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Dorothy Mckeegan, Siobhan Maya Abeyesinghe, Morven A Mcleman, John C Lowe, Theo G Demmers, Rodger P White, Roland Kranen, Hanneke van Bommel, Jeannette Ac Lankhaar, Christopher Michael Wathes

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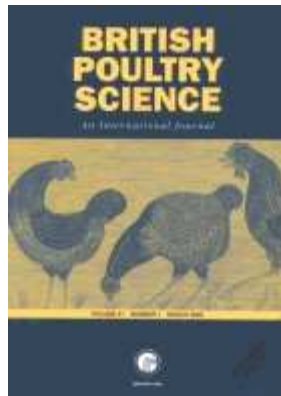
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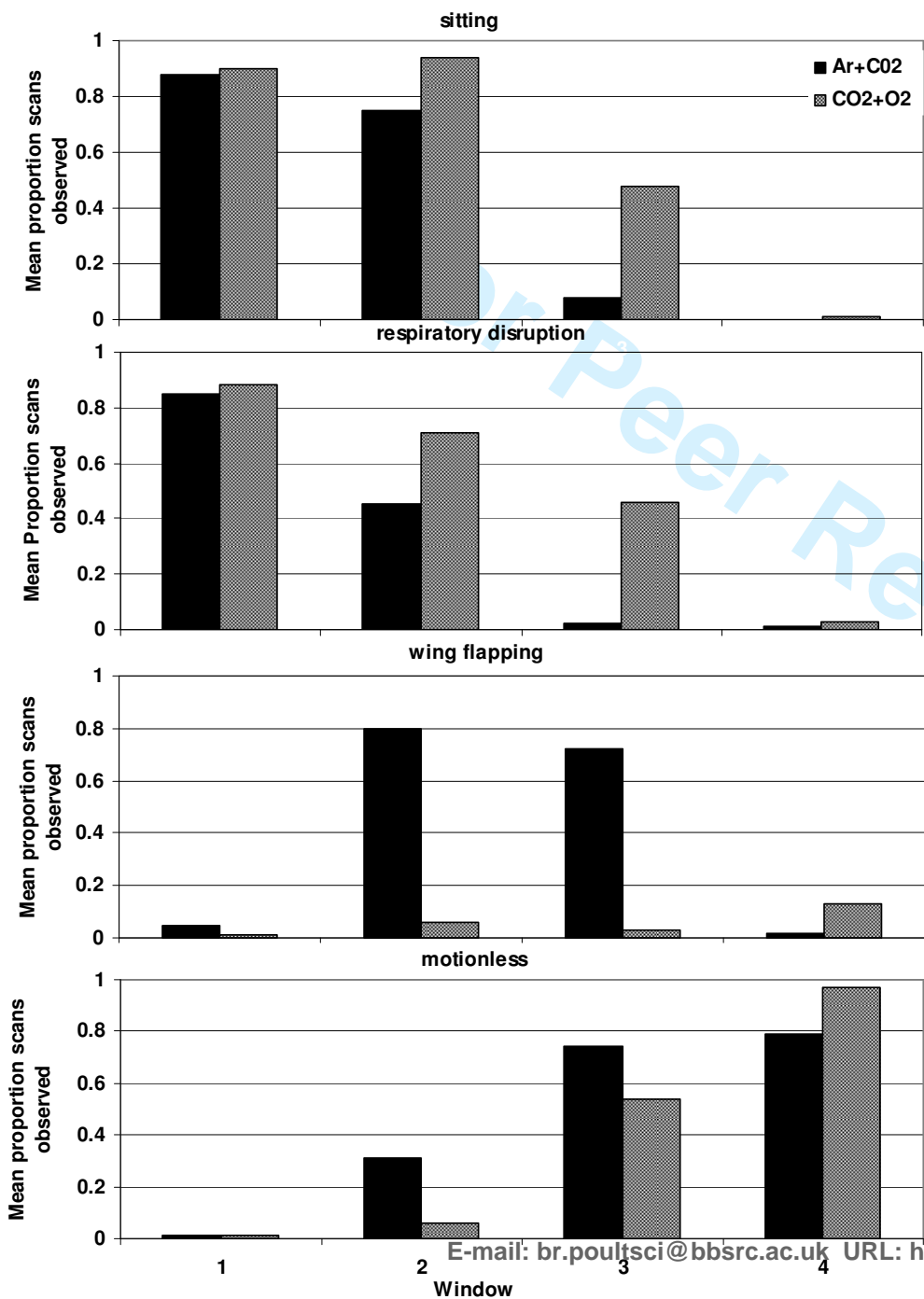
**Controlled atmosphere stunning of broiler chickens: II.
Effects on behaviour, physiology and meat quality in a
commercial processing plant**

