Multistage carbon dioxide gas stunning of broilers

M. A. Gerritzen,*[1] H. G. M. Reimert,* V. A. Hindle,* M. T. W. Verhoeven,* and W. B. Veerkamp†

*Wageningen UR Livestock Research, PO Box 65, 8200 AB Lelystad, the Netherlands; and †Meyn Food Processing Technology BV, PO Box 16, 1510 AA Oostzaan, the Netherlands

ABSTRACT The stunning quality of animals for slaughter remains under constant scrutiny. In response to previous research showing low stunning efficiency in poultry, the conventional water bath will be phased out in the Netherlands. Presently, the main practical alternative to water bath stunning of poultry is a 2-phased gas stunning method. Gas stunning methods are recognized by governments and animal welfare organizations across Europe. In this study, 3 sets of experiments were conducted on gas stunning methods using CO_2 in 2 phases. Two methods were examined to identify potential effects on bird behavior and investigate their practical implications: a 5-stage incremental CO_2 scheme lasting 6 min (treatment 1) and a 4-stage incremental CO_2 scheme lasting 4 min (treatment 2). The onset and duration of unconsciousness were specifically tested in experiment 2 by using 25 birds equipped with electrodes monitoring brain and heart activity. Behavioral responses were observed on 15 non-instrument-monitored birds kept in the same cages at that time. Results in all 3 sets of the experiments showed that multistage gas stunning was stable and consistent, and increases in CO_2 concentrations were rapid and reliable. Ambient temperatures and RH of the air remained within acceptable levels at all times. Induction of unconsciousness occurred below 40% CO_2 and did not significantly differ between treatments. Conscious birds were never exposed to high CO_2 concentrations (>40% CO_2), yet some birds showed signs of distress (e.g., head shaking, wing flapping) before losing consciousness. Discomfort experienced during exposure to low (<40%) CO_2 concentrations compares favorably with the experiences of handling, tilting, and or shackling of conscious birds when using alternative stunning methods, implying that multistage gas stunning has distinct advantages for bird welfare. Compared with the multibird water bath system, this method provides an opportunity to guarantee that all birds are properly stunned. The risk of convulsions, which was higher with treatment 2, leading to possible injuries, indicates a preference for the 5-stage treatment.

Key words: gas stunning, carbon dioxide, physiology, behavior, broiler

2013 Poultry Science 92:41–50
http://dx.doi.org/10.3382/ps.2012-02551

INTRODUCTION

In 2007, more than 54 billion poultry were slaughtered for meat production worldwide (CIWF, 2010). Dutch and European legislation (Ministerie van Landbouw, Natuurbeheer en Visserij, 1992; European Commission, 1993; European Union, 2009) provide laws and rules for the slaughter of farm animals. One of the laws describes the obligatory stunning of animals preslaughter to ensure unconsciousness and insensibility, to prevent unnecessary suffering of the animal (FAWC, 2003; EFSA, 2004). Unconsciousness in poultry is often elicited by applying an electric current or by using a gas consisting of (or a mixture of) Ar, N_2, or CO_2. After stunning, birds are slaughtered by exsanguination. Improvement in the quality of stunning animals intended for human consumption is under constant scrutiny. Effectiveness of a stun is receiving particular attention from society and politicians wishing to safeguard animal welfare. In reaction to previous research showing low stunning efficiency in poultry, the conventional water bath will be phased out in the Netherlands (ASG, 2009). Alternative electrical stunning systems are now being developed, but electrical stunning methods in current use or under development still rely on handling and shackling live and conscious birds. It is well known and accepted that shackling a conscious bird is an important detriment to bird welfare (Raj, 1998). To minimize such risks to bird welfare, handling of conscious birds should be reconsidered and avoided where possible. The only practical alternative to water bath stunning in use is a 2-phased gas (CO_2) system, of which only one type is used in the Netherlands (Morgenstern et al., 2009). Use of this controlled-atmosphere stunning (**CAS**) method (Ribeiro et al., 2009) has been recognized by govern-

Received June 20, 2012.
Accepted August 27, 2012.
[1]Corresponding author: Marien.gerritzen@wur.nl

ments and animal welfare organizations across Europe as an acceptable alternative to traditional water bath stunning (European Commission, 1993; European Union, 2009). Some of the welfare problems related to electrical stunning have been eliminated using CAS.

However, one of the problems associated with some CAS methods is that high concentrations of CO_2 are considered to be distressing to the birds (Raj and Gregory, 1994; Coenen et al., 1995; Hari et al., 1997). Addition of O_2 can reduce the negative effects of high initial CO_2 concentrations but can slow down the process to loss of consciousness (Coenen et al., 1995; Lambooij et al., 1999; Gerritzen et al., 2000). Using the 2-phased CO_2 method, the bird is brought under narcosis first with a low concentration of CO_2 (maximum of 40%) and a decreasing amount of O_2. Thereafter, the increase in CO_2 concentration up to 80% in the second phase is sufficient to induce a deeper state of unconsciousness, after which the arteries in the neck can be severed to kill the bird.

The challenge is to find an acceptable balance between the intensity and duration of the stun and the amount of distress for the birds. In this study, 3 scaled experiments were conducted to evaluate and present an alternative means of gas stunning by using a more gradual increase of CO_2 acceptable for European regulatory and market standards. In particular, 2 treatments were compared using CO_2 in 2 phases; a 5-stage incremental CO_2 scheme lasting for 6 min as developed by Praxair Inc. versus a 4-stage incremental CO_2 scheme lasting for 4 min. A dilemma arises in the choice of whether to allow a mild level of distress during a slightly longer stun or to provide a short stun with a considerably higher distress level. This study was an attempt to assess the consequences arising from both alternatives.

MATERIALS AND METHODS

The system used for the experiments consisted of a CO_2 supply tank, an evaporator, and a test chamber provided by Meyn Food Processing Technology BV (Oostzaan, the Netherlands). The same test chamber was used for all experiments and included the gas stunning technology licensed to Meyn Food Processing Technology BV by Praxair Inc. (Danbury, CT). This chamber is suitable for use in combination with most commercially applied transport (poultry) modules. During these experiments, Meyn modules were used. Small windows in the door and side wall of the chamber allowed for continuous observation of the stunning process from start to finish.

