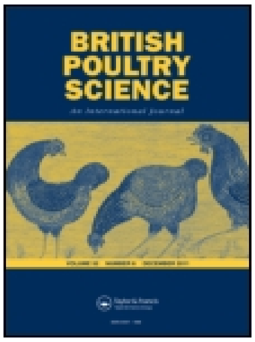

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Controlled atmosphere stunning of broiler chickens. II. Effects on behaviour, physiology and meat quality in a commercial processing plant

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Controlled atmosphere stunning of broiler chickens. II. Effects on behaviour, physiology and meat quality in a commercial processing plant

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Abstract 1. The effects of controlled atmosphere stunning on behavioural and physiological responses, and carcase and meat quality of broiler chickens were studied experimentally in a full scale processing plant.

2. The gas mixtures tested were a single phase hypercapnic anoxic mixture of 60% Ar and 30% CO_2 in air with <2% O_2, and a biphasic hypercapnic hyperoxygenation mixture, comprising an anaesthetic phase, 40% CO_2, 30% O_2, 30% N_2, followed by an euthanasia phase, 80% CO_2, 5% O_2, 15% N_2.

3. Birds stunned with $Ar + CO_2$ were more often observed to flap their wings earlier, jump, paddle their legs, twitch and lie dorsally (rather than ventrally) than those stunned with $CO_2 + O_2$. These behaviours indicate a more agitated response with more severe convulsions during hypercapnic anoxia, thereby introducing greater potential for injury.

4. Heart rate during the first 100 s of gas stunning was similar for both gases, after which it remained constant at ≈230 beats/min for $CO_2 + O_2$ birds whereas it declined gently for $Ar + CO_2$ birds.

5. In terms of carcase and meat quality, there appeared to be clear advantages to the processor in using $CO_2 + O_2$ rather than $Ar + CO_2$ to stun broiler chickens, for example, a much smaller number of fractured wings (1·6 *vs.* 6·8%) with fewer haemorrhages of the fillet.

6. This study supports the conclusions of both laboratory and pilot scale experiments that controlled atmosphere stunning of broiler chickens based upon a biphasic hypercapnic hyperoxygenation approach has advantages, in terms of welfare and carcase and meat quality, over a single phase hypercapnic anoxic approach employing 60% Ar and 30% CO_2 in air with <2% O_2.

INTRODUCTION

Within Europe and North America, electrical stunning using a constant voltage is the most common technique by which poultry are stunned prior to slaughter. However, despite the advantages of low cost and proven reliability, there are concerns (Raj and Tserveni-Gousi, 2000) about the humaneness of electrical stunning that can be overcome by the alternative technique of controlled atmosphere stunning (CAS). The major disadvantages of electrical stunning include manual handling, inversion, shackling and the variable current experienced, all of which adversely affect animal welfare.

There are many laboratory studies of the behavioural and physiological responses of fowl during CAS (Woolley and Gentle, 1988; Raj and Gregory, 1990; Raj *et al.*, 1998; Lambooij *et al.*, 1999; Coenen *et al.*, 2000; Gerritzen *et al.*, 2000; Webster and Fletcher, 2001; McKeegan *et al.*, 2006, 2007). In general, these support the

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contention that in terms of the overall system from arrival at the abattoir to death, CAS is more humane than electrical stunning but there are still doubts over the choice of gases to be used. Furthermore, the experimental conditions of laboratory studies differ in some key respects from those in processing plants, which may call into question the interpretation of laboratory findings, due to the presence of other birds or the atmosphere experienced.

In a comprehensive, coordinated project, we have examined the humaneness of CAS at various scales and degrees of refinement in the laboratory, a pilot scale CAS system and a full scale processing plant. This paper is the last in a series (McKeegan, 2004; McKeegan *et al*., 2006, 2007; Abeyesinghe *et al*., 2007; Lowe *et al*., 2007) reporting the results of this project. Here, we describe the behavioural and physiological reactions of broiler chickens to CAS under commercial conditions in a full scale processing plant. An assessment was also made of carcase and meat quality. Broiler chickens were stunned by one of two gas mixtures that tested alternative approaches to stunning: (i) a single phase hypercapnic anoxic mixture consisting of 60% Ar and 30% CO_2 in air with <2% O_2 ('Ar + CO_2'); and (ii) a biphasic hypercapnic hyperoxygenation mixture, comprising an anaesthetic phase with a gas mixture of 40% CO_2, 30% O_2, 30% N_2, followed by an euthanasia phase with 80% CO_2, 5% O_2, 15% N_2 ('$CO_2 + O_2$'). Both approaches are used commercially in continental Europe, though only Ar + CO_2 is permitted in the UK.

MATERIALS AND METHODS

Many of the materials and methods used in this experiment were similar to those reported in the accompanying paper (Abeyesinghe *et al*., 2007). Groups of mature broiler chickens were stunned by gas in a commercial processing plant using one of two gas mixtures. Detailed observations of behaviour, and measurements of heart and respiration rates were made prior to and during stunning. Carcase and meat quality were assessed after stunning. The experiment lasted 5 d; both treatments were applied on each day.

Full scale processing plant

The commercial processing plant was located in Belgium and ran at an average throughput of 9000 birds/h during the experiment, which took place between 30th August and 3rd September 2004. The CAS plant was manufactured by Stork Food Systems. Birds were loaded automatically from a transport crate on to a conveyor. They were then passed sequentially through a tunnel of two chambers in which the atmosphere could be controlled. The first chamber measured 9400 mm × 759 mm × 350 mm (l × w × h); the second chamber was divided into two parts, measuring 11 250 mm × 750 mm × 430 mm and 11 800 mm × 500 mm × 375 mm, respectively. The normal period of exposure was 64 and 118 s in the first and second chambers, respectively, but this was affected by the bird's movement on the conveyor. Gas concentration was controlled and monitored continuously by a Hydro MACS™ stunning control system (Yara International ASA, Oslo, Norway), measuring independently the concentration of CO_2 and O_2 within the flow in phase 1, and CO_2 in phase 2; the Ar concentration was inferred from the oxygen concentration. Additional measurement of the gas concentration was performed at a single location at the bird's head height within the chamber using a gas analyser (PBI Dansensor Checkpoint, Model 200548/210661). The mean (± SD) concentrations of CO_2 and O_2 in the first and second chambers were: (i) $CO_2 + O_2$ mixture, chamber 1—CO_2 40·0 ± 0·6%, O_2 30·0 ± 0·4%; chamber 2—CO_2 80·0 ± 0·7%; and (ii) Ar + CO_2 mixture, chamber 1 CO_2 30·1 ± 1·7%, O_2 1·3 ± 0·6%, Ar 58·6%. The atmosphere within the first chamber was humidified to 60 to 80 ± 10% relative humidity, following commercial practice.

After stunning, the birds were shackled by their legs and killed automatically by a unilateral cut to one carotid artery and one jugular vein in the neck, the whole process taking approximately 25 s. The carcases were then bled for 3 min, scalded at 53·5°C for 3 min and plucked. After evisceration, the carcases were chilled by air for 30 min at −0·5°C and then for a further 1·75 h at 0·5 to 1°C. Carcases, which were to be analysed for meat quality, were collected after leaving the chiller and stored at 0·5°C until manual de-boning, except for those which were examined at 1·5 h post mortem.

Behavioural and physiological observations

The methods used to observe the behavioural and physiological reactions of the birds to CAS are described by Abeyesinghe *et al*. (2007) and measurements were made daily for three days with each gas treatment applied on each day. The gas treatments were applied sequentially while the processing line was running at 9000 birds/h. A short break was needed between batches to allow the tunnel to be purged before a new gas mixture was introduced to the stunning chambers. All birds used in paired batches originated from the same flock.

