at the time it lost posture was noted. Other than STUN, the same measures were recorded for the retraining sessions, with the addition of latencies to enter the lower chamber (LCH) and to eat (EAT). If a hen did not perform one of the timed actions during a test or retraining session, a maximum latency of 150 s was assigned. Two hens on one occasion each did not leave the upper chamber during a test. These tests and the next respective retraining sessions were excluded from the data analysis because it could not be said that the hens had been exposed to the test atmosphere.

2.6. Statistical analyses

For each hen, the latency to perform each action was subtracted from the average latency for the hen to perform the same action during its last five training sessions (one hen: last four sessions because it did not leave the start in the fifth-to-last training). The latency data were subjected to logarithmic transformation before analysis. STOP and RETREAT were subjected to square root transformation prior to analysis. For the sake of presentation, the data to follow are shown untransformed. Analyses of variance were calculated using the general linear models (GLM) procedure of SAS (1999). For the behavioral tests, sources of variation were partitioned for gas atmosphere, test, and hen. For retraining sessions, sources of variation were gas atmosphere in the preceding behavioral test, retraining session, and hen. There were too few degrees of freedom available to calculate variability for interactions among model factors so these were pooled within the residual variation to form the error term for the analysis. When the analysis of variance indicated a significant effect for gas atmosphere, the PDIFF option within the GLM procedure was used to determine the significance of differences between the gas atmosphere least squares means. Fisher's exact probability tests were calculated to compare atmospheres on a paired basis regarding the number of hens stunned and the number of hens that entered the lower chamber. The probability level for statistical significance was considered to be $P < 0.05$.

3. Results

Fig. 1 illustrates the response performance level achieved during the last stage of training. The sound of gas being injected into the lower chamber during training initially slowed most

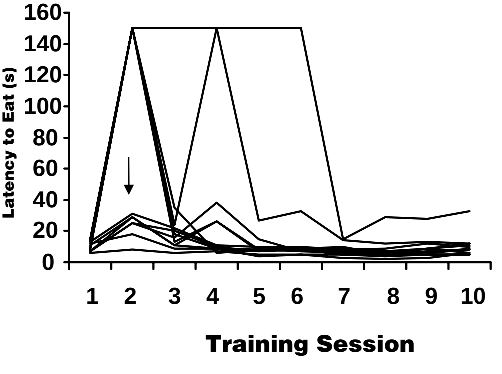

Fig. 1. Latency to eat (s) during the final training sessions as hens habituated to the sound of gas (compressed air) being injected into the lower chamber of the test apparatus. The first day of air injection is indicated by an arrow. Each line represents a different hen. A maximum latency of 150 s was assigned when a hen failed to eat during a training session.

Table 1
Behavior of hens during retraining sessions between approach-avoidance tests

Previous treatment	UCH (s)	LCH (s)	EAT (s)	STOP (*n*)	RETREAT (*n*)
Air	5.1 bc	4.7	4.0	0.5	0.2
30% CO_2-in-air	−1.3 bc	2.1	2.0	0.7	0.5
45% CO_2-in-air	34.5 a	34.7	34.9	1.1	0.2
60% CO_2-in-air	−2.6 c	4.4	4.7	0.8	0.3
70% Ar/30% CO_2	9.4 abc	12.7	13.8	0.9	0.2
Argon	24.4 ab	43.1	42.7	1.3	0.4
Pooled SEM	10.0	10.8	10.6	0.4	0.1

UCH, LCH, EAT = least squares means of latencies, respectively, to leave the upper chamber of the test apparatus, to enter the lower chamber of the test apparatus and to eat (the latency was calculated for each hen as the difference from its final average latency during training). STOP = number of times a hen stopped in the corridor during its approach to the lower chamber of the test apparatus. RETREAT = number of times a hen retreated toward the upper chamber during its approach to the lower chamber of the test apparatus. Means within columns sharing no common letters are significantly different ($P < 0.05$).

hens' approach toward feed, and caused several hens to avoid the lower chamber entirely. Many hens habituated quickly to the sound, but several took a number of days of training to adjust.