The outer dimensions of the test chamber were 2,600 × 2,200 × 2,500 mm (length × width × height), and this included the programmable logic control panel on one side of the chamber plus inlet and outlet channels on the other side. The stunning "recipes" were programmed in the programmable logic control, which was automatically activated as soon as the door was closed.

At the start of the process, a 7-kW blower on top of the chamber circulated the atmosphere horizontally through the chamber. The gas air inlet was layered at multiple levels for the poultry transport modules, whereas the outlet was on the same side of the chamber adjacent to the inlet. Gas concentration was measured in the outlet for process control, allowing CO_2 to be added through a diffuser. Temperature and RH sensors were mounted on top of the chamber. Strong circulation ensured that the CO_2 was thoroughly mixed and uniformly spread throughout the chamber.

At the beginning of each stage of the chosen treatment, CO_2 was injected for 8 to 10 s to attain the required concentration. At the end of the final stage, the exhaust valve was opened, and the blower flushed the chamber with outside air. The working principles of the multistage gas stunning system, CO_2 treatments, and bird welfare aspects were examined in 3 consecutive experiments, carefully scaled up by an order of magnitude each time from small to medium to commercial scale. All bird experiments were conducted with the approval of the Ethical Committee of Wageningen UR, Livestock Research. Measurements in a commercial setting (experiment 3) were conducted with the additional approval of the Belgian authorities.

Experiment 1 of this project was conducted to determine the working principle of the stunning system and to test different treatments and their effects on bird behavior. The proposed CO_2 recipes (concentration and exposure duration) were discussed with the companies involved and a selection of 2 recipes was tested. On the basis of this first experiment, a choice was made for further testing of 2 treatments and how to determine bird welfare aspects and stunning effectiveness. In the second experiment, 2 CO_2 treatments were tested for their effect on bird physiology and behavior. In the third experiment, implications for and aspects of practical application were examined under semicommercial conditions.

Experiment 1: Determination and Description of the Working Principle of Multistage Gas Stunning

The working principle of the stunning system, that is, controlling the stability of the gas, temperature, and RH during processing and registering the reactions of the birds when exposed to different stunner settings, were tested in 10 different runs. During each run, different CO_2 concentrations and different exposure times were examined. Measurements were performed for CO_2 concentration, temperature, and air RH. Behavioral responses of broilers during exposure to the stunning process were observed in 2 birds per run. Video recordings and live visual observations were used to determine different behavioral responses indicative of reduced bird welfare. Furthermore, the behavioral observations including more specific bird movement and convulsions were analyzed and taken into account when determining electrophysiological measurements in the second experiment.

Experiment 2: Bird Welfare Aspects

With the results obtained in experiment 1, the second experiment evaluated the induction of unconsciousness based on the exposure of individual broiler chickens to 2 treatments. Treatment 1 was a 5-stage incremental scheme for CO_2 gas stunning in 2 phases taking 6 min to complete. It involved exposure to CO_2 concentrations of 20, 30, 35, 40, and 64%, with every stage allowing for a 2% variation. Exposure times per stage were 60 s each, except for 120 s for the last one. Treatment 2 was a 4-stage incremental CO_2 scheme lasting for 4 min with exposure to CO_2 concentrations of 20, 35, 45, and 65%, with every stage allowing for a 2% variation. Exposure to the first 3 stages was 45 s each, whereas exposure in the last stage was 105 s in duration. Both treatments were tested using 25 birds in 25 runs in the multistage stunning system. All 25 birds were equipped with electrodes and a data logger to measure brain and heart activity. During 15 of these runs, a nonequipped bird accompanied the test bird, to observe behavioral responses such as gasping, head shaking, wing flapping, and convulsions. For these observations, nonequipped, free-moving birds were used to avoid effects from wearing the data logger.

Measuring Unconsciousness

On the basis of brain and heart activity, determining the correct method of application (working method) guaranteed an acceptable and reliable stun: 1) onset of unconsciousness, and 2) duration to onset of consciousness after expulsion from the stunner (recovery).

General activity of the brain, via electroencephalogram (**EEG**), was measured using 3 needle electrodes that were inserted into the skull immediately before exposure to the stunning process. Brain activity was recorded using a mobile data logger (Lowe et al., 2007) placed in a Lycra stretch jacket attached to the back of the birds. Traces were analyzed using Labchart analytical software (Labchart7 Pro, version 7.1.2; AD Instruments, Cologne, Germany). Changes in the EEG traces to a transitional, suppressed, and isoelectric signal were located in time and related to CO_2 concentrations. Change to the transitional (slow-wave) state indicated induction of reduced consciousness or the onset of unconsciousness. A suppressed EEG, with a strong frequency reduction, is generally accepted as an indicator of unconsciousness (Gerritzen et al., 2004, 2006; McKeegan et al., 2007). The change to an isoelectric EEG indicated very low or no brain activity, often resulting in brain death.

Heart activity, via an electrocardiogram (**ECG**), was measured using 2 adhesive patches (Unilect, 5 cm in diameter; Unomedical Ltd., Stonehouse, UK) placed on the left and right pectoralis muscles under the wing base. The ECG signals were used to assess when and how long it took for the birds to die. Parameters that were looked at were bradycardia [i.e., a slow heartbeat with a 40% reduction in relation to initial beats per minute (**bpm**)] and strong suppression of the heartbeat (i.e., a reduction of more than 75% of initial bpm together with rhythmic disorders). Additionally, changes in ECG signals associated with rhythmic disorders were used as indications of stress (i.e., distress).

Bird Behavior

Behavior of the free-moving birds, which were not equipped with a data logger and stretch jacket, was recorded using an infrared video camera. This camera was placed 1 m from the birds at the same height as the birds that were placed in the container. Recordings were analyzed for incidences of head shaking, deep breathing and or gasping, loss of posture, wing flapping, and convulsions.