Physiological measurements of heart and respiration rate were made on a total of 90 individuals (15 per treatment per d), each of which was fitted with a telemetric/logging system mounted on a body harness (Lowe *et al.*, 2007; see method described in Abeyesinghe *et al.*, 2007). Measurements were made for 60 s to establish a baseline while the bird was sitting on a table adjacent to the loading conveyor. The bird was then placed on the conveyor at the entrance to the tunnel, thereby rejoining a continuous stream of broiler chickens that were to be processed. Respiration rate was determined every 5 s from an average over the preceding 5 s. Heart rate was calculated from the electrocardiogram (ECG) trace at 5-s intervals for the baseline and stunning periods. During the recordings, the ECG waveform was occasionally obscured (sometimes for long periods) by electromyogram activity from the pectoral muscles and movement artefacts arising from a combination of either wing flapping and/or prolonged muscle contractions.

For the behavioural measurements, cameras were placed at 4 (out of 6) viewing windows along the length of the first chamber, which was lit artificially. This allowed an estimate to be made of the approximate time since entry to the tunnel. The positions and timings were: window 1, 0 to 8 s; window 2, 9 to 16 s; window 3, 17 to 24 s; and window 4, 33 to 40 s. It was not possible to view the birds in the second chamber because of the low light intensity. The number of birds being processed rendered it impossible to visually track harnessed individuals via these viewing stations so instead recordings were made continuously for the duration of all trials with each gas treatment.

Scan sampling at 3-min intervals was conducted on these continuously recorded data. For each scan, the observer selected the first observable bird passing nearest the window camera from which to record its behaviour, with the provisos that the chamber was full of birds and the conveyor was moving to maintain condition consistency. These provisos occasionally meant the scan was delayed by up to a maximum of 2 min, but on average only 5 s. The behaviour categories recorded were similar to those used by Abeyesinghe *et al.* (2007) in the pilot scale system with the addition of some new postural categories. The bird's posture was described as: (i) lie—bird lying (ventrally, dorsally or laterally) with body in contact with the conveyor and supine head; (ii) sit—bird sitting with sternum in contact with the conveyor with an erect head; and (iii) stand—bird standing upright on two legs. Several behaviours and the bird's posture could be recorded from each scan. In total, scans of 472 and 572 individual birds from the $Ar + CO_2$ and $CO_2 + O_2$ treatments, respectively, were observed.

Measurement of carcase and meat quality

A comprehensive set of measurements were made to determine the effects of stunning gas on carcase and meat quality; the methods are described in detail by Abeyesinghe *et al.* (2007). Table 1 shows the measurements that were made for each gas treatment on 6 flocks over 5 successive days, two days longer than the period

Table 1. *Measurements of carcase and meat quality in broiler chickens stunned by gas*

Measurement	Replicates per treatment per flock	Method, see Abeyesinghe *et al.* (2007)
Stunning, killing and bleeding		
Wing fracture	≈750 carcases	Palpation after stunning
Blood loss	10	Gravimetric
Evisceration and chilling		
Liver colour	50	Classification on a three-point scale
Plucking efficiency	24	Count
Skin damage	24	Classification on a two-point scale, summed separately by scratches and bruises on back, breast, legs, wing tips and tail
De-boning[1]		
Fillet pH[2]	6[3]	pH sensor
Fillet meat colour	6[3]	Spectrophotometric using CIELab classification
Shear force (t_0, N)	6[3]	Mechanical using a Warner Bratzler device
Haemorrhages	24	Classification on a three-point scale
Fractured bones	24	Palpation
Storage and cooking[4]		
Storage loss	6	Gravimetric
Cooking loss	6	Gravimetric
Shear force (t_{24}, N)	6	Mechanical using a Warner Bratzler device

[1]Carcases were de-boned at 15 min, 1·5, 3, 5, 7 and 24 h post mortem.
[2]$n = 20$ carcases at 15 min post mortem.
[3]Number of carcases per treatment per flock per de-boning time.
[4]Fillet halves were stored for 24 h until measurements were made.

over which behavioural and physiological measurements were made.

Statistical analysis

Genstat, release 6·2 (6th edn) for Windows© (1998 Lawes Agricultural Trust IACR), was used to perform the statistical analyses. For the behavioural data, Generalised Linear Mixed Model (GLMM: Marginal Model; Binomial distribution with logit link function) analyses were conducted on the proportion of total scans of each behaviour observed. All behavioural analyses used the treatment structure (in Genstat notation) of gas*window and a blocking structure of 'day' where gas described the two different gas mixtures ($Ar + CO_2$ and $CO_2 + O_2$), 'window' indicated the viewing window at which behaviour was observed and 'day' represented d 1 to 3 of the experiment. All behavioural data were transformed using the logit. For the physiological data, arithmetic means and SEs were calculated and visual comparisons made.

The carcase and meat quality data were analysed using ANOVA or regression analysis. For measurements made at a single time, the treatment structure was 'gas' with a blocking structure of flock/bird, where 'flock' indicates the source of the birds and bird indicates the individual. Where measurements were taken at various de-boning times, the treatment structure was gas*time; 'time' was log-transformed and linear and quadratic trends were determined using low order (up to second) polynomial contrasts. Transforming de-boning time made the steps between de-boning times more linear. Shear force data were transformed using logarithms. The blocking structure was flock/gas/time/bird. Analysis of deviance with a logit link was also used for variates with a binomial distribution, such as liver colour and wing fractures.

RESULTS

Behaviour

In total 1044 scans of behaviour were recorded; 143 scans per window on the $CO_2 + O_2$ treatment and 118 scans per window on the $Ar + CO_2$ treatment. The posture and behaviour categories are discussed individually. The transformed means, SEDs, Wald statistic and *P*-value for relevant treatment comparisons are given in parentheses for each behaviour; Figures 1 to 4 inclusive show the back-transformed means for each behaviour (either for the two gas mixtures or for the four windows, in order). The birds remained in the first chamber for approximately 75 s, which was defined as the first phase of exposure, while the second phase lasted from about 75 to 185 s.

Standing

Standing was recorded on only 3% of scans in total. There was no difference in occurrence between the gas mixtures (Figure 2: mean $Ar + CO_2$ and $CO_2 + O_2 = -3{\cdot}86$ and $-7{\cdot}03$, SED $= 4{\cdot}97$, $W_1 = 0{\cdot}83$, $P = 0{\cdot}362$) and, as expected, it sharply declined after the first window (Figure 3: mean $= -2{\cdot}25$, $-3{\cdot}60$, $-7{\cdot}97$ and $-7{\cdot}97$, SED $= 0{\cdot}46$, $W_3 = 16{\cdot}82$, $P < 0{\cdot}001$).

Sitting

Sitting was the most frequently recorded posture, occurring on 51% of scans. There was an interactive effect of gas type and duration in the chamber (Figure 1: mean $Ar + CO_2 = 2{\cdot}01$, $1{\cdot}08$, $-2{\cdot}50$ and $-10{\cdot}98$ and mean $CO_2 + O_2 = 2{\cdot}22$, $2{\cdot}83$, $-0{\cdot}07$ and $-4{\cdot}26$, SED $= 4{\cdot}84$, $W_3 = 10{\cdot}0$, $P = 0{\cdot}018$) with a decline in sitting across windows (as birds progressed) in both gas mixtures but this occurred more steeply in $Ar + CO_2$ than $CO_2 + O_2$.