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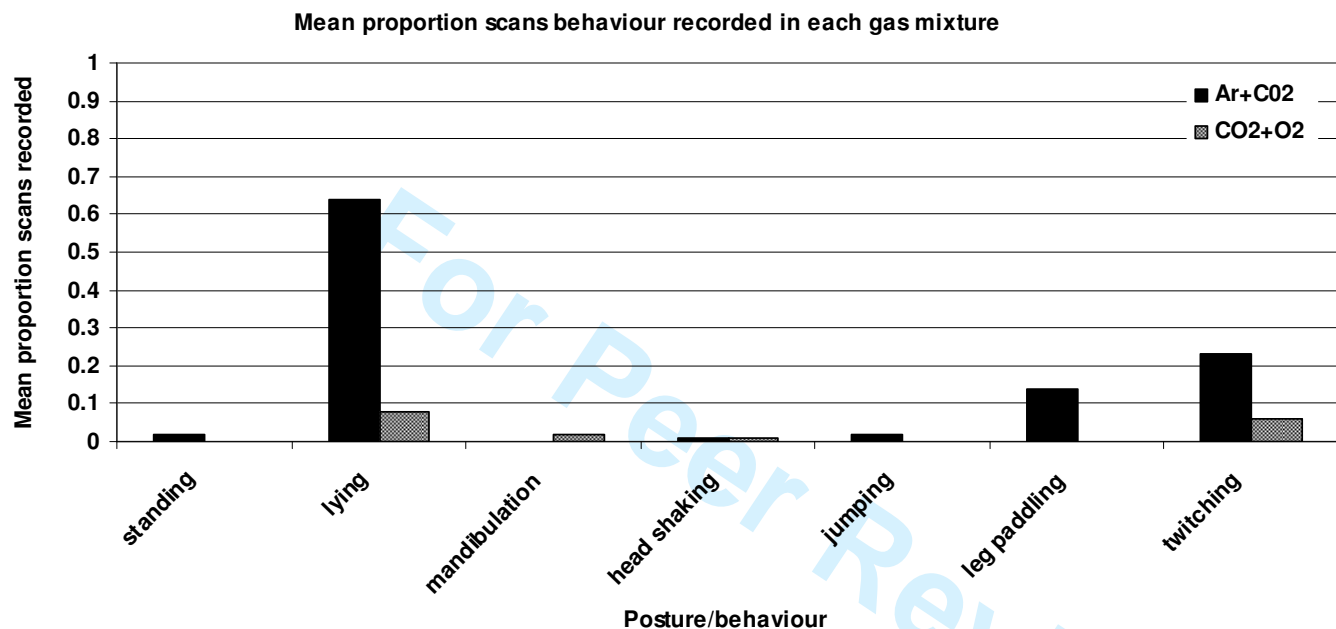
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14 **Figure 1.** Interactive effects of gas mixture and section of gas chamber (windows 1-4) on mean proportion of scans of posture/behaviour observed and based
15 on back-transformed means (*n*= 143 and 118 scans per window for CO₂+O₂ and AR+CO₂, respectively). The transformed means on the logit scale, s.e.d.s,
16 Wald statistics and p-values referring to the transformed data are quoted in the text.
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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, s.e.d.s, Wald statistics and p-values referring to the transformed data are quoted in the text.

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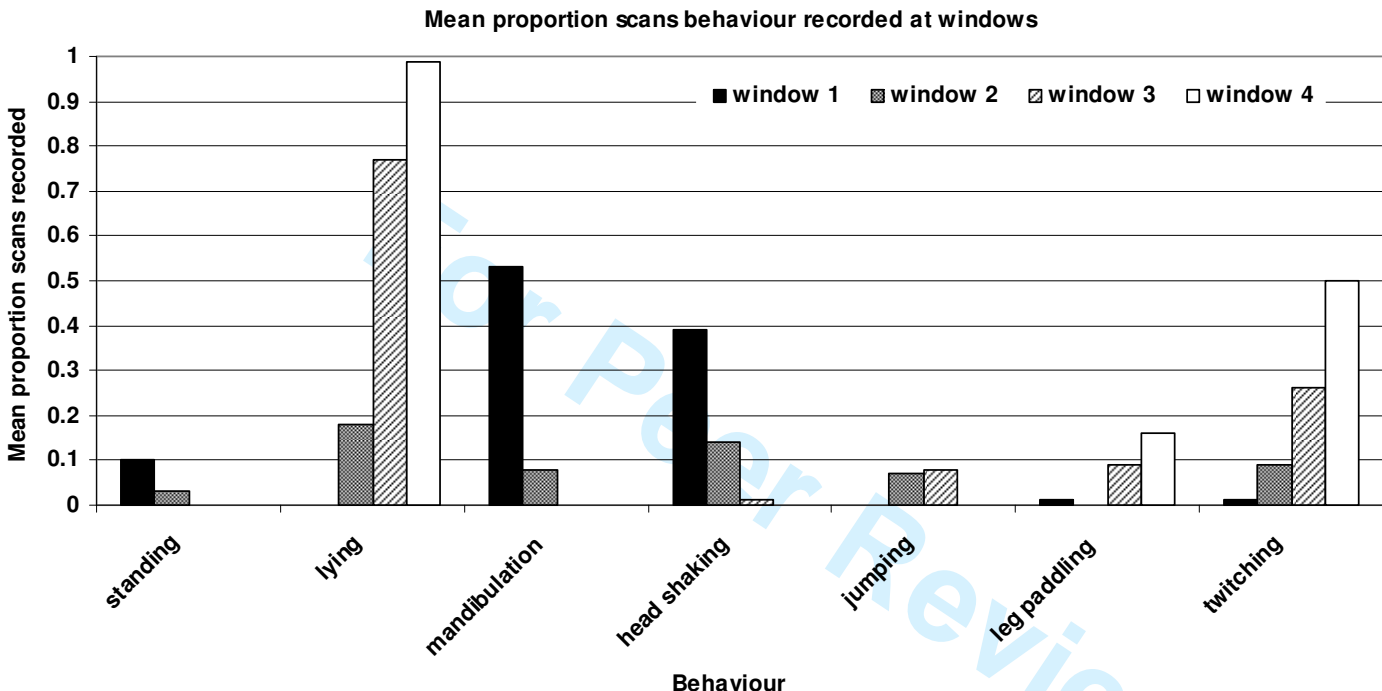


Figure 3. Main effects of window on mean proportion of scans of posture/behaviour observed based on back-transformed means. Zero scans were recorded for some measurements ($n= 143$ and 118 scans per window for CO_2+O_2 and $AR+CO_2$, respectively). The transformed means on the logit scale, s.e.d.s, Wald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