Experiment 3: Performance Under Practical Conditions

Performance of the multistage gas stunning system was tested in a commercial slaughter plant under semicommercial conditions. The aim of this third experiment was to examine the efficacy of the 2 different gas stunning treatments, as tested in experiment 2, under a semicommercial setup based on the behavior of the birds in fully loaded transport containers. Distribution and measurement of the CO_2 levels were monitored up to and including the moment that the required concentration had been established in the system. After each test, the system controller was checked with a sensor.

During a standard working day at the slaughter plant, fully loaded commercial containers were selected from the regular supply of birds. Fully loaded containers contained on average 250 to 350 birds distributed over 4 or 5 tills per container. Containers consisting of 4 tills measured 2,400 × 1,200 × 1,176 mm (350 kg) and containers with 5 tills measured 2,400 × 1,200 × 1,433 mm (400 kg). Before exposure to the stunning unit, each till was checked for dead birds. To prevent dead birds from entering the system, all birds found dead before entry were removed from the crates.

Three different groups of broilers were exposed to the system, namely, heavy (2,200 to 2,800 g), light (±1,600 g), and free-range broilers. After stunning, the containers were emptied and the birds entered the regular slaughter process.

Measurements

Control of CO_2 injection and correct O_2 concentration were facilitated by continual measurement in the system outlet. To measure the distribution of CO_2

between the birds, 4 O_2 sensors (ST400 CE; Buveco, Bleiswijk, the Netherlands) connected to a logger (Eurotherm 6100; Invensys Eurotherm B.V., Alphen a.d. Rijn, the Netherlands) were placed in a crossed line ranging from the upper rear corner to the lower front corner of the container. Ambient temperature and air RH were measured in the outlet during each run.

The following behavioral characteristics of the birds were observed and registered continuously during the gas stunning procedure: alerting response: looking around, head drawn upright, nervous reactions; sitting: speed at which standing birds change to a position in which the torso is vertically directed and the body is supported at the rear end; head shaking: short and fast shaking movements of the head; gasping: deep breathing accompanied by neck stretching; loss of posture: falling over on the side or laying down with head in a relaxed position (prostrate) on the floor or on top of other birds; wing flapping: bouts of fast, short flapping; and convulsions: strong, uncontrolled muscle movements of the wings and legs.

Statistical Analysis

Data were analyzed using the statistical package Genstat (VSN International, Hemel Hempstead, UK). Each bird represented an experiment with a probability, P, that the bird was unconscious during the general epileptiform insult after stunning. For n birds, which were treated independently, the number x (unconscious) followed a binomial distribution with total n and probability P. The confidence interval could be calculated for the probability, P, based on the relationship between the binomial and β distribution. The number of effective stuns followed a binomial distribution. A 95% confidence limit on the probability of an effective stun could be obtained by means of the well-known relationship with the β distribution (Johnson and Kotz, 1969).

RESULTS

Experiment 1: Technical Results

Gas concentrations remained stable and consistently followed the preset treatment. Increases in gas concentration between levels were accomplished in very short intervals. Moderate variations in the gas concentration never exceeded 1 to 2% of the preset value. Compartment temperature development during gas stunning remained stable. The average temperature during processing was 20°C. Differences in temperature between the start and finish never exceeded 3°C. Because of the very low density bird loading (<1%, 2 birds in a chamber with a 250- to 350-bird capacity) in the large commercial-scale test chamber used in these small-scale experiments, the RH decreased from 58 to 18% during the first run. This was not indicative of commercial RH conditions, as verified in experiment 3 when using reasonable bird loading rates. As the bird loading was increased in the subsequent larger-scale experiments, acceptable air RH values recorded in the system remained stable within each run.

Bird Behavior

Behavioral observations were conducted during each trial. Most birds showed alerting reactions, expressed as short, abrupt head movements, a few moderate head flicks, and looking around in an alert, upright position.

Within approximately 30 s after exposure to 18 to 20% CO_2, birds started to lose balance, followed rapidly by attempts to maintain balance. Attempts to maintain balance were expressed by walking and some short, moderate leg-stretching movements while in a standing position. Before and during the induction to loss of posture, indicative of inducing unconsciousness, no signs of panic, fear, and breathlessness were observed. After the loss of posture, some moderate wing flapping occurred. However, no convulsions were observed during these trials.

Experiment 2: Technical Results

Results from runs with the 2 different CO_2 treatments are presented in Figure 1. Gas concentrations remained stable and consistently followed the preset treatment (Table 1). Gas concentrations adjusted rapidly toward the subsequent increases in the "set" levels. Variations in the gas concentration were moderate (i.e., never more than 1 to 2% of the preset value). Temperature development during gas stunning was very stable. The average temperature during processing was 20°C. The difference in temperature between the start and finish did not exceed 3°C for all tests.

As expected, with very sparse bird loading (<10%, 25 birds in a test chamber with a 250- to 350-bird capacity) in the large commercial test chamber used for the medium-scale experiment (experiment 2), the average air RH at the beginning was 45%, which decreased simultaneously with increased CO_2 concentrations. At the maximum CO_2 concentrations (i.e., during the last stage of both treatments), the air RH fell below 30%. Again, this was not indicative of commercial RH conditions, as verified in experiment 3 when using reasonable bird loading rates in the gas stunning chamber.