Lying

Overall, lying was recorded on 46% of scans. There was no difference in occurrence between the gas mixtures (Figure 2: mean $Ar + CO_2$ and $CO_2 + O_2 = 0{\cdot}58$ and $-2{\cdot}44$, SED $= 3{\cdot}95$, $W_1 = 0{\cdot}03$, $P = 0{\cdot}858$), however, further analysis indicated lying posture was affected by the gas treatment; more birds were recorded lying dorsally in $Ar + CO_2$ (Figure 4: mean $Ar + CO_2$ and $CO_2 + O_2 = 0{\cdot}11$ and $-4{\cdot}6$, SED $= 0{\cdot}75$, $W_1 = 39{\cdot}34$, $P < 0{\cdot}001$) and more lying ventrally in $CO_2 + O_2$ (Figure 4: mean $Ar + CO_2$ and $CO_2 + O_2 = -0{\cdot}83$ and $0{\cdot}42$, SED $= 0{\cdot}25$, $W_1 = 25{\cdot}58$, $P < 0{\cdot}001$). Observations of birds lying laterally did not differ between gases (Figure 4: mean $Ar + CO_2$ and $CO_2 + O_2 = -1{\cdot}48$ and $-7{\cdot}13$, SED $= 11{\cdot}54$, $W_1 = 0{\cdot}24$, $P = 0{\cdot}627$). As expected, a sharp increase in lying occurred across windows, particularly at window 3 (Figure 3: mean $= -7{\cdot}43$, $-2{\cdot}02$, $1{\cdot}23$ and $4{\cdot}51$, SED $= 0{\cdot}32$, $W_3 = 178{\cdot}50$, $P < 0{\cdot}001$).

Respiratory disruption

Respiratory disruption was recorded on 43% of scans overall. It occurred progressively less as birds moved along the chamber (from windows 1 to 4) in both gas mixtures, with little difference between treatments at window 1, but continued at a high occurrence for longer in $CO_2 + O_2$ than $Ar + CO_2$ (Figure 1: mean $Ar + CO_2 = 1{\cdot}71$,

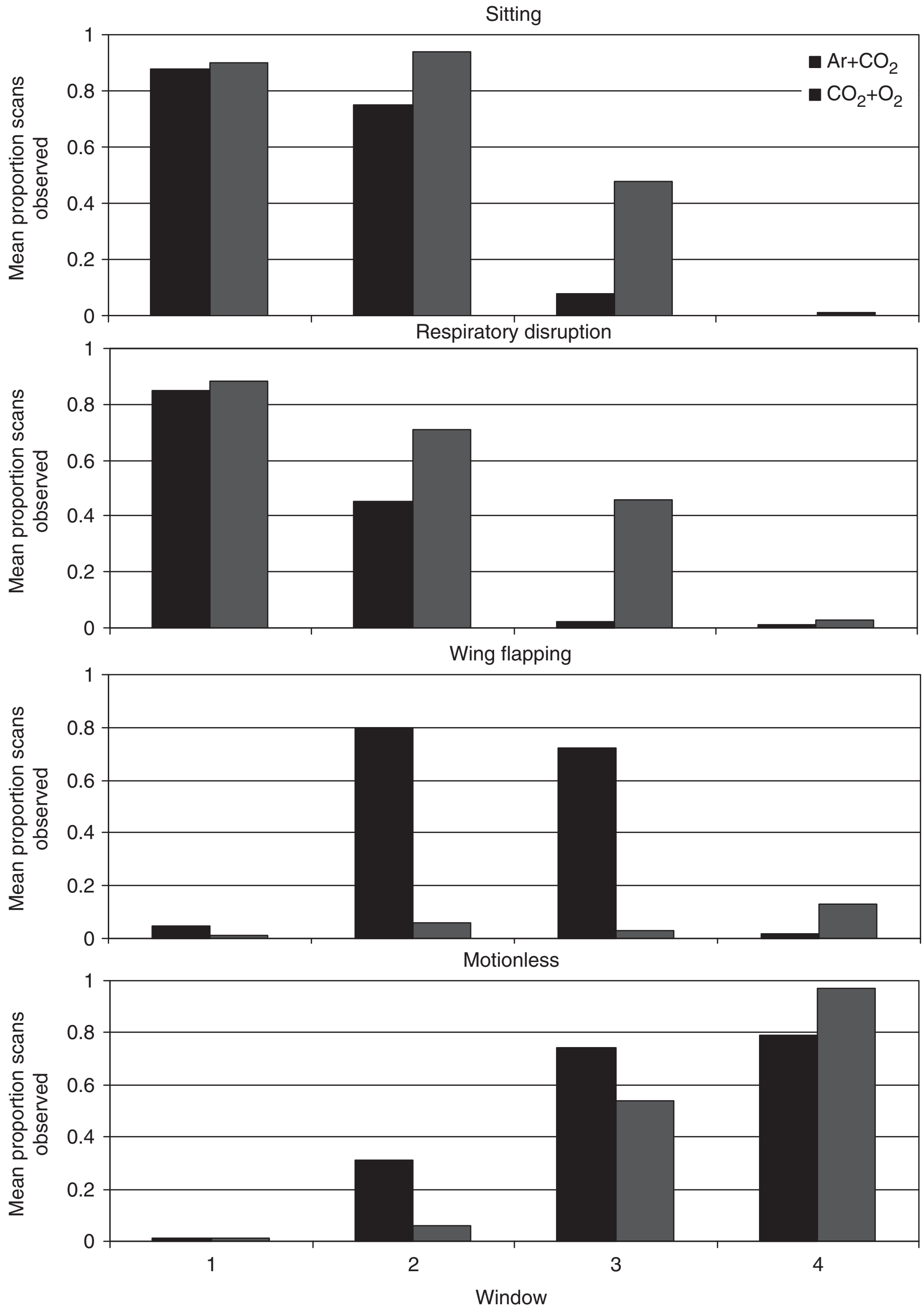

Figure 1. *Interactive effects of gas mixture and section of gas chamber (windows 1 to 4) on mean proportion of scans of posture/behaviour observed and based on back-transformed means ($n = 143$ and 118 scans per window for $CO_2 + O_2$ and $Ar + CO_2$, respectively). The transformed means on the logit scale, SEDs, Wald statistics and P-values referring to the transformed data are quoted in the text.*

−0·21, −4·06 and −4·76 and mean $CO_2 + O_2 = 2{\cdot}01$, 0·92, −0·14 and −3·54, SED = 0·86, $W_3 = 10{\cdot}7$, $P = 0{\cdot}014$).

Mandibulation

Mandibulation occurred on only 16% of scans overall and was equally apparent in both mixtures (Figure 2: mean $Ar + CO_2$ and $CO_2 + O_2 = -6{\cdot}28$ and −3·98, SED = 6·60, $W_1 = 0{\cdot}23$, $P = 0{\cdot}630$), declining in occurrence sharply after the first window (Figure 3: mean = 0·12, −2·43, −7·14 and −11·06, SED = 0·31, $W_3 = 108{\cdot}07$, $P < 0{\cdot}001$).