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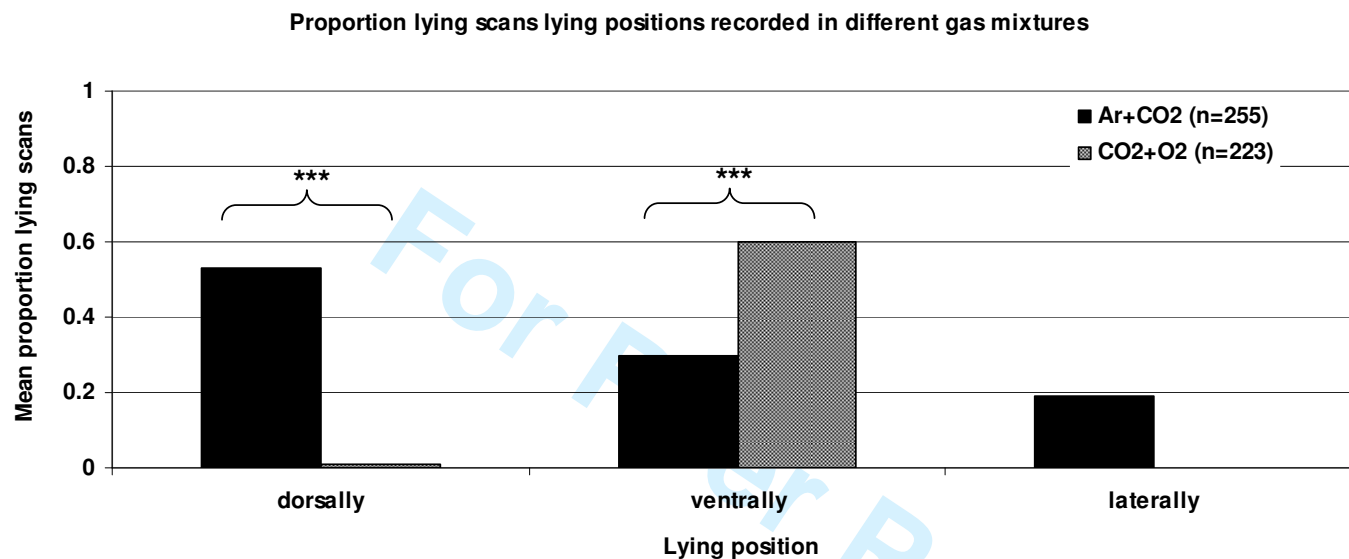


Figure 4. Effects of gas treatment on mean proportion of scans recorded as lying shown as back-transformed means; birds were observed lying dorsally, ventrally and laterally. Zero scans were recorded for some measurements ($n= 143$ and 118 scans per window for CO_2+O_2 and $AR+CO_2$, respectively). The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

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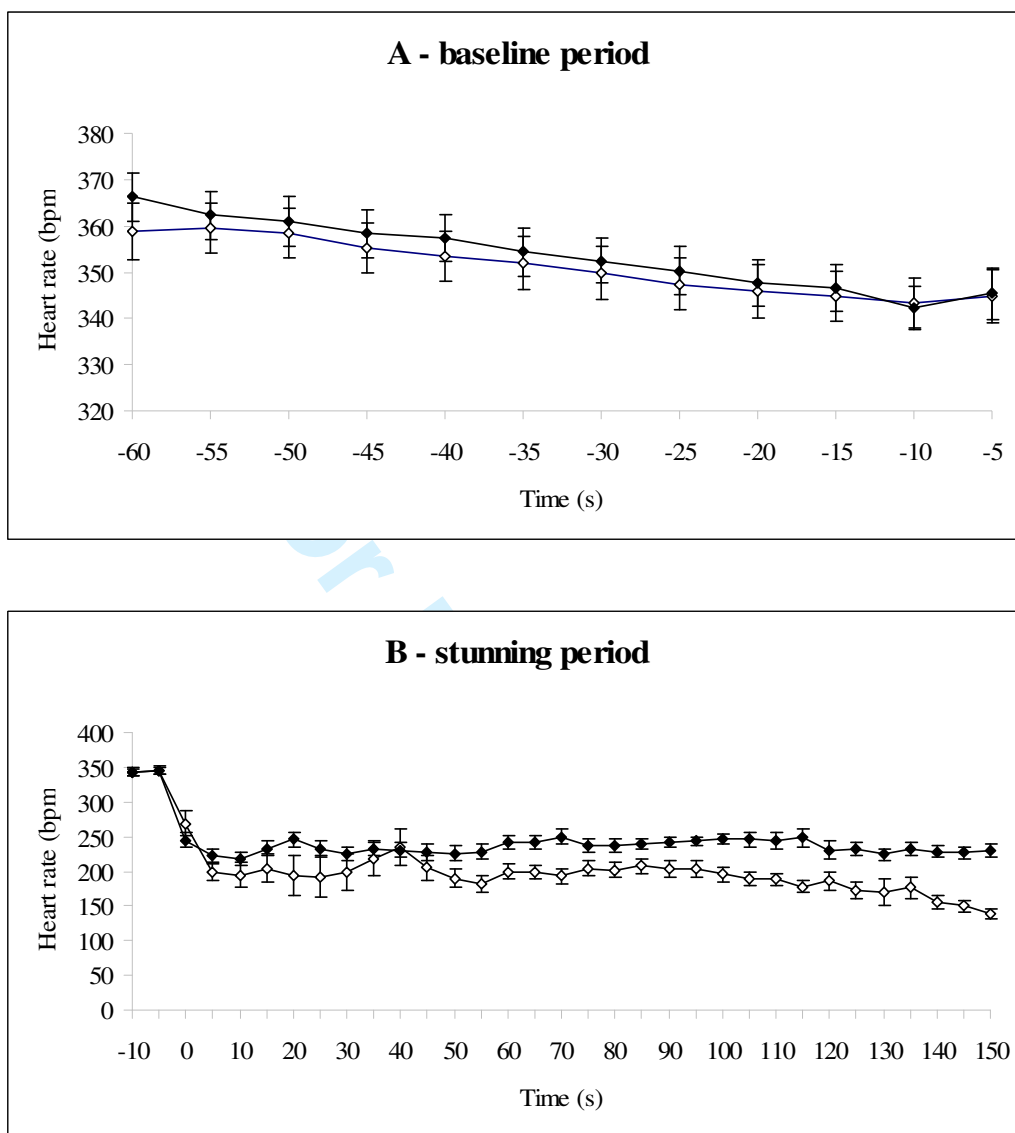


Figure 5. Mean heart rate over time for A) the baseline period and B) the first 150 s of gas stunning (CO₂+O₂: closed circles; Ar+CO₂: open circles). Error bars represent standard errors. n=45 birds for each gas treatment.