Bird Behavior

Because of differences in the starting position of the birds, loss of posture could not be scored objectively from the video recordings and was therefore excluded from the analysis. Furthermore, in most recordings, it was impossible to distinguish between sitting and loss of posture. During both treatments, head shaking and deep breathing commenced shortly after increasing CO_2 concentration (Table 2). Initial behavioral reactions to

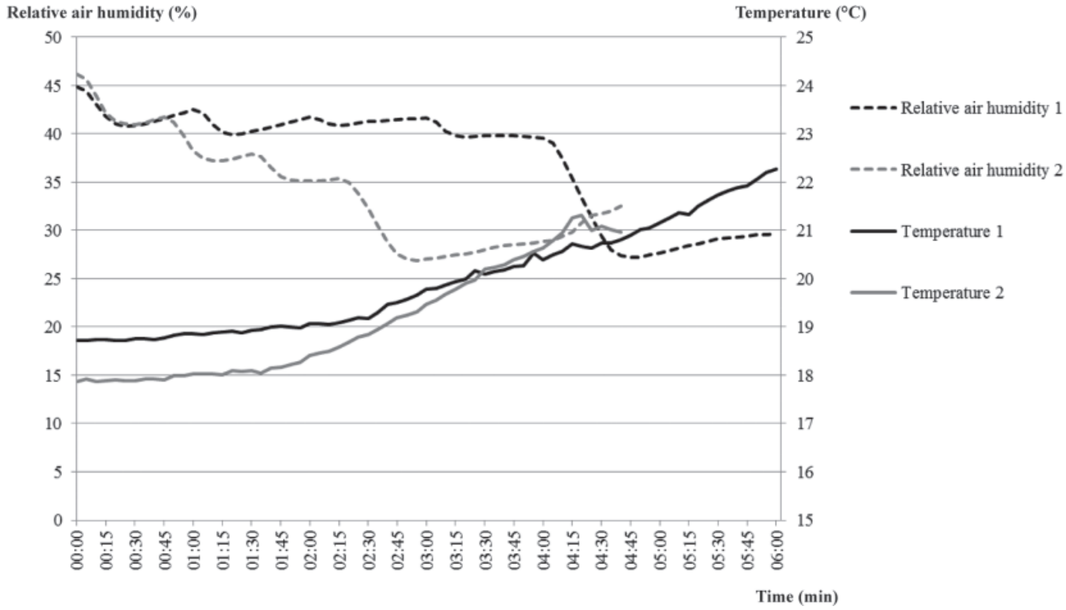

Figure 1. Temperature (solid lines) and air RH (dashed lines) measured as averages of 22 runs (treatment 1: 5-stage) and 25 runs (treatment 2: 4-stage) in experiment 2.

the increasing CO_2 concentrations were 1 to 3 short, rapid head-shaking movements. Before this, the birds had shown no reaction to placement in the stunner and initial gas flow. Immediately after head shaking, birds started to breathe more heavily, with a gradual transition into gasping. Deep breathing continued repeatedly on average every 4 to 5 s. No difference was observed between treatments 1 and 2 in the onset and incidence of head shaking and deep breathing.

After some time, the birds showed 1 to 3 moderate bouts of wing flapping. Wing flapping occurred earlier with birds exposed to treatment 2 than for those in treatment 1. In a few cases, flapping bouts were followed by convulsive movements. During treatment 1, only 1 bird displayed 2 moderate convulsive movements. During treatment 2, only 3 of the 15 birds showed 1 to 3 convulsive movements.

Table 1. Carbon dioxide concentration per stage (maximum percentage measured during all runs) compared with programmed treatments during experiment 2

	Treatment 1[1]		Treatment 2[2]	
Stage	Setting	Measured	Setting	Measured
1	18	19	20	21
2	28	29	35	36
3	33	33	45	46
4	38	39	65	65
5	62	65		

[1]Treatment 1: a 5-stage incremental scheme for CO_2 supply taking 6 min to complete, involving exposure to CO_2 concentrations of 20, 30, 40, 50, and 60%, with every stage allowing for a 2% variation.

[2]Treatment 2: a 4-stage incremental CO_2 scheme lasting for 4 min, involving exposure to CO_2 concentrations of 20, 35, 45, and 65%, with every stage allowing for a 2% variation.

Brain Activity

Times taken for the induction of a transitional, a suppressed, and an isoelectric EEG in treatments 1 and 2 are presented in Figure 2. Transitional EEG (i.e., slow-wave activity) occurred on average at 36 ± 12 s after the start of CO_2 inflow in treatment 1 and at 29 ± 10 s for treatment 2. Suppressed EEG (i.e., loss of α and β waves) occurred on average at 60 ± 23 s and 50 ± 24 s on, respectively, treatments 1 and 2. However, these differences between treatments 1 and 2 were not significant. Suppression of the EEG to minimal activity or a near-isoelectric signal occurred significantly slower in treatment 1 (4:07 ± 1:04 min) compared with treatment 2 (3:27 min ± 50 s).

Heart Activity

Suppression of the heartbeat (i.e., average bpm) is presented in Figure 3 as bradycardia, strong suppression, and cessation of the heartbeat (isoelectric). Bradycardia occurred shortly after exposure to increasing CO_2 concentrations and before a transitional ECG. Time taken to induce bradycardia did not differ significantly between treatment 1 (22 ± 13 s) and treatment 2 (29 ± 27 s). Strong suppression of the heartbeat occurred earlier in broilers exposed to treatment 2 (3:27 ± 1:04 min) than in those exposed to treatment 1 (4:46 ± 1:03 min). Isoelectric ECG or those approaching isoelectric occurred earlier in broilers in treatment 2 (4:04 ± 1:03 min) than in those in treatment 1 (5:54 ± 1:39 min). However, it should be noted that isoelectric ECG occurred in only 40% of the runs analyzed. No birds survived these experiments.

Table 2. Onset of behavioral events (mean ± SD, in seconds) after beginning the CO_2 gassing procedure, as observed in experiment 2

	Behavioral event			
Treatment	Head shaking	Deep breathing	Wing flapping	Convulsions
1	18 ± 13	21 ± 9	215 ± 84	304[1]
2	30 ± 22	36 ± 21	153 ± 69	143 ± 23[2]

[1]n = 1.
[2]n = 3.

Experiment 3: Technical Results

Distribution of the CO_2 was even throughout the entire gas stunning unit (Figures 4 and 5). No significant differences were observed between O_2 concentrations and, consequently, in CO_2 concentrations at the top, at the bottom, or inside the container. No differences in CO_2 concentrations were observed between containers loaded with heavy or light birds.

During the stunning cycle, the atmospheric air circulated at a high velocity throughout the chamber and along the system sensors. At the beginning of each stage, CO_2 injection was very short, but was constantly mixed and circulated during all stages.