Head shaking

Head shaking occurred on only 13% of scans in total and followed a similar pattern to mandibulation, with no difference between gas mixtures

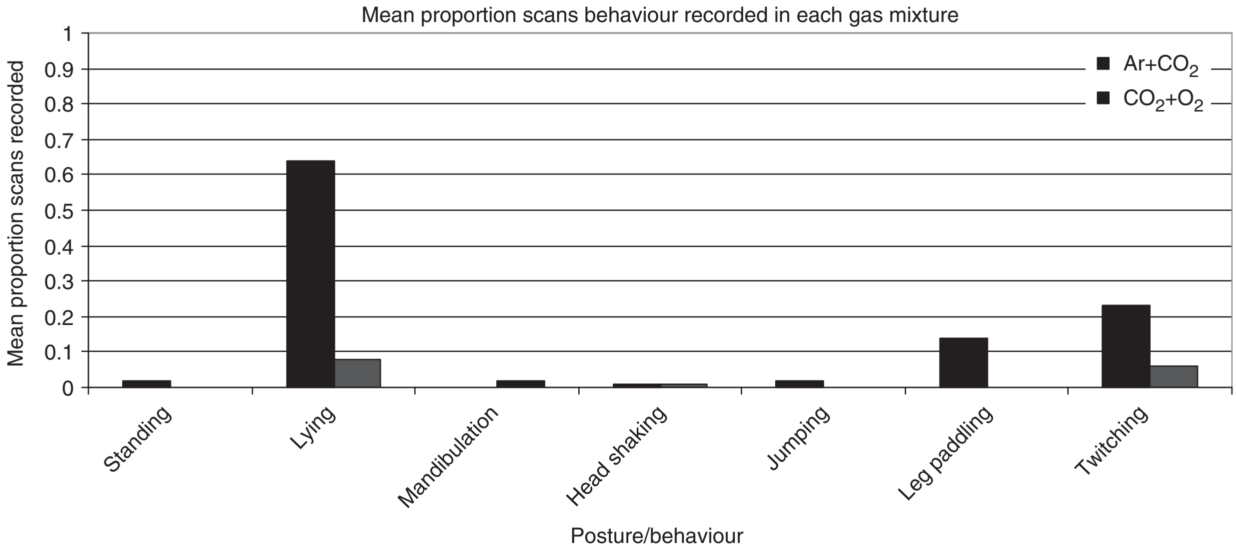

Figure 2. *Main effects of gas mixture on mean proportion of scans of posture/behaviour observed based on back-transformed means. Zero scans were recorded for some measurements (n = 143 and 118 scans per window for $CO_2 + O_2$ and $Ar + CO_2$, respectively). The transformed means on the logit scale, SEDs, Wald statistics and P-values referring to the transformed data are quoted in the text.*

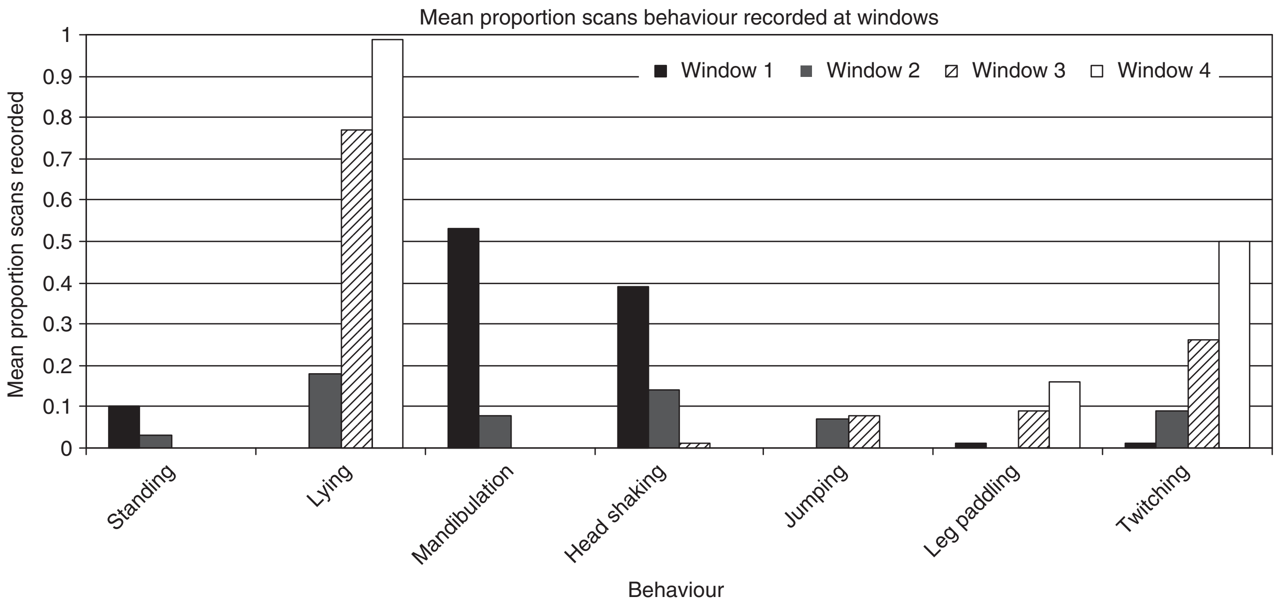

Figure 3. *Main effects of window on mean proportion of scans of posture/behaviour observed based on back-transformed means. Zero scans were recorded for some measurements (n = 143 and 118 scans per window for $CO_2 + O_2$ and $Ar + CO_2$, respectively). The transformed means on the logit scale, SEDs, Wald statistics and P-values referring to the transformed data are quoted in the text.*

(Figure 2: mean $Ar + CO_2$ and $CO_2 + O_2 = -4{\cdot}59$ and $-4{\cdot}33$, SED = 6·73, $W_1 = 0{\cdot}30$, $P = 0{\cdot}585$) and a decline across the first two windows, with few birds observed head shaking after window 2 (Figure 3: mean = −0·44, −1·82, −4·51 and −11·08, SED = 0·31, $W_3 = 38{\cdot}40$, $P < 0{\cdot}001$).

Wing flapping

Wing flapping occurred on 21% of all scans. Significantly more wing flapping was recorded in $Ar + CO_2$ than $CO_2 + O_2$ and this mostly occurred in the second and third windows, tailing off sharply at the final viewing point (Figure 1: mean $Ar + CO_2 = -2{\cdot}93$, 1·37, 0·95 and −4·06 and mean $CO_2 + O_2 = -4{\cdot}96$, −2·83, −3·55 and −1·88, SED = 0·89, $W_3 = 38{\cdot}3$, $P < 0{\cdot}001$).

Jumping

Jumping was recorded on approximately 10% of all scans, almost exclusively in $Ar + CO_2$ (Figure 2: mean $Ar + CO_2$ and $CO_2 + O_2 = -3{\cdot}78$ and $-5{\cdot}88$, SED = 0·37, $W_1 = 32{\cdot}71$, $P < 0{\cdot}001$). It was generally associated with wing