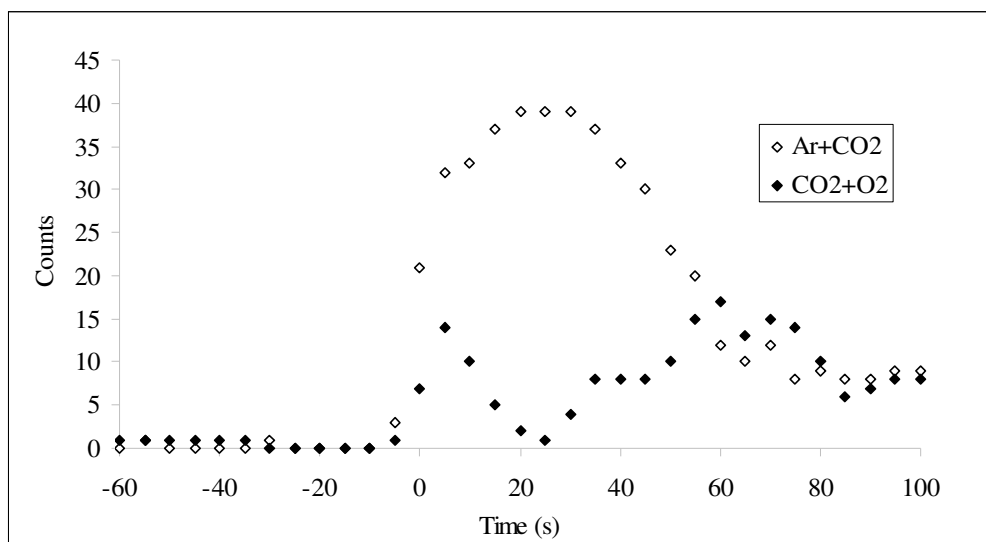


Figure 6. Number of segments of obscured ECG traces for broiler chickens ($n=45$ *birds* per treatment) stunned by gas for each 5 s time period.

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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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Short title: Controlled atmosphere stunning of broilers. II

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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% CO₂ in air with <2% O₂, and a biphasic hypercapnic hyperoxygenation mixture, comprising an anaesthetic phase, 40% CO₂, 30% O₂, 30% N₂, followed by an euthanasia phase: 80% CO₂, 5% O₂, 15% N₂.

3. Birds stunned with Ar+CO₂ were more often observed to flap their wings earlier, jump, paddle their legs, twitch, and lie dorsally (rather than ventrally) than those stunned with CO₂+O₂. 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 \approx 230 beats/min for CO₂+O₂ birds whereas it declined gently for Ar+CO₂ birds.

5. In terms of carcase and meat quality, there appeared to be clear advantages to the processor in using CO₂+O₂ rather than Ar+CO₂ 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% CO₂ in air with <2% O₂.

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 the fowl during CAS (Woolley and Gentle, 1988; Raj and Gregory, 1990; Raj *et al.*, 1998; Lambooi *et al.*, 1999; Coenen *et al.*, 2000; Gerritzen *et al.*, 2000; Webster and Fletcher, 2001; and McKeegan *et al.*, 2006). In general, these support the 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 (Abeyesinghe *et al.*, 2007; Lowe *et al.*, 2007; McKeegan, 2004; McKeegan *et al.*, 2006; 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 carcass 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% CO₂ in air with <2% O₂ ('Ar+CO₂'); and ii) a biphasic hypercapnic hyperoxygenation mixture, comprising an anaesthetic phase with a gas mixture of 40% CO₂, 30% O₂, 30% N₂, followed by an euthanasia phase with 80% CO₂, 5% O₂, 15% N₂ ('CO₂+O₂'). Both approaches are used commercially in continental Europe, though only Ar+CO₂ is permitted in the United Kingdom.

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 the behaviour, and measurements of heart and respiration rates were made

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3 prior to and during stunning. Carcase and meat quality were assessed after stunning. The
4 experiment lasted 5 d; both treatments were applied on each day.
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7 **Full-scale processing plant**

8 The commercial processing plant was located in Belgium and ran at an average throughput of
9 9000 birds/h during the experiment, which took place between August 30 and September 3
10 2004. The CAS plant was manufactured by Stork Food Systems. Birds were loaded
11 automatically from a transport crate on to a conveyor. They were then passed sequentially
12 through a tunnel of two chambers in which the atmosphere could be controlled. The first
13 chamber measured 9400 x 759 x 350 mm (l x w x h); the second chamber was divided into
14 two parts, measuring 11250 x 750 x 430 and 11800 x 500 x 375 mm, respectively. The
15 normal period of exposure was 64 and 118 s in the first and second chambers respectively, but
16 this was affected by the bird's movement on the conveyor. Gas concentration was controlled
17 and monitored continuously by a Hydro MACS™ stunning control system (Yara International
18 ASA, Oslo, Norway), measuring independently the concentration of CO₂ and O₂ within the
19 flow in phase 1, and CO₂ in phase 2; the Ar concentration was inferred from the oxygen
20 concentration. Additional measurement of the gas concentration was performed at a single
21 location at the bird's head height within the chamber using a gas analyser (PBI Dansensor
22 Checkpoint, Model 200548/210661). The mean (\pm s.d.) concentrations of CO₂ and O₂ in the
23 first and second chambers were: (i) CO₂+O₂ mixture, chamber 1 - CO₂ 40.0 \pm 0.6 %, O₂ 30.0
24 \pm 0.4%; chamber 2 - CO₂ 80.0 \pm 0.7%; and (ii) Ar + CO₂ mixture, chamber 1 CO₂ 30.1 \pm
25 1.7%, O₂ 1.3 \pm 0.6%, Ar 58.6%. The atmosphere within the first chamber was humidified to
26 60-80 \pm 10% relative humidity, following commercial practice.
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40 After stunning, the birds were shackled by their legs and killed automatically by a
41 unilateral cut to one carotid artery and one jugular vein in the neck, the whole process taking
42 approximately 25 s. The carcasses were then bled for 3 min, scalded at 53.5°C for 3 min, and
43 plucked. After evisceration, the carcasses were chilled by air for 30 min at -0.5°C and then for
44 a further 1.75 h at 0.5 to 1°C. Carcasses, which were to be analysed for meat quality, were
45 collected after leaving the chiller and stored at 0.5°C until manual de-boning, except for those
46 which were examined at 1.5 h post mortem.
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51 **Behavioural and physiological observations**

52 The methods used to observe the behavioural and physiological reactions of the birds to CAS
53 are described by Abeyesinghe *et al.* (2007) and measurements were made daily for three days
54 with each gas treatment applied on each day. The gas treatments were applied sequentially
55 while the processing line was running at 9000 birds/h. A short break was needed between
56 batches to allow the tunnel to be purged before a new gas mixture was introduced to the
57 stunning chambers. All birds used in paired batches originated from the same flock.
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Physiological measurements of heart and respiration rate were made on a total of 90 individuals (15 per treatment per day), each of which was fitted with a telemetric/logging system mounted on a body harness (Lowe *et al.*, 2007; 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 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, 0-8 s; window 2, 9-16 s; window 3, 17-24 s; and window 4, 33-40 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+CO₂ and CO₂+O₂ treatments, respectively were observed.