Evaluation of the system sensor and controller in the laboratory environment confirmed that the system control did not deviate by more than 2.5% at the lower CO_2 concentrations and by <1% from the actual value at 60% CO_2. The air RH was stable during CO_2 gas stunning (approximately 90%) and declined to approximately 80% during the last injection cycle during these full-scale experiments. The air RH remained well above the advised minimum acceptable percentage of 60% for the first stages. On average, the temperature declined from 27 to 22°C during gas stunning with both treatments.

Bird Behavior

Times taken until the onset of behavioral events of the birds in the stunning systems are presented in Table 3. The only differences that occurred were the onset and severity of convulsions and the occurrence of wing flapping in the 4-stage procedure. Convulsions in the 5-stage treatment were mild and occurred on only a few occasions, whereas convulsions in the 4-stage treatment were more severe and occurred for a larger number of birds in each trial. In general, behavioral expressions of the birds were the same during both procedures. The birds were more alert between 11 to 17 s at average concentrations of 10 to 15% CO_2. This was identified as standing up, moving, and looking around, with an occasional head flick or sneezing movement. Shortly thereafter, the birds expressed gasping at CO_2 concentrations between 15 and 20% in the inhaled air, followed shortly by head shaking. Wing flapping began after,

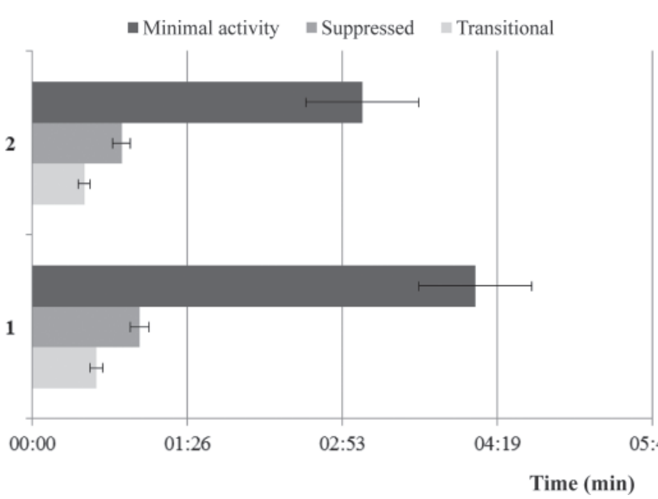

Figure 2. Average time (mean ± SE, in minutes) taken for the induction of a transitional, a suppressed, and an isoelectric electroencephalogram in treatments 1 and 2 during experiment 2. Treatment 1 was a 5-stage incremental scheme for CO_2 supply taking 6 min to complete, involving exposure to CO_2 concentrations of 20, 30, 40, 50, and 60%, with every stage allowing for a 2% variation. Treatment 2 was a 4-stage incremental CO_2 scheme lasting for 4 min, involving exposure to CO_2 concentrations of 20, 35, 45, and 65%, with every stage allowing for a 2% variation.

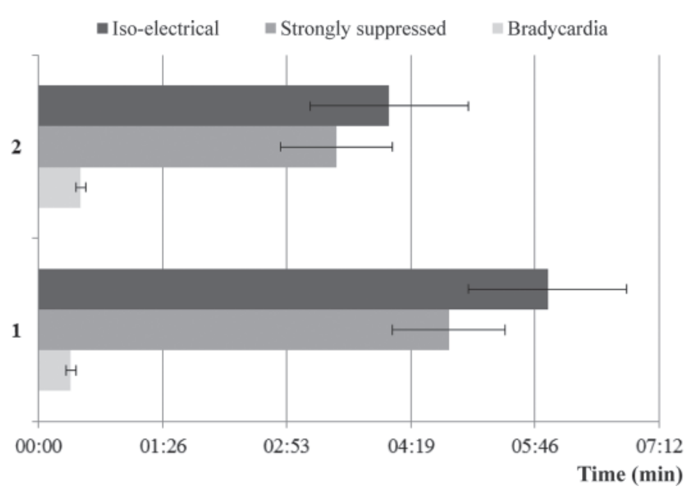

Figure 3. Average time (mean ± SE, in minutes) elapsed to a change in heart activity (via electrocardiogram) during exposure to the 2 different multistage CO_2 treatments. Treatment 1 was a 5-stage incremental scheme for CO_2 supply taking 6 min to complete, involving exposure to CO_2 concentrations of 20, 30, 40, 50, and 60%, with every stage allowing for a 2% variation. Treatment 2 was a 4-stage incremental CO_2 scheme lasting for 4 min, involving exposure to CO_2 concentrations of 20, 35, 45, and 65%, with every stage allowing for a 2% variation.