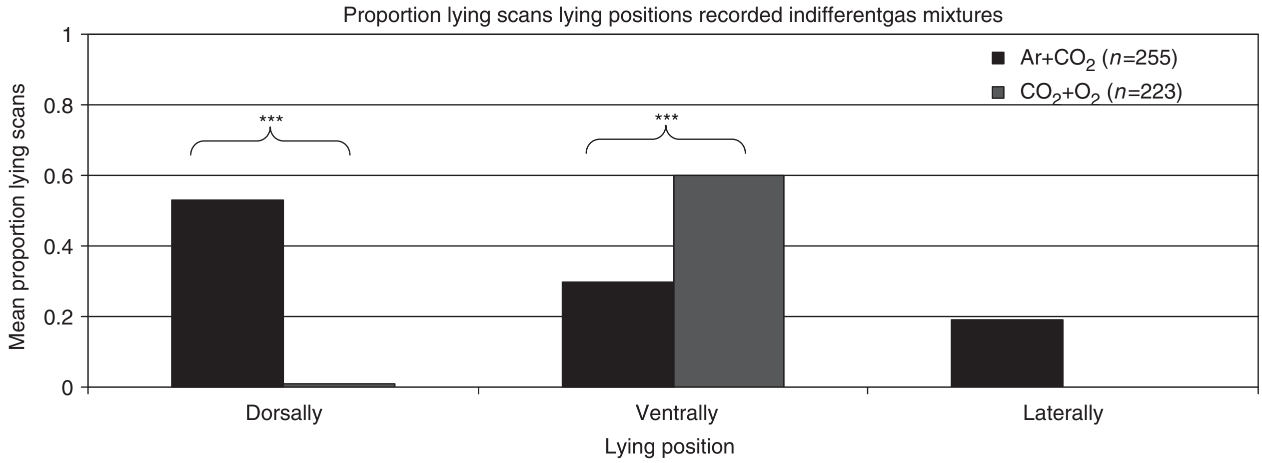

Figure 4. *Effects of gas treatment on mean proportion of scans recorded as lying shown as back-transformed means; birds were observed lying dorsally, ventrally and laterally. Zero scans were recorded for some measurements (n = 143 and 118 scans per window for $CO_2 + O_2$ and $Ar + CO_2$, respectively). The transformed means on the logit scale, SEDs, Wald statistics and P-values referring to the transformed data are quoted in the text.*

flapping, occurring largely in the two mid-windows (Figure 3: mean = −7·26, −2·58, −2·46 and −7·03, SED = 0·916, $W_3 = 17{\cdot}97$, $P < 0{\cdot}001$) and indicates a greater severity of convulsion.

Leg paddling

Leg paddling was recorded on 11% of all scans, again almost exclusively in $Ar + CO_2$ (Figure 2: mean $Ar + CO_2$ and $CO_2 + O_2$ = −1·82 and −5·80, SED = 0·85, $W_1 = 21{\cdot}68$, $P < 0{\cdot}001$) and increasing in occurrence across windows (Figure 3: mean = −4·50, −6·76, −2·29 and −1·68, SED = 0·68, $W_3 = 20{\cdot}14$, $P < 0{\cdot}001$).

Twitching

Twitching occurred on 23% of all scans and was observed significantly more in $Ar + CO_2$ than $CO_2 + O_2$ (Figure 2: mean $Ar + CO_2$ and $CO_2 + O_2$ = −1·24 and −2·69, SED = 0·53, $W_1 = 7{\cdot}64$, $P = 0{\cdot}006$). Its occurrence also increased across windows (Figure 3: mean = −4·50, −2·33, −1·03 and 0·01, SED = 0·29, $W_3 = 43{\cdot}88$, $P < 0{\cdot}001$).

Motionless

Motionless increased later in $CO_2 + O_2$ than $Ar + CO_2$, but a significantly greater proportion of scans recorded motionless in the final window for $CO_2 + O_2$ compared with $Ar + CO_2$ (Figure 1: mean $Ar + CO_2$ = −4·75, −0·81, 1·05 and 1·33 and mean $CO_2 + O_2$ = −4·96, −2·83, 0·15 and 3·54, SED = 1·38, $W_3 = 10{\cdot}5$, $P = 0{\cdot}15$). This may have been due to the more frequent occurrence of other behaviours seen in $Ar + CO_2$.

Heart rate and respiration rate

Figure 5 shows the mean heart rate of the birds during the baseline period and the first 150 s of stunning. Heart rate fell slightly from about 360 beats/min when the birds were caught and fitted with a harness to about 350 beats/min when they were placed on the conveyor to enter the gas tunnel. Once they had entered the tunnel (at 0 s), heart rate fell markedly over the next 10 s before stabilising at about 204 and 236 beats/min for $Ar + CO_2$ and $CO_2 + O_2$, respectively. Inspection of the data shows that the difference between the treatments was minor. The mean heart rate for the $CO_2 + O_2$ birds remained at about 230 beats/min for a further 50 s and declined slightly for the $Ar + CO_2$ birds. The number of obscured segments of ECG trace was much greater for $Ar + CO_2$ birds than $CO_2 + O_2$ birds, reflecting the vigorous movements of the former and/or the permanent loss of the sensor ($Ar + CO_2$ 8/45 birds; $CO_2 + O_2$ 1/45 birds; Figure 6).

The respiration rate in the baseline period was similar for birds from both treatments and declined slowly as the birds recovered from handling from about 53 breaths/min, just after the harness was fitted, to 50 breaths/min prior to placement on the conveyor.

Carcase and meat quality

Table 2 shows the results of the measurements of carcase and meat quality. Some, but not all, of the measures were affected significantly by the gas used to stun the birds. Blood loss (approximately 3% by weight), skin damage and liver colour were similar in both treatments. Overall, the number of carcases with fractured wings ($P < 0{\cdot}001$) and the mean score for fillet

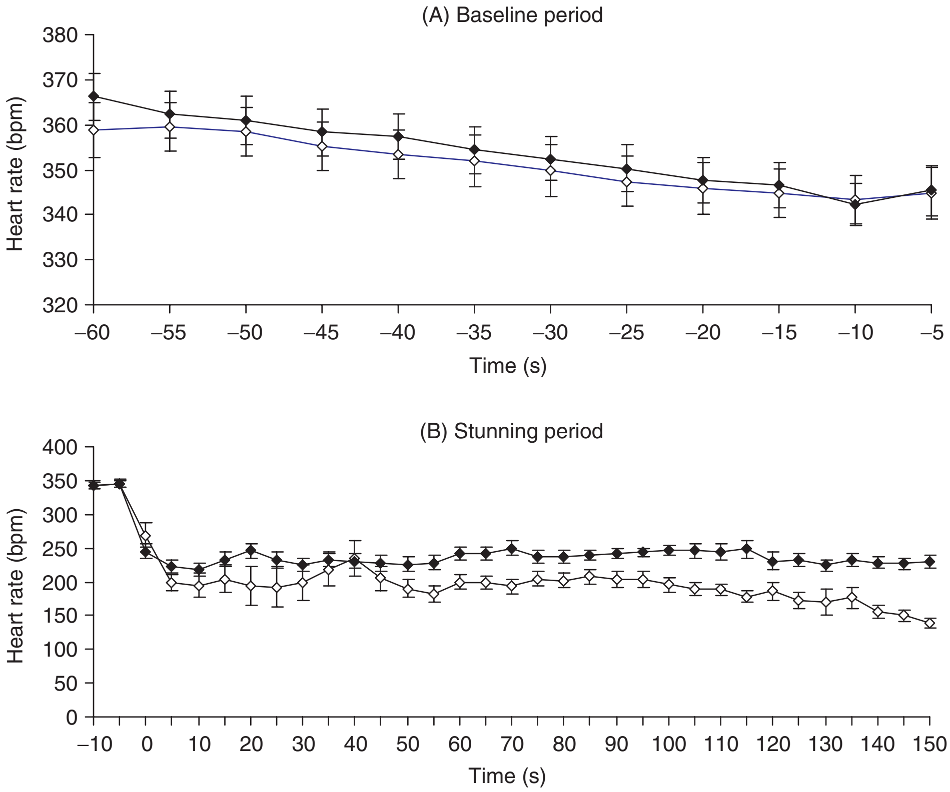

Figure 5. *Mean heart rate over time for (A) the baseline period and (B) the first 150 s of gas stunning ($CO_2 + O_2$: closed circles; $Ar + CO_2$: open circles). Error bars represent standard errors. n = 45 birds for each gas treatment.*