Table 1 shows the behavior of hens during retraining sessions after exposure to the indicated gas atmospheres in immediately previous approach-avoidance tests. Latencies to travel through the test apparatus appeared to be prolonged for hens that had been exposed to 45% CO_2-in-air or Ar in the previous test, but the variability of the data was such that statistically significant differences were evident only for UCH. UCH was greatest when hens had been exposed to 45% CO_2-in-air in the previous test, with the next greatest time occurring when Ar was the previous treatment. None of the remaining gas treatments differed from one another in their effects on behavior during retraining sessions. Hens left the upper chamber during a retraining session most quickly when the atmosphere in the previous test had been 60% CO_2-in-air. There were no statistically significant differences due to the last atmosphere experienced for STOP and RETREAT, but the numbers of stops were numerically highest when the previous gas exposure had been to 45% CO_2-in-air and Ar.

Data for the behavior of hens during the approach-avoidance tests are shown in Table 2. There were no significant differences among any of the atmospheres for UCH, i.e., the time taken for a hen to leave the upper chamber after having been placed into the test apparatus. There were, however, significant differences among atmospheres for STOP and RETREAT. The fewest stops and retreats occurred when compressed air or Ar was in the lower chamber. All the other gas treatments, i.e., those for which CO_2 was part of the atmosphere, increased a hen's tendency to stop in the corridor on approach to the lower chamber, but not differently so among these treatments. Numerically, the highest number of retreats occurred in tests with 45% CO_2-in-air and 70% Ar/30% CO_2.

Every hen but one was stunned on its first encounter with an atmosphere containing CO_2. The one hen that was not stunned during this initial encounter approached but did not enter the lower chamber during a 60% CO_2-in-air test, and then retreated in stages to the upper chamber. All hens were stunned in at least one CO_2-enriched atmosphere. Similarly,

Table 2
Behavior of hens during approach-avoidance tests involving different gas atmospheres

Gas treatment	UCH (s)	STOP (*n*)	RETREAT (*n*)	STUN (%)	LCH1 (%)	LCH2 (%)
Air	1.2	0.5 b	0.0 b	0 b	100 a	NA
30% CO_2-in-air	2.8	1.6 a	1.0 ab	82 a	73 ab	89 a
45% CO_2-in-air	2.4	1.7 a	1.6 a	67 a	33 b	50 ab
60% CO_2-in-air	1.1	1.8 a	1.0 ab	82 a	27 b	33 b
70% Ar/30% CO_2	1.8	1.8 a	1.7 a	73 a	45 b	62 ab
Argon	2.7	0.5 b	0.2 b	91 a	82 a	90 a
Pooled SEM	1.4	0.3	0.5	–	–	–

UCH, STOP and RETREAT defined as in Table 1. STUN, LCH1, LCH2 = percentages, respectively, of hens that lost posture, of hens that entered the lower chamber, and of hens which lost posture that entered the lower chamber. Means within columns sharing no common letters are significantly different ($P < 0.05$).

every hen but one was stunned in Ar. The hen not stunned in Ar, a different bird from the one not stunned in its initial encounter with a CO_2-enriched atmosphere, approached the lower chamber and retreated without stopping back to the upper chamber. No significant differences were evident among the atmospheres capable of stunning hens in the percentage of hens actually stunned (Table 2). All of the hens entered the lower chamber when tested with air. Most, but not all, hens entered the lower chamber when tested with 30% CO_2-in-air or Ar. The percentage of hens entering the lower chamber was significantly reduced when they were tested with the higher levels of CO_2-in-air or with 70% Ar/30% CO_2. These percentages did not differ significantly from one another. Similarly, among the hens that became stunned during tests, most entered the lower chamber before losing posture when tested with 30% CO_2-in-air or Ar. The fewest entered the lower chamber when it contained 60% CO_2-in-air, and intermediate percentages did so in the presence of 45% CO_2-in-air or 70% Ar/30% CO_2.