Measurement of carcass and meat quality

A comprehensive set of measurements were made to determine the effects of stunning gas on carcass 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

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3 successive days, two days longer than the period over which behavioural and physiological
4 measurements were made.
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8 Table 1 here
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10 11 **Statistical analysis**

12 Genstat, release 6.2 (6th edition) for Windows© (1998 Lawes Agricultural Trust IACR), was
13 used to perform the statistical analyses. For the behavioural data, Generalised Linear Mixed
14 Model (GLMM: Marginal Model; Binomial distribution with logit link function) analyses
15 were conducted on the proportion of total scans of each behaviour observed. All behavioural
16 analyses used the treatment structure (in Genstat notation) of gas*window and a blocking
17 structure of 'day' where gas described the two different gas mixtures (Ar+CO₂ and CO₂+O₂),
18 'window' indicated which the viewing window at which behaviour was observed and 'day'
19 represented days 1-3 of the experiment. All behavioural data were transformed using the logit.
20 For the physiological data, arithmetic means and s.e.'s were calculated and visual
21 comparisons made.
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24 The carcass and meat quality data were analysed using ANOVA or regression analysis.
25 For measurements made at a single time, the treatment structure was 'gas' with a blocking
26 structure of flock/bird, where 'flock' indicates the source of the birds and bird indicates the
27 individual. Where measurements were taken at various de-boning times, the treatment
28 structure was gas*time; 'time' was log-transformed and linear and quadratic trends were
29 determined using low order (up to second) polynomial contrasts. Transforming de-boning
30 time made the steps between de-boning times more linear. Shear force data were transformed
31 using logarithms. The blocking structure was flock/gas/time/bird. Analysis of deviance with a
32 logit link was also used for variates with a binomial distribution, such as liver colour and
33 wing fractures.
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36 37 38 39 40 41 42 43 44 45 46 47 48 **RESULTS**

49 **Behaviour**

50 In total 1044 scans of behaviour were recorded; 143 scans per window on the CO₂+O₂
51 treatment and 118 scans per window on the Ar+CO₂ treatment. The posture and behaviour
52 categories are discussed individually. The transformed means, s.e.d.'s, Wald statistic and *P*-
53 value for relevant treatment comparisons are given in parentheses for each behaviour; Figures
54 1 to 4 inclusive show the back-transformed means for each behaviour (either for the two gas
55 mixtures or for the four windows, in order). The birds remained in the first chamber for
56 approximately 75 s, which was defined as the first phase of exposure, while the second phase
57 lasted from about 75 to 185 s.
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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+CO₂ and CO₂+O₂ = -3.86 and -7.03, s.e.d. = 4.97, $W_1 = 0.83$, $P = 0.362$) and, as expected it sharply declined after the first window (Figure 3: mean = -2.25, -3.60, -7.97 and -7.97, s.e.d. = 0.46, $W_3 = 16.82$, $P < 0.001$).

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+CO₂ = 2.01, 1.08, -2.50 and -10.98 and mean CO₂+O₂ = 2.22, 2.83, -0.07 and -4.26, s.e.d. = 4.84 $W_3 = 10.0$, $P = 0.018$) with a decline in sitting across windows (as birds progressed) in both gas mixtures but this occurred more steeply in Ar+CO₂ than CO₂+O₂.

Lying

Overall, lying was recorded on 46% of scans. There was no difference in occurrence between the gas mixtures (Figure 2: mean Ar+CO₂ and CO₂+O₂ = 0.58 and -2.44, s.e.d. = 3.95, $W_1 = 0.03$, $P = 0.858$), however further analysis indicated lying posture was affected by the gas treatment; more birds were recorded lying dorsally in Ar+CO₂ (Figure 4: mean Ar+CO₂ and CO₂+O₂ = 0.11 and -4.6, s.e.d. = 0.75, $W_1 = 39.34$, $P < 0.001$), and more lying ventrally in CO₂+O₂ (Figure 4: mean Ar+CO₂ and CO₂+O₂ = -0.83 and 0.42, s.e.d. = 0.25, $W_1 = 25.58$, $P < 0.001$). Observations of birds lying laterally did not differ between gases (Figure 4: mean Ar+CO₂ and CO₂+O₂ = -1.48 and -7.13, s.e.d. = 11.54, $W_1 = 0.24$, $P = 0.627$). As expected, a sharp increase in lying occurred across windows, particularly at window 3 (Figure 3: mean = -7.43, -2.02, 1.23 and 4.51, s.e.d. = 0.32 $W_3 = 178.50$, $P < 0.001$).

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 CO₂+O₂ than Ar+CO₂ and (Figure 1: mean Ar+CO₂ = 1.71, -0.21, -4.06 and -4.76 and mean CO₂+O₂ = 2.01, 0.92, -0.14 and -3.54, s.e.d. = 0.86, $W_3 = 10.7$, $P = 0.014$).

Mandibulation

Mandibulation occurred on only 16% of scans overall and was equally apparent in both mixtures (Figure 2: mean Ar+CO₂ and CO₂+O₂ = -6.28 and -3.98, s.e.d. = 6.60, $W_1 = 0.23$, $P = 0.630$), declining in occurrence sharply after the first window (Figure 3: mean = 0.12, -2.43, -7.14 and -11.06, s.e.d. = 0.31, $W_3 = 108.07$, $P < 0.001$).

Head shaking

Head shaking, occurring on only 13% of scans in total and followed a similar pattern to mandibulation, with no difference between gas mixtures (Figure 2: mean Ar+CO₂ and

CO₂+O₂ = -4.59 and -4.33, s.e.d. = 6.73, $W_1 = 0.30$, $P = 0.585$) 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, s.e.d. = 0.31, $W_3 = 38.40$, $P < 0.001$).

Wing flapping

Wing flapping occurred on 21% of all scans. Significantly more wing flapping was recorded in Ar+CO₂ than CO₂+O₂ and this mostly occurred in the second and third windows, tailing off sharply at the final viewing point (Figure 1: mean Ar+CO₂ = -2.93, 1.37, 0.95 and -4.06 and mean CO₂+O₂ = -4.96, -2.83, -3.55 and -1.88, s.e.d. = 0.89, $W_3 = 38.3$, $P < 0.001$).