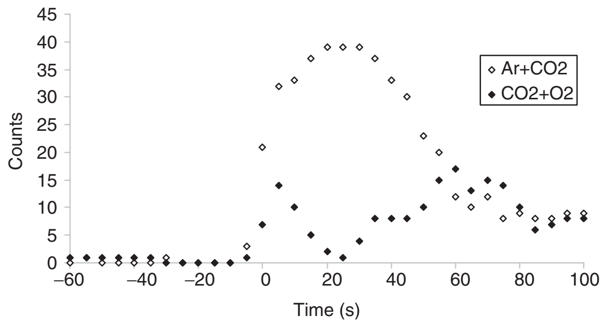

Figure 6. *Number of segments of obscured ECG traces for broiler chickens (n = 45 birds per treatment) stunned by gas for each 5-s time period.*

haemorrhages ($P = 0{\cdot}05$) were significantly higher for $Ar + CO_2$ than $CO_2 + O_2$. The number of wings examined was 9110 and 9930 for the $Ar + CO_2$ and $CO_2 + O_2$ treatments, respectively. By far the most common fractures were of the wrist and elbow joint ($Ar + CO_2$ 3·9 and 2·4%; $CO_2 + O_2$ 0·8 and 0·8%); there were few fractures of the humerus and radius/ulna and none of the wing tips. In terms of fractured wings, the prevalence of breakages with or without perforation of the skin was between 5 and 6 times higher for $Ar + CO_2$ than $CO_2 + O_2$; overall, the prevalence of fractured wings of both types was 6·8 and 1·6%, respectively.

Various measures of meat quality over time are shown in Table 3. There was a significant interaction between time and gas for fillet pH but it could not be explained by a simple linear or quadratic trend ($P < 0{\cdot}001$). Fillet pH rose initially for $CO_2 + O_2$, but not $Ar + CO_2$, carcases and final pH was reached 15 min after post mortem for $Ar + CO_2$ but not until 5 h for $CO_2 + O_2$. There were main effects of gas and time on shear force, measured immediately after de-boning (t_0) and after 24 h storage (t_{24}); shear force fell non-linearly with time, was highest in $CO_2 + O_2$ and did not stabilise until about 7 h post mortem. Neither storage nor cooking losses were affected by the gas treatment nor did they change with time; they were about 0·5 and 25%, respectively.

The results of the measurements of the colour of the fillets are given in Table 4 using the CIELab convention. There were no interactions between gas and time. The distal surface of the fillets was darkest for $Ar + CO_2$ ($P = 0{\cdot}003$) and there was a similar trend for the proximal surface (NS). Some changes were also observed over time; the distal surface of the fillets became paler and less red, while the proximal surface became less yellow.

DISCUSSION

In general, these experimental findings support those of the accompanying paper (Abeyesinghe

Table 2. *Effects of stunning gas on blood loss, liver colour, carcase quality and muscle haemorrhages in broiler chickens stunned by gas during various stages of processing*

	$Ar + CO_2$	$CO_2 + O_2$	Gas	
			P	SED
Blood loss, g per bird[1]				
At 90 s after stunning	59·1	57·1	NS	2·46
At 150 s after stunning	65·7	65·9	NS	2·09
Liver colour, mean score per carcase[2]	2·23	2·17	NS	n/a
Plucking efficiency, proportion of carcases with ≥2 feathers remaining per bird	0·85	0·81	NS	0·044
Skin damage, mean score per carcase				
Carcase scratches, $\log_e$	1·03	1·13	NS	0·114
Carcase bruises	8·18	8·11	NS	0·713
Wing fractures, mean proportion of wings[3]				
Breakage	0·047	0·012)		
Breakage with skin perforation	0·021	0·004)	<0·001	0·0049
Intact	0·932	0·984)		
Other fractures, mean proportion of carcases				
Wishbone with haemorrhaging	0·275	0·183	NS	0·054
Coracoid	0·008	0	NS	0·0083
Shoulder blade	0·008	0	NS	0·0083
Haemorrhages, mean score				
Fillet (distal surface)	0·32	0·19	0·05	0·056
Tenderloin (distal surface)	0·05	0·04	NS	–
Thigh	0·33	0·30	NS	0·044
Shoulder joint	0·25	0·28	NS	0·034

[1]Using live weight as a covariate.
[2]Comparison of frequency distributions after logit transformation.
[3]Analysis of deviance, SEDs are approximate where given.
NS = not significant; n/a = not applicable; number of replicates given in Table 1.

Table 3. *Dynamic changes in fillet pH, shear force, and storage and cooking losses in broiler chickens stunned by gas*

	Time post slaughter, h						Gas		Time		Time. Gas interaction	
	0·25	1·5	3	5	7	24	SED	*P*	SED	*P*	SED	*P*
Fillet pH												
$Ar + CO_2$	6·00	6·04	5·99	5·97	5·98	5·97	0·021	<0·001	0·039	<0·001	0·055	<0·001
$CO_2 + O_2$	6·32	6·48	6·38	6·04	5·97	5·95						
Shear force (t_0, N), ln												
$Ar + CO_2$	–	4·94	4·73	4·28	3·67	2·95	0·043	0·05	0·076	<0·001	0·105	NS
$CO_2 + O_2$	–	4·86	4·93	4·53	3·74	3·07						
Shear force (t_{24}, N), ln												
$Ar + CO_2$	–	3·95	3·60	3·32	3·05	2·87	0·028	0·002	0·073	<0·001	0·097	NS
$CO_2 + O_2$	–	4·14	3·91	3·52	3·18	2·85						
Storage loss,%												
$Ar + CO_2$	–	0·55	0·53	0·44	0·40	0·56	0·019	NS	0·063	NS	0·081	NS
$CO_2 + O_2$	–	0·51	0·50	0·49	0·35	0·50						
Cooking loss,%												
$Ar + CO_2$	–	27·4	25·6	25·0	24·7	24·7	0·455	NS	1·486	NS	1·934	NS
$CO_2 + O_2$	–	26·1	26·0	24·8	24·9	23·7						

Number of replicates given in Table 1.

et al., 2007) on the reactions and responses of broiler chickens to CAS. This study employed the same techniques as the latter but extended the scope from a pilot scale system to the full scale conditions of the commercial processing plant, while still employing a common scientific approach. To the best of our knowledge this is the first report of the effects of CAS on broiler chickens in the processing plant on such a large scale.

The behavioural responses of the birds followed a logical sequence in terms of their physiological and behavioural reactions to CAS, though it should be noted that our observations were restricted to the first 40 s of stunning. Most birds were sitting on entry to the gas tunnel and

Table 4. *Dynamic changes in the colour of fillets of broiler chickens stunned by gas*

	Time post slaughter, h					Gas		Time		Time. Gas interaction	
	1·5	3	5	7	24	SED	*P*	SED	*P*	SED	*P*
L* proximal											
$Ar + CO_2$	57·0	57·7	57·4	58·5	57·4						
$CO_2 + O_2$	57·7	58·6	59·4	59·3	59·8	0·424	NS	0·678	NS	0·952	NS
L* distal											
$Ar + CO_2$	58·5	59·8	60·9	61·1	60·7						
$CO_2 + O_2$	59·3	60·8	61·8	62·1	63·1	0·222	0·003	0·559	<0·001	0·740	NS
a* proximal											
$Ar + CO_2$	4·82	4·52	4·30	4·89	4·80						
$CO_2 + O_2$	4·79	4·40	4·23	4·51	4·16	0·212	NS	0·209	NS	0·339	NS
a* distal											
$Ar + CO_2$	4·08	3·45	3·30	4·17	3·91						
$CO_2 + O_2$	3·88	3·44	3·31	3·38	3·32	0·172	NS	0·177	0·004	0·282	NS
b* proximal											
$Ar + CO_2$	12·8	12·5	12·0	12·6	11·1						
$CO_2 + O_2$	13·7	13·3	12·6	12·6	12·0	0·374	NS	0·423	0·003	0·649	NS
b* distal											
$Ar + CO_2$	9·9	10·2	10·2	10·5	10·0						
$CO_2 + O_2$	10·9	11·2	10·7	11·1	10·8	0·119	0·001	0·308	NS	0·407	NS