4. Discussion

With hens being exposed to more than one stunning gas atmosphere, there is the possibility that effects of a given stunning atmosphere could carry over to influence the results of a test with another atmosphere. Carryover effects might be due to failure to fully recover physiologically between tests, or due to learned responses arising from perceptions associated with recovery from unconsciousness or from exposure to the stunning atmosphere itself. It seems unlikely that hens would not have recovered physiologically between tests. Zeller et al. (1988) observed substantial changes in partial pressures of O_2 and CO_2, pH, and potassium of arterial blood, and in EEG record when chickens were stunned with mixtures of CO_2 in air ranging from 30 to 60%, but these measures returned to normal 10 min after the birds were returned to fresh air. Recovery from anoxia in Ar may be even faster than recovery from hypercapnia in stunning mixtures of CO_2 in air (Mohan Raj and Gregory, 1990a,b). Adding O_2 to CO_2-enriched atmospheres to induce anesthesia or euthanasia has been found to cause lung damage in rats and mice (Danneman et al., 1997; Ambrose et al., 2000). Such an effect of O_2 does not appear to have been investigated in chickens. Simple mixing of CO_2 in air to produce a stunning atmosphere, as was done in the present study, is much less likely to cause lung damage in mice, probably because the damage appears to stem from effects of high partial pressures of O_2 in the lungs (Ambrose et al., 2000).

UCH, i.e., the latency to leave the upper chamber, measured the willingness of a hen to move toward the lower chamber before it made contact with the atmosphere below. A change in UCH shown during retraining after a test could indicate aversion learned during exposure to a given atmosphere. It is possible that learned effects did carry over from exposure to 45% CO_2-in-air and Ar because UCH appeared elevated in retraining sessions after tests with these atmospheres and similar patterns were noted in other behavioral measures, although these were not statistically significant. The reason for such a carryover effect with these atmospheres and not with the other stunning atmospheres is not known. The retraining appears to have extinguished any carryover effect because UCH was small during tests and did not differ between treatments. This indicates that hens were not influenced at the start of the test by whatever gas atmosphere was in the lower chamber at the time, and that they were not anticipating an aversive experience upon leaving the upper chamber.

During tests with the modified gas atmospheres, the number of stops made by hens on their way down the corridor was greater for atmospheres that contained CO_2. Retreats were increased for the 45% CO_2-in-air and 70% Ar/30% CO_2 atmospheres. Hens, thus, were able to detect the presence of CO_2 and tended to hesitate as a result. McKeegan et al. (2003) observed an aversion threshold to CO_2 of 24% in air for chickens. All the CO_2-enriched atmospheres in the present study were above this threshold so the behavior observed could be explained as aversion-related responses to the CO_2. What the hen perceived to generate this aversion is not known at this point. McKeegan et al. (2003) noted that evidence of aversion did not depend on the stimulus being perceived as painful. Tests by Anton et al. (1992) revealed pain thresholds in the human nasal mucosa at average CO_2 concentrations of 47%. If the pain threshold in comparable tissue of the chicken is similar, two CO_2-enriched atmospheres in this study would be below the threshold concentration, one would be near it, and one would be above the pain threshold. However, discomfort due to nociception did not appear to govern the observed aversion to CO_2-enriched atmospheres in the present study because the hens' responses did not differ greatly between the different atmospheres. It is possible that the behavior of the chickens might have reduced the potential nociceptive differences of the CO_2-enriched atmospheres. Measurements of nociception caused by CO_2 have generally involved stimulation of nasal mucosa (Cain and Murphy, 1980; Kobal, 1985; Anton et al., 1992). Chickens quickly start mouth breathing in CO_2-enriched atmospheres (personal observation), which might minimize nociception in the nasal tissue.

The physiological effects of the stunning atmospheres probably interacted with the tendency to hesitate to determine the location where hens lost posture. The mixtures of CO_2 in air provided different levels of hypercapnic hypoxia, the mixture of Ar and CO_2 provided hypercapnic anoxia, and Ar provided anoxia. Hypercapnia produces anesthesia (Danneman et al., 1997; Coenen et al., 2000), and gives a bird the appearance of being sedated during the process of stunning (Webster and Fletcher, 2001). Since the timelines of physiological effects caused by CO_2 are dose related (Zeller et al., 1988), an increase in CO_2 concentration would increase the likelihood that a hen would become sedated and hypoxic once it paused, and thus unable to move thereafter. The percentages of hens that entered the lower chamber in CO_2-in-air atmospheres are consistent with this idea. Although the tendency of hens to pause and retreat did not differ greatly in 30, 45, and 60% CO_2-in-air mixtures, they entered the lower chamber when tested with 30% CO_2-in-air at a frequency approaching that when tested with Ar, which gas appeared to have little inhibitory effect on the birds. Hens in 60% CO_2-in-air tended to become stunned in the corridor before reaching the lower chamber, and the percentages for hens tested in 45% CO_2-in-air were intermediate. Although the percentage of hens entering the lower chamber in the 70% Ar/30% CO_2 atmosphere did not differ significantly from the results for any of the CO_2-in-air atmospheres, numerically, the percentage fell between those for 30 and 45% CO_2-in-air, perhaps indicating that lack of O_2 had some effect on forward progress if a hen hesitated in response to the CO_2 component of the atmosphere.