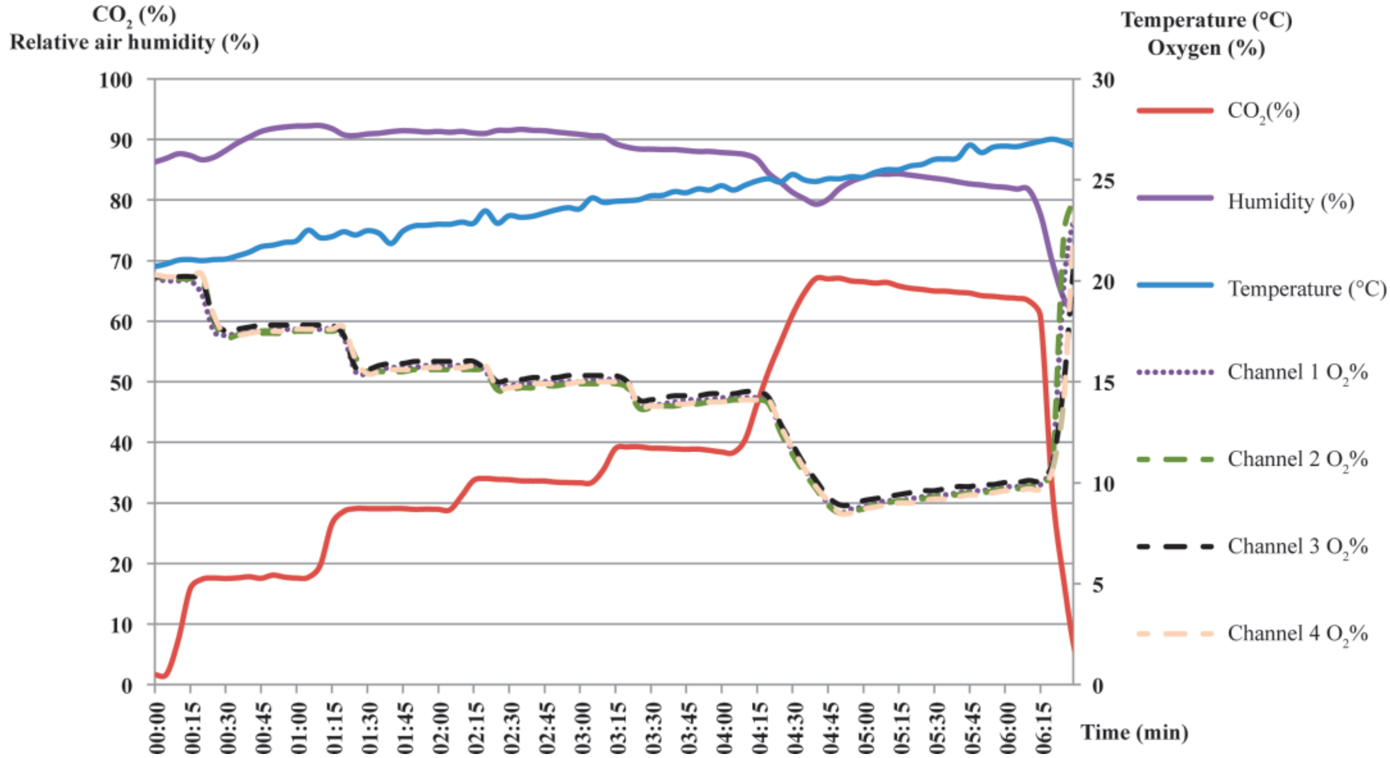

Figure 4. Course of CO_2 concentration, O_2, air RH, and temperature during a single run lasting 6 min, as observed in experiment 3. Color version available in the online PDF.

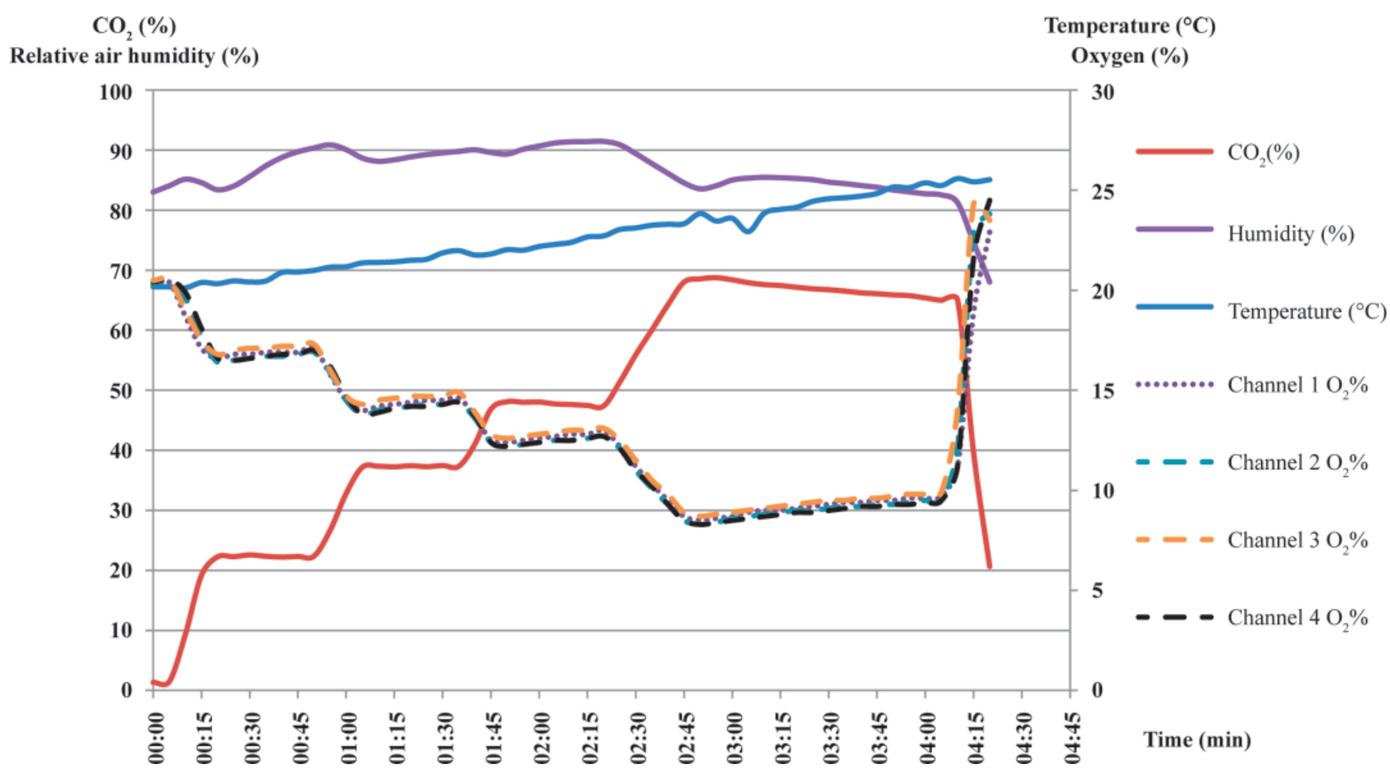

Figure 5. Course of CO_2 concentration, O_2, air RH, and temperature during a single run lasting 4 min, as observed in experiment 3. Color version available in the online PDF.