Number of replicates given in Table 1.

their initial reaction to the stunning gas was mandibulation and head shaking, showing that they had detected the gas either directly via chemo-receptors or indirectly via consequential physiological effects. Subsequently—and in an approximate order of occurrence—they sat down, showed signs of respiratory disruption, flapped their wings, lay down, paddled their legs, jumped and twitched before becoming motionless. However, this sequence differed in some aspects for the two gases. Birds stunned with $Ar + CO_2$ were more often observed to flap their wings earlier, jump, paddle their legs, twitch and lie dorsally (rather than ventrally) than those stunned with $CO_2 + O_2$. These behaviours have the potential to cause distress if the birds are conscious; similar observations were made by Lambooij *et al.* (1999) and Abeyesinghe *et al.* (2007). In particular, our findings for lying position, wing flapping and jumping indicate more severe convulsions during hypercapnic anoxia, thereby introducing greater potential for injury for both the bird itself and its companions. Indeed, the high prevalence of wing fractures in $Ar + CO_2$ demonstrates the force of the bird's movements and convulsions in this gas mixture. This is a welfare issue only if birds were sensible at any time during these convulsions; our laboratory findings (McKeegan *et al.*, 2007) describing electroencephalogram (EEG) signals during this period suggest that this is a possibility.

It would have been advantageous to have recorded the vocalisations made by the birds because these would have been indicative of their state of anxiety, fear or distress, but to the best of our knowledge there are no published measurements of vocalisations during CAS. It is not known whether the sight, sound and smell of one bird dying are distressing to another (conscious) bird although the physical proximity of others may result in disturbance or even injury; sheep are apparently unaffected when a conspecific is killed in their presence (Anil *et al.*, 1996). Respiratory disruption was more common and continued for longer in $CO_2 + O_2$ than $Ar + CO_2$, but the interpretation of this finding is complicated by the fact that one of the mixtures ($Ar + CO_2$) was both hypercapnic and anoxic and thus may have induced both types of disruption that were included in this behavioural category. However, McKeegan *et al.* (2006, 2007) reported increased respiratory disruption in response to hypercapnic hyperoxygenation, an observation supported by the current findings. Overall, our observations of behaviour indicate a slower but somewhat smoother transition to a motionless state in the biphasic hypercapnic hyperoxygenation treatment compared with the hypercapnic anoxic treatment.

During the baseline period, heart rate fell steadily and respiration rate fell slightly as the birds recovered from handling. It was not possible to continue the measurement of respiration rate once the birds had entered the gas tunnel owing to the number of immeasurable traces that disrupted the recording system, caused by wing flapping, leg paddling, tonic pectoral muscle contractions and jostling by other birds. Heart rate throughout the first

100 s of gas stunning was similar for both gases and its dynamic change over time followed a similar pattern to that observed for both individuals and groups of birds in the pilot scale system (Abeyesinghe *et al.*, 2007). More detailed analysis of the ECG traces in the latter study showed evidence of bradycardia and arrhythmia with both gas treatments. The loss of traces was greater for $Ar + CO_2$ than $CO_2 + O_2$, probably due to the loss of the sensor and signals and supporting the conclusion that experience of an anoxic hypercapnic gas mixture causes a more vigorous behavioural response in broiler chickens. Such intense and long lasting artefacts in the trace may indicate strong isometric muscle contractions which are likely to be painful and which occur during a period when consciousness cannot be excluded (McKeegan *et al.*, 2007).

In terms of carcase quality there appears to be clear advantages to the processor and the consumer in using $CO_2 + O_2$ rather than $Ar + CO_2$ to stun broiler chickens, particularly because of a much smaller number of fractured wings. Based upon a commercially relevant number of carcases that were examined for wing fracture (≈19 000), the overall prevalence of wing fractures was 4 times worse in $Ar + CO_2$ than $CO_2 + O_2$. Notably, a large proportion of these fractures led to skin perforation, which can be attributed to the more violent movements and convulsions of birds stunned with $Ar + CO_2$ compared with $CO_2 + O_2$. If these fractures occurred when the birds were conscious, then welfare as well as carcase quality would be severely compromised.

CAS has been shown to produce meat of superior quality when compared with electrical stunning (Raj *et al.*, 1997; Uijttenboogaart, 1997; Hoen and Lankhaar, 1999). Compared with $Ar + CO_2$, $CO_2 + O_2$ stunning induced fewer haemorrhages in the fillets. Differences between the gases in the fall in fillet pH post mortem appear to be associated with the behaviour of the birds during stunning, confirming the results of the pilot plant study (Abeyesinghe *et al.*, 2007). The more pronounced peri-mortem struggles, such as wing flapping and leg paddling, in $Ar + CO_2$ accelerated glycolysis and lactate accumulation, as shown by the low pH 15 min after slaughter. These results contrast with our earlier study (Abeyesinghe *et al.*, 2007), where the rate of fall of pH decline was similar in $CO_2 + O_2$ and $Ar + CO_2$ stunned birds. This may be due to the very rapid decline in pH observed for $Ar + CO_2$ in this study, which was probably the result of vigorous wing flapping. Storage and cooking losses were similar to those recorded in our pilot study and were unaffected by stunning treatment (Abeyesinghe *et al.*, 2007).

Raj *et al.* (1991) found that the majority of broilers stunned by argon attained rigor mortis within 20 min, enabling carcases to be filleted 2 h post mortem without affecting meat texture. The low pH values measured after 15 min post mortem for $Ar + CO_2$ are in accordance with these results. However, our measurements of toughness (shear force, t_0) indicate that for both stunning CAS methods, carcases took up to 7 h to attain full rigor mortis. In commercial in-line poultry processing, carcases are portioned and de-boned after 2 to 3 h post mortem, with a risk of tough meat when CAS is used. This problem can be overcome by electrical stimulation of the carcase to allow in-line de-boning, typically 3 h post mortem, without the risk of tough meat (Kranen, 2003). This solution is adopted commercially in many European abattoirs as one stage in processing broiler carcases for meat.

The lightness of the meat is indicated by its L* value, which is inversely proportional to muscle pH (Qiao *et al.*, 2001). This was indeed true for the distal (exterior) surface of $CO_2 + O_2$ fillets, which became lighter over time post slaughter as pH fell. However, this relationship did not appear to hold for $Ar + CO_2$ fillets, indicating that muscle colour is not only determined by muscle pH.

The results of this study have direct application to the practical conditions of the commercial processing plant. Unlike laboratory experiments, the birds experienced the full range of environmental and social stressors during stunning. These relate to the presence of other birds in the gas tunnel, which may cause physical interference or whose behaviour, vocalisations and other reactions may be perceived perhaps adversely; process control of the atmospheric and thermal environment, which is so influential in ensuring rapid exposure to the correct concentration of gases at an appropriate temperature and humidity; and the experience of the birds during transport from the farm to the lairage. Indeed, the methods employed here could be used to determine the causes of poor welfare and meat quality from the farm to the abattoir, identifying the risk factors responsible and suggesting improvements. Risk factors that might be addressed include: the merits of gas stunning within or outside transport crates; and the benefits and drawbacks of electrical stunning *vs.* CAS. The advantages of biphasic CAS over electrical stunning of poultry were also recognised at a symposium on this topic (Barton Gade *et al.*, 2001). It is only by integration of studies in the laboratory and commercial plants using techniques from a range of scientific and engineering disciplines that progress can be made to the benefit of the bird, processor and consumer alike (Wathes, 2005).