Jumping

Jumping was recorded on approximately 10% of all scans, almost exclusively in Ar+CO₂ (Figure 2: mean Ar+CO₂ and CO₂+O₂ = -3.78 and -5.88, s.e.d. = 0.37, $W_1 = 32.71$, $P < 0.001$). It was generally associated with wing flapping, occurring largely in the two mid-windows (Figure 3: mean = -7.26, -2.58, -2.46 and -7.03, s.e.d. = 0.916, $W_3 = 17.97$, $P < 0.001$) and indicates a greater severity of convulsion.

Leg paddling

Leg paddling was recorded on 11% of all scans, again almost exclusively in Ar+CO₂ (Figure 2: mean Ar+CO₂ and CO₂+O₂ = -1.82 and -5.80, s.e.d. = 0.85, $W_1 = 21.68$, $P < 0.001$) and increasing in occurrence across windows (Figure 3: mean = -4.50, -6.76, -2.29 and -1.68, s.e.d. = 0.68, $W_3 = 20.14$, $P < 0.001$).

Twitching

Twitching occurred on 23% of all scans and was observed significantly more in Ar+CO₂ than CO₂+O₂ (Figure 2: mean Ar+CO₂ and CO₂+O₂ = -1.24 and -2.69, s.e.d. = 0.53, $W_1 = 7.64$, $P = 0.006$). Its occurrence also increased across windows (Figure 3: mean = -4.50, -2.33, -1.03 and 0.01, s.e.d. = 0.29, $W_3 = 43.88$, $P < 0.001$).

Motionless

Motionless increased later in CO₂+O₂ than Ar+CO₂, but a significantly greater proportion of scans recorded motionless in the final window for CO₂+O₂ compared with Ar+CO₂ (Figure 1: mean Ar+CO₂ = -4.75, -0.81, 1.05 and 1.33 and mean CO₂+O₂ = -4.96, -2.83, 0.15 and 3.54, s.e.d. = 1.38, $W_3 = 10.5$, $P = 0.15$). This may have been due to the more frequent occurrence of other behaviours seen in Ar+CO₂.

Figs 1-4 here

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

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3 the gas tunnel. Once they had entered the tunnel (at 0 s), heart rate fell markedly over the next
4 10 s before stabilising at about 204 and 236 beats/min for Ar+CO₂ and CO₂+O₂, respectively.
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6 Inspection of the data shows that the difference between the treatments was minor. The mean
7 heart rate for the CO₂+O₂ birds remained at about 230 beats/min for a further 50 s and
8 declined slightly for the Ar+CO₂ birds. The number of obscured segments of ECG trace was
9 much greater for Ar+CO₂ birds than CO₂+O₂ birds, reflecting the vigorous movements of the
10 former and/or the permanent loss of the sensor (Ar+CO₂ 8/45 birds; CO₂+O₂ 1/45 birds;
11 Figure 6).
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16 The respiration rate in the baseline period was similar for birds from both treatments and
17 declined slowly as the birds recovered from handling from about 53 breaths/min, just after the
18 harness was fitted, to 50 breaths/min prior to placement on the conveyor.
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23 Figs 5 and 6 here
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26 **Carcase and meat quality**

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28 Table 2 shows the results of the measurements of carcass and meat quality. Some, but not all,
29 of the measures were affected significantly by the gas used to stun the birds. Blood loss
30 (approximately 3% by weight), skin damage and liver colour were similar in both treatments.
31 Overall, the number of carcasses with fractured wings ($P < 0.001$) and the mean score for fillet
32 haemorrhages ($P = 0.05$) was significantly higher for Ar+CO₂ than CO₂+O₂. The number of
33 wings examined was 9110 and 9930 for the Ar+CO₂ and CO₂+O₂ treatments, respectively. By
34 far the most common fractures were of the wrist and elbow joint (Ar+CO₂ 3.9 and 2.4%;
35 CO₂+O₂ 0.8 and 0.8%); there were few fractures of the humerus and radius/ulna and none of
36 the wing tips. In terms of fractured wings, the prevalence of breakages with or without
37 perforation of the skin was between 5 and 6 times higher for Ar+CO₂ than CO₂+O₂; overall,
38 the prevalence of fractured wings of both types was 6.8 and 1.6%, respectively.
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47 Table 2 here
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51 Various measures of meat quality over time are shown in Table 3. There was a significant
52 interaction between time and gas for fillet pH but it could not be explained by a simple linear
53 or quadratic trend ($P < 0.001$). Fillet pH rose initially for CO₂+O₂, but not Ar+CO₂, carcasses
54 and final pH was reached 15 min after post mortem for Ar+CO₂ but not until 5 h for CO₂+O₂.
55 There were main effects of gas and time on shear force, measured immediately after de-
56 boning (t_0) and after 24 h storage (t_{24}); shear force fell non-linearly with time, was highest in
57 CO₂+O₂ and did not stabilise until about 7 h post mortem. Neither storage nor cooking losses
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3 were affected by the gas treatment nor did they change with time; they were about 0.5% and
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5 25% respectively.
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11 The results of the measurements of the colour of the fillets are given in Table 4 using the
12 CIELab convention. There were no interactions between gas and time. The distal surface of
13 the fillets was darkest for Ar+CO₂ ($P = 0.003$) and there was a similar trend for the proximal
14 surface (ns). Some changes were also observed over time; the distal surface of the fillets
15 became paler and less red, while the proximal surface became less yellow.
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24 DISCUSSION