Table 3. Onset of behavioral events (in seconds) after beginning the CO_2 gassing procedure for treatment 1 (5-stage treatment) and treatment 2 (4-stage treatment), as observed in experiment 3

	Behavioral event							
Treatment	Alert	Gasp	Head shake	Sit	Wing flapping	LOP[1] first	LOP all	Convulsions
1	17	13	18	35	—	66	93	170
2	11	17	21	35	42	57	80	85

[1]LOP = loss of posture.

respectively, 57 and 66 s (4- and 5-stage treatments) and birds began to lose posture, which was indicative of the loss of consciousness. After 80 s (4-stage treatment) and 93 s (5-stage treatment), all birds had lost posture and were assumed to be unconscious. On the 4-stage treatment at 85 s, which is immediately after the loss of posture, convulsions occurred. In this treatment, a relatively large number of broilers displayed these uncontrolled muscle movements. However, during the 5-stage treatment, only a few birds displayed mild convulsive movements. No differences were observed between heavy, light, or free-range chickens.

DISCUSSION

In 3 consecutively scaled-up experiments, this study examined the potential effects of a newly developed gas stunning method and system on the behavior of poultry. Tests were run with 2 different gas treatments: a 5-stage incremental CO_2 scheme lasting for a total of 6 min versus a 4-stage incremental scheme lasting for 4 min. Implications for practical application were also examined under semicommercial conditions.

It was concluded from all the experiments conducted in this study that the multistage gas stunning system is stable and consistent in function. Different gas stunning treatments are easy to conduct. Increases in CO_2 concentrations to subsequent levels during processing were accomplished rapidly, and it was reliable in both the experimental and the semicommercial setup (i.e., experiment 3). It appeared that the flow of CO_2 gas was strong enough to penetrate between the birds without delay; therefore, the stunning conditions realized were uniform for all birds, even in a fully loaded container system.

Ambient temperatures remained stable and within the thermal comfort zone of the birds, and it decreased by no more than 5°C per run. Air RH above 60% at the beginning and during the first 2 stages of both treatments was advised as a minimum acceptable percentage to avoid negative effects on the mucous membrane of the respiratory tract. Air RH below 60% can precipitate a more painful stimulus of the mucous membrane and presumably have a negative effect on CO_2 exchange within the respiratory pathway. This phenomenon has a detrimental influence on the stunning efficiency of some CO_2 systems (Hoenderken et al., 1994). However, when using the multistage CO_2 system under practical commercial conditions, the containers were fully loaded with birds; therefore, the resulting air RH was higher than during the first 2 experiments, and it stayed well above the recommended 60% at all times.

The 2-phase gas stunning method and system have been legally approved since 2007 in the Netherlands and even earlier in other European countries (European Union, 2009; Morgenstern et al., 2009). In this system, no concentrations >40% may be reached during the first phase to avoid aversive reactions of the birds. Carbon dioxide concentrations in all experiments remained below 40% in the first stages, as imposed by law, which makes this system legally applicable for the Dutch market.

All broilers were stunned and killed during runs with either the 6- or 4-min treatment. It was concluded, for both treatments, that CO_2 concentration did not exceed 40% before all the birds expressed loss of posture, the behavioral indication of unconsciousness. In addition, in brain activity measured, the loss of α and β waves leading to a strongly suppressed EEG confirmed the induction of unconsciousness before birds were exposed to CO_2 concentrations above 40%. Although birds were not exposed to high levels of CO_2 (>40%) while still conscious, it was clear that the birds experienced some discomfort and stress before loss of consciousness. This discomfort was expressed by alerting reactions, gasping, and head shaking while the birds were clearly conscious. The behavioral observations showed the occurrence of head shaking in all experiments. It is well known that birds, including broiler chickens, detect and respond to CO_2 in very low concentrations in the air they inhale (McKeegan et al., 2005). Head shaking has been interpreted as a reaction to new or sudden events (Hughes, 1983), but also as a response to reduce stress in unknown circumstances (Black and Hughes, 1974). It has been identified as a tool to measure aversion in poultry to gas mixtures (Sandilands et al., 2011). Inhalation of high concentrations of CO_2 was found to be aversive in multiple species, and a more gradual increase in CO_2 is therefore recommended (Coenen et al., 1995; Raj and Gregory, 1995; McKeegan, 2006). However, from comparison of the discomfort experienced during exposure to CO_2 concentrations slightly below 40% with experiences during other alternative stunning systems requiring handling, tilting, and or shackling of fully conscious birds (as occurs with water bath stunning and some CO_2 tunnel stunning systems), it would appear that the presented multistage gas stunning system has distinct advantages for bird welfare. A major advantage of this method is that the birds are stunned in their transport containers; therefore, handling of conscious birds is greatly reduced or eliminated. These results are consistent with the potential reduction in broken wings and internal hemorrhages as compared with alternate stunning systems, such as electrical water bath stunning and a low atmospheric pressure system (Vizzier-Thaxton et al., 2010).

Compared with other CO_2 stunning methods, multistage CO_2 stunning of birds in stationary cages provides the highest level of control of the environment surrounding the birds. This new system demonstrated a uniform and accurate controlled atmosphere in the entire chamber, which is extremely difficult to achieve with other CO_2 stunning systems, especially tunnels. The 6-min 5-stage cycle, in combination with the fact that the birds are stunned in a closed environment instead of in an open tunnel, resulted in an optimized process not only for bird welfare, but also for product quality.

In comparison with the common practice of multiple-bird water bath stunning, it appears that this new method provides a better guarantee that all birds will be properly stunned, even though the time taken to lose consciousness is substantially longer than with electrical water bath stunning. On the basis of the lesser expression of convulsions after a loss of posture, it is recommended that the 5-stage treatment developed by Praxair Inc. be followed rather than the 4-stage treatment.

In conclusion, the results presented in this study showed that stunning poultry by exposure to gradually increasing concentrations of CO_2 in 2 phases by using a multistage gas stunning method was effective in all birds. The stunning system proved to be stable and consistent with preprogrammed settings, in which temperature and RH values remained within the recommended ranges. Although the induction of unconsciousness occurred when the CO_2 concentration was below 40%, some discomfort was nevertheless observed.

It should be taken into account that birds are considered to run greater risks of distress during unloading of the crates, handling, restraining, and shackling, which makes this system an improvement to bird welfare compared with present conventional water bath stunning methods. The 5-stage treatment is preferred to the 4-stage treatment because of the slightly increased risk of convulsions when using the 4-stage stunning method.