It is relevant to ask, in light of the aversion hens showed to some of the gas mixtures tested, if the severity of the aversion makes it important to avoid the use of certain of these gas mixtures for stunning/killing chickens in commercial settings. Most hens, given modest motivation, were not sufficiently deterred from entering any of the CO_2-enriched atmospheres to avoid being stunned, and their likelihood of being stunned did not differ significantly among these atmospheres. Similarly, Gerritzen et al. (2000) found that among those broilers that overcame inhibition to leave the starting point of a test apparatus, none were further deterred from approaching social stimuli by encountering stunning gas atmospheres, including those containing CO_2 at concentrations up to 60%. However, in the present study there was a fairly wide range of percentages of hens stunned in the different atmospheres (67–82%), leaving room for the possibility that some differences in willingness to enter such atmospheres do exist. This study confirms and adds to other research which indicates that from a behavioral standpoint there does not appear to be a welfare disadvantage to the use of mixtures of up to 60% CO_2-in-air to stun/kill chickens compared to a mixture of Ar with 30% CO_2 (Lambooij et al., 1999; Gerritzen et al., 2000; Webster and Fletcher, 2001).

Although the percentage of hens stunned in Ar did not differ significantly from the other stunning gas mixtures, this percentage was numerically the highest. Ar also caused less hesitation in hens on their approach to the lower chamber. These results may indicate a welfare advantage for Ar. It is too soon to conclude, however, that Ar is markedly superior to CO_2-enriched atmospheres for modified atmosphere stunning/killing of chickens. The possible welfare advantage indicated for Ar in this study is not so substantial that it could not readily be balanced by other welfare related or non-welfare related considerations. It has been proposed that the head shaking and deep breathing that chickens and other poultry manifest in CO_2-enriched atmospheres reflect respiratory distress (Raj and Gregory, 1994), which if true would give a substantial advantage to Ar. This proposition might be correct, but it has not yet been substantiated by research. An alternative interpretation of the behavior has been suggested by Webster and Fletcher (2001). The true welfare significance of these behaviors needs to be clarified. If CO_2-enriched atmospheres were greatly distressing to chickens, one might expect to see a high rate of refusal to enter these atmospheres when given a choice. Alternatively, behavior indicative of arousal, such as an increase in activity, alertness and escape behavior, or the manifestation of a hypoesthetic state would be predictable if birds were immersed in a noxious atmosphere (Woolley and Gentle, 1987; Gentle and Hill, 1987; Gentle and Hunter, 1991). These actions are not typically shown by chickens for CO_2 concentrations up to 60% in air. At a higher concentration of CO_2 (72%), a somewhat greater degree of aversion was observed in turkeys, but even then, most of the birds did not refuse to enter the CO_2-enriched atmosphere (Raj, 1996). Heart rate measurements in birds undisturbed by restraint might shed light on arousal caused by modified atmospheres used for stunning. Although the animals were not unrestrained, CO_2-enriched atmospheres were not observed to cause heart rate increases in chickens or pigs beyond what might have been caused by restraint and handling (Coenen et al., 2000; Martoft et al., 2001). Thus far, the published data on modified atmosphere stunning give the impression that any welfare advantage for Ar is relatively minor in regard to chickens.