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26 In general, these experimental findings supports those of the accompanying paper
27 (Abeyesinghe *et al.*; 2007) on the reactions and responses of broiler chickens to CAS. This
28 study employed the same techniques as the latter but extended the scope from a pilot scale
29 system to the full scale conditions of the commercial processing plant, while still employing a
30 common scientific approach. To the best of our knowledge this is the first report of the effects
31 of CAS on broiler chickens in the processing plant on such a large scale.
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34 The behavioural responses of the birds followed a logical sequence in terms of their
35 physiological and behavioural reactions to CAS, though it should be noted that our
36 observations were restricted to the first 40 s of stunning. Most birds were sitting on entry to
37 the gas tunnel and their initial reaction to the stunning gas was mandibulation and head
38 shaking, showing that they had detected the gas either directly via chemo-receptors or
39 indirectly via consequential physiological effects. Subsequently – and in an approximate order
40 of occurrence - they sat down, showed signs of respiratory disruption, flapped their wings, lay
41 down, paddled their legs, jumped, and twitched before becoming motionless. However, this
42 sequence differed in some aspects for the two gases. Birds stunned with Ar+CO₂ were more
43 often observed to flap their wings earlier, jump, paddle their legs, twitch, and lie dorsally
44 (rather than ventrally) than those stunned with CO₂+O₂. These behaviours have the potential
45 to cause distress if the birds are conscious; similar observations were made by Lambooij *et al.*
46 (1999) and Abeyesinghe *et al.* (2007). In particular, our findings for lying position, wing
47 flapping and jumping indicate more severe convulsions during hypercapnic anoxia, thereby
48 introducing greater potential for injury for both the bird itself and its companions. Indeed, the
49 high prevalence of wing fractures in Ar+CO₂ demonstrates the force of the bird's movements
50 and convulsions in this gas mixture. This is a welfare issue only if birds were sensible at any
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3 time during these convulsions; our laboratory findings McKeegan *et al.* (2006b) describing
4 EEG signals during this period suggest that this is a possibility.
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7 It would have been advantageous to have recorded the vocalisations made by the birds
8 because these would have been indicative of their state of anxiety, fear or distress, but to the
9 best of our knowledge there are no published measurements of vocalisations during CAS. It is
10 not known whether the sight, sound and smell of one bird dying are distressing to another
11 (conscious) bird although the physical proximity of others may result in disturbance or even
12 injury; sheep are apparently unaffected when a conspecific is killed in their presence (Anil *et al.*
13 1996). Respiratory disruption was more common and continued for longer in CO₂+O₂ than
14 Ar+CO₂, but the interpretation of this finding is complicated by the fact that one of the
15 mixtures (Ar+CO₂) was both hypercapnic and anoxic and thus may have induced both types
16 of disruption that were included in this behavioural category. However, McKeegan *et al.*
17 (2006; 2007) reported increased respiratory disruption in response to hypercapnic
18 hyperoxygenation, an observation supported by the current findings. Overall, our observations
19 of behaviour indicate a slower but somewhat smoother transition to a motionless state in the
20 biphasic hypercapnic hyperoxygenation treatment compared with the hypercapnic anoxic
21 treatment.
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24 During the baseline period, heart rate fell steadily and respiration rate fell slightly as the
25 birds recovered from handling. It was not possible to continue the measurement of respiration
26 rate once the birds had entered the gas tunnel owing to the number of immeasurable traces
27 that disrupted the recording system, caused by wing flapping, leg paddling, tonic pectoral
28 muscle contractions and jostling by other birds. Heart rate throughout the first 100 s of gas
29 stunning was similar for both gases and its dynamic change over time followed a similar
30 pattern to that observed for both individuals and groups of birds in the pilot scale system
31 (Abeyesinghe *et al.*, 2007). More detailed analysis of the ECG traces in the latter study
32 showed evidence of bradycardia and arrhythmia with both gas treatments. The loss of traces
33 was greater for Ar+CO₂ than CO₂+O₂, probably due to the loss of the sensor and signals and
34 supporting the conclusion that experience of an anoxic hypercapnic gas mixture causes a
35 more vigorous behavioural response in broiler chickens. Such intense and long lasting
36 artefacts in the trace may indicate strong isometric muscle contractions which are likely to be
37 painful and which occur during a period when consciousness cannot be excluded (McKeegan
38 *et al.*, 2006).
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41 In terms of carcase quality there appears to be clear advantages to the processor and the
42 consumer in using CO₂+O₂ than Ar+CO₂ to stun broiler chickens, particularly because of a
43 much smaller number of fractured wings. Based upon a commercially relevant number of
44 carcasses that were examined for wing fracture (\approx 19,000), the overall prevalence of wing
45 fractures was 4 times worse in Ar+CO₂ than CO₂+O₂. Notably, a large proportion of these
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3 fractures led to skin perforation, which can be attributed to the more violent movements and
4 convulsions of birds stunned with Ar+CO₂ compared with CO₂+O₂. If these fractures
5 occurred when the birds were conscious, then welfare as well as carcass quality would be
6 severely compromised.
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10 CAS has been shown to produce meat of superior quality when compared with electrical
11 stunning (Raj *et al.*, 1997; Uijttenboogaart *et al.*, 1997; Hoen and Lankhaar, 1999). Compared
12 with Ar+CO₂, CO₂+O₂ stunning induced fewer haemorrhages in the fillets. Differences
13 between the gases in the fall in fillet pH post mortem appear to be associated with the
14 behaviour of the birds during stunning, confirming the results of the pilot plant study
15 (Abeyesinghe *et al.*, 2007). The more pronounced peri mortem struggles, such as wing
16 flapping and leg paddling, in Ar+CO₂ accelerated glycolysis and lactate accumulation, as
17 shown by the low pH 15 min after slaughter. These results contrast with our earlier study
18 (Abeyesinghe *et al.*, 2007), where the rate of fall of pH decline was similar in CO₂+O₂ and
19 Ar+CO₂ stunned birds. This may be due to the very rapid decline in pH observed for Ar+CO₂
20 in this study, which was probably the result of vigorous wing flapping. Storage and cooking
21 losses were similar to those recorded in our pilot study and were unaffected by stunning
22 treatment (Abeyesinghe *et al.*, 2007).
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26 Raj *et al.* (1991) found that the majority of broilers stunned by argon attained rigor mortis
27 within 20 min, enabling carcasses to be filleted 2 h post mortem without affecting meat
28 texture. The low pH values measured after 15 min post mortem for Ar+CO₂ are in accordance
29 with these results. However, our measurements of toughness (shear force, t₀) indicate that for
30 both stunning CAS methods, carcasses took up to 7 h to attain full rigor mortis. In commercial
31 in-line poultry processing, carcasses are portioned and de-boned after 2 to 3 h post mortem,
32 with a risk of tough meat when CAS is used. This problem can be overcome by electrical
33 stimulation of the carcass to allow in-line de-boning, typically 3 h post mortem, without the
34 risk of tough meat (Kranen, 2003). This solution is adopted commercially in many European
35 abattoirs as one stage in processing broiler carcasses for meat.
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39 The lightness of the meat is indicated by its L* value, which is inversely proportional to
40 muscle pH (Qiao *et al.*, 2001). This was indeed true for the distal (exterior) surface of
41 CO₂+O₂ fillets, which became lighter over time post slaughter as pH fell. However, this
42 relationship did not appear to hold for Ar+CO₂ fillets, indicating that muscle colour is not
43 only determined by muscle pH.
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47 The results of this study have direct application to the practical conditions of the
48 commercial processing plant. Unlike laboratory experiments, the birds experienced the full
49 range of environmental and social stressors during stunning. These relate to the presence of
50 other birds in the gas tunnel, which may cause physical interference or whose behaviour,
51 vocalisations and other reactions may be perceived perhaps adversely; process control of the
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3 atmospheric and thermal environment, which is so influential in ensuring rapid exposure to
4 the correct concentration of gases at an appropriate temperature and humidity; and the
5 experience of the birds during transport from the farm to the lairage. Indeed, the methods
6 employed here could be used to determine the causes of poor welfare and meat quality from
7 the farm to the abattoir, identifying the risk factors responsible and suggesting improvements.
8 Risk factors that might be addressed include: the merits of gas stunning within or outwith
9 transport crates; and the benefits and drawbacks of electrical stunning versus CAS. The
10 advantages of biphasic CAS over electrical stunning of poultry were also recognised at a
11 symposium on this topic (Barton Gade *et al.*, 2000). It is only by integration of studies in the
12 laboratory and commercial plants using techniques from a range of scientific and engineering
13 disciplines that progress can be made to the benefit of the bird, processor and consumer alike
14 (Wathes, 2005).