ACKNOWLEDGMENTS

This project was commissioned by Meyn Food Processing Technology (Oostzaan, the Netherlands) and Praxair Inc. (Danbury, CT). The input, technology, and technical know-how of these companies made this project possible. The semicommercial tests were conducted at the Plukon poultry slaughter plant in Moeskroen, Belgium. The authors thank the staff at Moeskroen for their hospitality, which made testing under commercial conditions possible. Finally, the authors are very grateful to the Belgian government for approval for commercial testing.

REFERENCES

ASG (Animal Sciences Group). 2009. Elektrisch verdoven van pluimvee. Een evaluatie van de praktijk situatie in Nederland, Onderzoek naar elektrische alternatieven. ASG Rep. 200. Anim. Sci. Group, Wageningen UR, Lelystad, the Netherlands.

Black, A. J., and B. O. Hughes. 1974. Patterns of comfort behaviour and activity in domestic fowls—A comparison between cages and pens. Br. Vet. J. 130:23–33.

CIWF (Compassion in World Farming). 2010. Slaughter. Compassion in World Farming, Godalming, Surrey, UK.

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

EFSA (European Food Safety Authority). 2004. Welfare aspects of stunning and killing methods. EFSA-Q-2003-093. Scientific Report of the Animal Health and Welfare Panel (AHAW/04-027). Eur. Food Saf. Auth., Parma, Italy.

European Commission. 1993. Council directive 93/119/EC of 22 December 1993 on the protection of animals at the time of slaughter and killing. Eur. Commun. Off. J. L 340:0021–0034.

European Union. 2009. Council regulation (EC) no. 1099/2009 of 24 September 2009 on the protection of animals at the time of killing. Off. J. Eur. Union L 303:1–30.

FAWC (Farm Animal Welfare Council). 2003. Report of the welfare of farmed animals at slaughter or killing. Part 1: Red meat animals. Farm Anim. Welf. Counc., Defra Publications, London, UK.

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

Gerritzen, M. A., E. Lambooij, H. G. M. Reimert, B. M. Spruijt, and J. A. Stegeman. 2006. Susceptibility of duck and turkey to severe hypercapnic hypoxia. Poult. Sci. 85:1055–1061.

Gerritzen, M. A., B. Lambooij, H. Reimert, A. Stegeman, and B. Spruijt. 2004. On farm euthanasia of broiler chickens: Effects of different gas mixtures on behavior and brain activity. Poult. Sci. 83:1294–1301.

Hari, R., K. Portin, B. Kettenmann, V. Jousmäki, and G. Kobal. 1997. Right-hemisphere preponderance of responses to painful CO_2 stimulation of the human nasal mucosa. Pain 72:145–151.

Hoenderken, R., B. Lambooij, A. E. J. M. van den Boogaard, and S. J. W. Hillebrand. 1994. Tierschutzgerechte gasbetäubung von Geflügel. Fleischwirtsch 74:497–500.

Hughes, B. O. 1983. Headshaking in fowls—The effect of environmental stimuli. Appl. Anim. Ethol. 11:45–53.

Johnson, N. L., and S. Kotz. 1969. Discrete Distributions. John Wiley, New York, NY.

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

Lowe, J. C., S. M. Abeyesinghe, T. G. M. Demmers, C. M. Wathes, and D. E. F. McKeegan. 2007. A novel telemetric logging system for recording physiological signals in unrestrained animals. Comput. Electron. Agric. 57:74–79.

McKeegan, D. E. F., J. A. McIntyre, T. G. M. Demmers, J. C. Lowe, C. M. Wathes, P. L. C. van den Broek, A. M. L. Coenen, and M. J. Gentle. 2007. Physiological and behavioural responses of broilers to controlled atmosphere stunning: Implications for welfare. Anim. Welf. 16:409–426.

McKeegan, D. E. F., J. McIntyre, T. G. M. Demmers, C. M. Wathes, and R. B. Jones. 2006. Behavioural responses of broiler chickens during acute exposure to gaseous stimulation. Appl. Anim. Behav. Sci. 99:271–286.

McKeegan, D. E. F., F. S. Smith, T. G. M. Demmers, C. M. Wathes, and R. B. Jones. 2005. Behavioural correlates of olfactory and trigeminal gaseous stimulation in chickens, *Gallus domesticus*. Physiol. Behav. 84:761–768.

Ministerie van Landbouw, Natuurbeheer en Visserij. 1992. Gezondheids- en welzijnswet voor dieren. Ministerie van Landbouw, Natuurbeheer en Visserij, Den Haag, the Netherlands.

Morgenstern, P.P., E. Lambooij, and W.H.M. Baltussen. 2009. Integrale beoordeling verdovingstechnieken pluimveeslachterijen. RIVM—Rapport 609021080/2009. LEI and Animal Sciences Group, Wageningen UR, Lelystad, the Netherlands. (in Dutch)

Raj, A. B. M., and N. G. Gregory. 1995. Welfare implications of the gas stunning of pigs 1. Determination of aversion to the initial inhalation of carbon dioxide or argon. Anim. Welf. 4:273–280.

Raj, M. 1998. Welfare during stunning and slaughter of poultry. Poult. Sci. 77:1815–1819.

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

Ribeiro, L. M., D. A. Ferreira, S. Brás, A. Castro, C. A. Nunes, P. Amorim, and L. M. Antunes. 2009. Correlation between clinical signs of depth of anaesthesia and cerebral state index responses in dogs during induction of anaesthesia with propofol. Res. Vet. Sci. 87:287–291.

Sandilands, V., A. B. M. Raj, and L. Baker. 2011. Aversion of chickens to various lethal gas mixtures. Anim. Welf. 20:253–262.

Vizzier-Thaxton, Y., K. D. Christensen, M. W. Schilling, R. J. Buhr, and J. P. Thaxton. 2010. A new humane method of stunning broilers using low atmospheric pressure. J. Appl. Poult. Res. 19:341–348.