Chickens immersed in an Ar-enriched atmosphere do not show the apparent sedation that is characteristically seen in chickens immersed in CO_2-enriched atmospheres, and appear to remain alert almost to loss of posture (Webster and Fletcher, 2001). These chickens may also be more likely to retain some degree of sensation beyond the initiation of convulsions compared to birds stunned in some CO_2-enriched atmospheres (Mohan Raj et al., 1992b). Convulsive, atypical head shaking behavior commonly occurs in chickens before loss of posture in an Ar-enriched atmosphere (Webster and Fletcher, 2001). The induction of this behavior may be unpleasant to a chicken and may occur at a time when the bird is unable to produce a volitional response to avoid the situation. Finally, modified atmosphere stunning/killing of poultry at commercial processing plants is done with many birds grouped together. In Ar-induced anoxia, many chickens manifest vigorous convulsions after loss of posture (Mohan Raj et al., 1991, 1992b; Poole and Fletcher, 1995), which involve leg movements and wing flapping powerful enough to throw the bird against other birds and walls (personal observation). The variation in time to loss of posture is such that some birds would undergo convulsions while others would still be conscious. The experience of seeing other birds convulse and of being struck by bodies and flapping wings might have a negative, though probably brief, impact on the welfare of chickens. Stunning chickens with relatively low concentrations of CO_2 can greatly reduce convulsive activity (Lambooij et al., 1999; Gerritzen et al., 2000; Webster and Fletcher, 2001). The judgment of which atmosphere is best from a welfare standpoint for modified atmosphere stunning is not simple, and clearly more work needs to be done to reach an informed conclusion.

High concentration of O_2 has been reported to diminish behavioral signs of agitation and asphyxia in rats immersed in CO_2-enriched atmospheres (Coenen et al., 1995), and some researchers have recently expressed preference for a 40% CO_2/30% N_2/30% O_2 atmosphere for stunning chickens (Coenen et al., 2000). The present study did not include tests of such an atmosphere, so nothing definitive can be said about how hens would have reacted to it. The possibility of causing lung damage made it inappropriate to use an O_2-enriched atmosphere in the current study since hens were to be repeatedly exposed to stunning atmospheres. The approach-avoidance tests by Gerritzen et al. (2000) showed no advantage for the 40% CO_2/30% N_2/30% O_2 atmosphere over 60% CO_2-in-air or 70% Ar/30% CO_2 atmospheres.

Acknowledgements

We would like to thank Massimiliano Petracci for his assistance with the training of hens. This study was supported by a grant from the US Poultry and Egg Association and by state and HATCH funds allocated to the University of Georgia Agricultural Experiment Station, Athens, GA.

References

Ambrose, N., Wadham, J., Morton, D., 2000. Refinement of euthanasia. In: Ball, M., van Zeller, A.-M., Halder, M.E. (Eds.), Proceedings of the Third World Congress on Alternatives and Animal Use in the Life Sciences. Progress in the Reduction, Refinement and Replacement of Animal Experimentation.

Anton, F., Euchner, I., Handwerker, H.O., 1992. Psychophysical examination of pain induced by defined CO_2 pulses applied to the nasal mucosa. Pain 49, 53–60.

Blackshaw, J.K., Fenwick, D.C., Beattie, A.W., Allan, D.J., 1988. The behaviour of chickens, mice and rats during euthanasia with chloroform, carbon dioxide and ether. Lab. Anim. 22, 67–75.

Cain, W.S., Murphy, C.L., 1980. Interaction between chemoreceptive modalities of odour and irritation. Nature 284, 255–257.

Coenen, A.M.L., Drinkenburg, W.H.I.M., Hoenderken, R., van Luijtelaar, E.L.J.M., 1995. Carbon dioxide euthanasia in rats: oxygen supplementation minimizes signs of agitation and asphyxia. Lab. Anim. 29, 262–268.

Coenen, A., Smit, A., Zhonghua, L., van Luijtelaar, G., 2000. Gas mixtures for anaesthesia and euthanasia in broiler chickens. World Poult. Sci. 56, 225–234.

Danneman, P.J., Stein, S., Walshaw, S.O., 1997. Humane and practical implications of using carbon dioxide mixed with oxygen for anesthesia or euthanasia of rats. Lab. Anim. Sci. 47, 376–385.

Forslid, A., Ingvar, M., Rosen, I., Ingvar, D.H., 1986. Carbon dioxide narcosis: influence of short-term high concentration carbon dioxide inhalation on EEG and cortical evoked responses in the rat. Acta Physiol. Scand. 127, 281–287.

Gentle, M.J., Hill, F.L., 1987. Oral lesions in the chicken: behavioural responses following nociceptive stimulation. Physiol. Behav. 40, 781–783.

Gentle, M.J., Hunter, L.N., 1991. Physiological and behavioural responses associated with feather removal in *Gallus gallus* var *domesticus*. Res. Vet. Sci. 50, 95–101.