This and the accompanying paper (Abeyesinghe *et al.*, 2007) form part of a coordinated series of experiments (McKeegan, 2004; McKeegan *et al.*, 2006, 2007; Lowe *et al.*, 2007) that have examined the effects of CAS on broiler chickens. We have studied the three distinct approaches that can be used in controlled atmosphere stunning of broiler chickens, namely, anoxia, hypercapnic anoxia and a combination of hypercapnic hyperoxygenation anaesthesia and hypercapnic euthanasia. The strengths of this coordinated study lie in firstly, the comprehensive range of techniques employed to measure behavioural and physiological responses that are indicative of welfare, and physico-chemical properties of the carcase and meat that describe the organoleptic and aesthetic appeal of chicken meat; secondly, the location and scale of study from the laboratory to the processing plant; and thirdly, the different physiological approaches used to stun the birds. Interpretation of the behavioural and physiological data is complex and we do not believe that it is wise to speculate on which of our findings are indicative of pain without direct measurements. Such measurements are either extremely difficult or impossible to undertake in a commercial abattoir at present. Fractures are clearly likely to be painful if experienced by a conscious bird, and although we cannot tell from this experiment when they occurred, they were 4 times more prevalent in $Ar + CO_2$ than $CO_2 + O_2$. Measurements relating to loss of consciousness were not carried out in this experiment but in all other respects the results support the findings of our laboratory studies (McKeegan *et al.*, 2007) which suggested that early onset wing flapping during anoxia occurs during periods in which a form of consciousness cannot be excluded. In terms of the two CAS approaches investigated here, we conclude that a biphasic approach based upon consecutive phases of anaesthesia and euthanasia is potentially more humane than an anoxic hypercapnic approach. It is also superior in terms of carcase and meat quality.

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REFERENCES

ABEYESINGHE, S.M., MCKEEGAN, D.E.F., MCLEMAN, M.A., LOWE, J.C., DEMMERS, T.G.M., WHITE, R.P., KRANEN, R.W., BEMMEL, H. VAN, LANKHAAR, J.A.C. & WATHES, C.M. (2007) Controlled atmosphere stunning of broiler chickens. I. Effects on behaviour, physiology and meat quality in a pilot scale system at a processing plant. *British Poultry Science*, **48**: 406–423.

ANIL, M.H., PRESTON, J., MCKINSTRY, J.L., RODWAY, R.G. & BROWN, S.N. (1996) An assessment of stress caused in sheep by watching slaughter of other sheep. *Animal Welfare*, **5**: 435–441.

BARTON GADE, P., HOLLEBEN, K. VAN & WENZLAWOWICZ, M. VON (2001) Animal welfare and controlled atmosphere stunning of poultry using mixtures of carbon dioxide and oxygen. *World's Poultry Science Journal*, **57**: 189–200.

COENEN, A., SMIT, A., ZHONGHUA, L. & LUIJTELAAR, G. VAN (2000) Gas mixtures for anaesthesia and euthanasia in broiler chickens. *World's Poultry Science Journal*, **56**: 225–234.

GERRITZEN, M.A., LAMBOOIJ, E., HILLEBRAND, S.J.W., LANKHAAR, J. & PIETERSE, C. (2000) Behavioural responses of broilers to different gaseous atmospheres. *Poultry Science*, **79**: 928–933.

HOEN, T. & LANKHAAR, J. (1999) Controlled atmosphere stunning of poultry. *Poultry Science*, **78**: 287–289.

Kranen R.W. (2003) Rapid rigor: the way to accelerate in-line poultry processing. *Proceedings of the XVIth European Symposium on the Quality of Poultry Meat. Vol. II Quality of Poultry Meat*, Saint-Brieuc, France pp. 666–671.

LAMBOOIJ, E., GERRITZEN, M.A., ENGEL, B., HILLEBRAND, S.J.W., LANKHAAR, J. & PIETERSE, C. (1999) Behavioural responses during exposure of broiler chickens to different gas mixtures. *Applied Animal Behaviour Science*, **62**: 255–265.

LOWE, J.C., ABEYESINGHE, S.M., DEMMERS, T.G.M., WATHES, C.M. & MCKEEGAN, D.E.F. (2007) A novel telemetric logging system for recording physiological signals in unrestrained animals. *Computers and Electronics in Agriculture*, **57**: 74–79.

MCKEEGAN, D.E.F. (2004) Mechano-chemical nociceptors in the avian trigeminal mucosa. *Brain Research Reviews*, **46**: 146–154.

MCKEEGAN, D.E.F., MCINTYRE, J., DEMMERS, T.G.M., WATHES, C.M. & JONES, R.B. (2006) Behavioural responses of broiler chickens during acute exposure to gaseous stimulation. *Applied Animal behaviour Science*, **99**: 271–286.

MCKEEGAN, D.E.F., MCINTYRE, J., DEMMERS, T.G.M., LOWE, J.C., WATHES, C.M., BROEK, P.L.C. Van Den, COENEN, A.M.L. & GENTLE, M.J. (2007) Physiological and behavioural responses of broilers to controlled atmosphere stunning—implications for welfare. *Animal Welfare* (in press).

QIAO, M., FLETCHER, D.L., SMITH, D.P. & NORTHCUTT, J.K. (2001) The effect of broiler breast meat colour on pH, moisture, water-holding capacity, and emulsification capacity. *Poultry Science*, **80**: 676–680.

RAJ, A.B.M. & GREGORY, N.G. (1990) Investigation into the batch stunning/killing of chickens using carbon dioxide or argon-induced hypoxia. *Research in Veterinary Science*, **49**: 363–366.

RAJ, A.B.M. & TSERVENI-GOUSI, A. (2000) Stunning methods for poultry. *World's Poultry Science Journal*, **56**: 291–304.

RAJ, A.B.M., GREY, T.C. & GREGORY, N.G. (1991) Effect of early filleting on the texture of breast muscle of broilers stunned with argon-induced anoxia. *British Poultry Science*, **32**: 319–325.

RAJ, A.B.M., WILKINS, L.J., RICHARDSON, R.I., JOHNSON, S.P. & WOTTON, S.B. (1997) Carcase and meat quality in broilers either killed with a gas mixture or stunned with an electrical current under commercial processing conditions. *British Poultry Science*, **38**: 169–174.

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. *British Poultry Science*, **39**: 686–695.

UIJTTENBOOGAART, T.G. (1997) Effects of gas and electrical stunning methods on meat quality, in: LAMBOOIJ, E. (Ed.) *Proceedings Symposium: Alternative Stunning Methods for Poultry*, pp. 25–33 (the Netherlands, Utrecht). ID-DLO nr. 97.037.

WATHES, C.M. (2005) Report of a workshop on controlled atmosphere stunning of poultry: an integrated approach. *Animal Welfare*, **14**: 85–86.

WEBSTER, A.B. & FLETCHER, D.L. (2001) Reactions of laying hens and broilers to different gas used for stunning poultry. *Poultry Science*, **80**: 1371–1377.

WOOLLEY, S.C. & GENTLE, M.J. (1988) Physiological and behavioural responses of the domestic hen to hypoxia. *Research in Veterinary Science*, **45**: 377–382.