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23 This and the accompanying paper (Abeyesinghe *et al.*, 2007) form part of a coordinated
24 series of experiments (Lowe *et al.*, 2007; McKeegan, 2004; McKeegan *et al.*, 2006) that have
25 examined the effects of CAS on broiler chickens. We have studied the three distinct
26 approaches that can be used in controlled atmosphere stunning of broiler chickens, *viz* anoxia,
27 hypercapnic anoxia and a combination of hypercapnic hyperoxygenation anaesthesia and
28 hypercapnic euthanasia. The strengths of this coordinated study lie in firstly, the
29 comprehensive range of techniques employed to measure behavioural and physiological
30 responses that are indicative of welfare, and physico-chemical properties of the carcass and
31 meat that describe the organoleptic and aesthetic appeal of chicken meat; secondly, the
32 location and scale of study from the laboratory to the processing plant; and thirdly, the
33 different physiological approaches used to stun the birds. Interpretation of the behavioural and
34 physiological data is complex and we do not believe that it is wise to speculate on which of
35 our findings are indicative of pain without direct measurements. Such measurements are
36 either extremely difficult or impossible to undertake in a commercial abattoir at present.
37 Fractures are clearly likely to be painful if experienced by a conscious bird, and although we
38 cannot tell from this experiment when they occurred, they were 4 times more prevalent in
39 Ar+CO₂ than CO₂+O₂. Measurements relating to loss of consciousness were not carried out in
40 this experiment but in all other respects the results support the findings of our laboratory
41 studies (McKeegan *et al.* 2006) which suggested that early onset wing flapping during anoxia
42 occurs during periods in which a form of consciousness cannot be excluded. In terms of the
43 two CAS approaches investigated here, we conclude that a biphasic approach based upon
44 consecutive phases of anaesthesia and euthanasia is potentially more humane than an anoxic
45 hypercapnic approach. It is also superior in terms of carcass and meat quality.

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

Measurement	Replicates per treatment per flock	Method, see Abeyesinghe <i>et al.</i> (2007)
<u>Stunning, killing and bleeding</u>		
Wing fracture	≈ 750 carcasses	Palpation after stunning
Blood loss	10	Gravimetric
<u>Evisceration and chilling</u>		
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
<u>De-boning</u> 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
<u>Storage and cooking</u> ⁴		
Storage loss	6	Gravimetric
Cooking loss	6	Gravimetric
Shear force (t ₂₄ , N)	6	Mechanical using a Warner Bratzler device

¹ Carcasses were de-boned at 15 min, 1.5, 3, 5, 7 and 24 h post mortem .

² n=20 carcasses at 15 min post mortem.

³ Number of carcasses per treatment per flock per de-boning time.

⁴ Fillet halves were stored for 24 h until measurements were made.

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

	Ar+CO ₂	CO ₂ +O ₂	Gas	
			P	s.e.d.
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 carcass ²	2.23	2.17	ns	na
Plucking efficiency, proportion of carcasses with >= 2 feathers remaining per bird	0.85	0.81	ns	0.044
Skin damage, mean score per carcass				
carcass scratches, log _e	1.03	1.13	ns	0.114
carcass 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 carcasses				
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 liveweight as a covariate.

² Comparison of frequency distributions after logit transformation.

³ Analysis of deviance, s.e.d.'s are approximate where given.

ns – not significant; na – 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	s.e.d.	<i>P</i>	s.e.d.	<i>p</i>	s.e.d.	<i>p</i>
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.

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	s.e.d.	P	s.e.d.	P	s.e.d.	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	4.82	4.52	4.30	4.89	4.80						
Ar+CO ₂	4.79	4.40	4.23	4.51	4.16	0.212	ns	0.209	ns	0.339	ns
CO ₂ +O ₂											
a* distal	4.08	3.45	3.30	4.17	3.91						
Ar+CO ₂	3.88	3.44	3.31	3.38	3.32	0.172	ns	0.177	0.004	0.282	ns
CO ₂ +O ₂											
b* proximal	12.8	12.5	12.0	12.6	11.1						
Ar+CO ₂	13.7	13.3	12.6	12.6	12.0	0.374	ns	0.423	0.003	0.649	ns
CO ₂ +O ₂											
b* distal	9.9	10.2	10.2	10.5	10.0						
Ar+CO ₂	10.9	11.2	10.7	11.1	10.8	0.119	0.001	0.308	ns	0.407	ns
CO ₂ +O ₂											

Number of replicates given in Table 1.