Gerritzen, M.A., Lambooij, E., Hillebrand, S.J.W., Lankhaar, J.A.C., Pieterse, C., 2000. Behavioral responses of broilers to different gaseous atmospheres. Poult. Sci. 79, 928–933.

Kobal, G., 1985. Pain-related electrical potentials of the human nasal mucosa elicited by chemical stimulation. Pain 22, 151–163.

Lambooij, E., Gerritzen, M.A., Engel, B., Hillebrand, S.J.W., Lankhaar, J., Pieterse, C., 1999. Behavioral responses during exposure of broiler chickens to different gas mixtures. Appl. Anim. Behav. Sci. 62, 255–265.

Martoft, L., Lomholt, L., Kolthoff, C., Rodriquez, B.E., Jensen, E.W., Jørgensen, F., Pedersen, H.D., Forslid, A., 2001. Effects of CO_2 anaesthesia on central nervous system activity in swine. Lab. Anim. 36, 115–126.

McKeegan, D.E.F., Demmers, T.G.M., Wathes, C.M., Jones, R.B., 2003. Chemosensory responses to gaseous pollutants and carbon dioxide: implications for poultry welfare. Poult. Sci. 82 (Suppl. 1), 16.

Mohan Raj, A.B., Gregory, N.G., 1990a. Effect of rate of induction of carbon dioxide anaesthesia on the time of onset of unconsciousness and convulsions. Res. Vet. Sci. 49, 360–363.

Mohan Raj, A.B., Gregory, N.G., 1990b. Investigation into the batch stunning/killing of chickens using carbon dioxide or argon-induced hypoxia. Res. Vet. Sci. 49, 364–366.

Mohan Raj, A.B., Gregory, N.G., 1991. Preferential feeding behaviour of hens in different gaseous atmospheres. Br. Poult. Sci. 32, 57–65.

Mohan Raj, A.B., Gregory, N.G., Wilkins, J.L., 1992a. Survival rate and carcase downgrading after the stunning of broilers with carbon dioxide–argon mixtures. Vet. Rec. 130, 325–328.

Mohan Raj, A.B., Gregory, N.G., Wotton, S.B., 1991. Changes in the somatosensory evoked potentials and spontaneous electroencephalogram of hens during stunning in argon-induced anoxia. Br. Vet. J. 147, 322–330.

Mohan Raj, A.B., Wotton, S.B., Gregory, N.G., 1992b. Changes in the somatosensory evoked potentials and spontaneous electroencephalogram of hens during stunning with a carbon dioxide and argon mixture. Br. Vet. J. 148, 147–156.

Poole, G.H., Fletcher, D.L., 1995. A comparison of argon, carbon dioxide, and nitrogen in a broiler killing system. Poult. Sci. 74, 1218–1223.

Raj, A.B.M., 1996. Aversive reactions of turkeys to argon carbon dioxide and a mixture of carbon dioxide and argon. Vet. Rec. 138, 592–593.

Raj, M., Gregory, N.G., 1993. Time to loss of somatosensory evoked potentials and onset of changes in the spontaneous electroencephalogram of turkeys during gas stunning. Vet. Rec. 133, 318–320.

Raj, M., Gregory, N.G., 1994. An evaluation of humane gas stunning methods for turkeys. Vet. Rec. 135, 222–223.

Raj, A.B.M., Whittington, P.E., 1995. Euthanasia of day-old chicks with carbon dioxide and argon. Vet. Rec. 136, 292–294.

Raj, A.B.M., Wotton, S.B., McKinstry, J.L., Hillebrand, S.J.W., Pieterse, C., 1998. Changes in the somatosensory evoked potentials and spontaneous electroencephalogram of broiler chickens during exposure to gas mixtures. Br. Poult. Sci. 39, 686–695.

SAS, 1999. SAS Proprietary Software Release 8.2. SAS Institute, Inc., Cary, NC.

Webster, A.B., Fletcher, D.L., 2001. Reactions of laying hens and broilers to different gases used for stunning poultry. Poult. Sci. 80, 1371–1377.

Woolley, S.C., Gentle, M.J., 1987. Physiological and behavioural responses in the hen (*Gallus domesticus*) to nociceptive stimulation. Comp. Biochem. Physiol. A 88, 27–31.

Zeller, W., Mettler, D., Schatzmann, U., 1988. Studies into the stunning of slaughter poultry with carbon dioxide. Fleischwirtschaft 68, 1308–1312.