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Licensing poultry CO_2 gas-stunning systems with regard to animal welfare: investigations under practical conditions

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Abstract

Carbon dioxide stunning of broilers is not permitted in Germany. However, the competent authority can license a system for testing, during which scientific evaluation with regard to animal welfare is required. Between 2004 and 2011 several aspects of three systems have been evaluated in Germany and Italy under practical conditions including: (i) supply to the stunning system; (ii) induction conditions; (iii) stunning effectiveness; and (iv) process control. The systems were: (i) LINCO progressive gas-stunning system in which broilers in their transport crates are lowered stepwise into a pit filled with CO_2 and exposed to slowly increasing concentrations of CO_2 in air up to between 50 and 65% with total dwell times between 275 and 440 s depending on birds' weight; (ii) Stork PMT two-phase gas-stunning system (40% CO_2/30% O_2/30%N_2 for 1 min/ 80% CO_2 for 2 min) in which broilers are tipped onto a belt, on which they pass through the gas atmospheres; and (iii) Anglia Autoflow two-phase CO_2-stunning system, in which the birds are exposed to the atmosphere in their crates. Results on the third system are pending as the investigation is still ongoing. In systems (i) and (ii) analysis of behaviour showed that birds were only exposed to high CO_2 concentration (> 40%) after becoming unconscious. Stunning effectiveness was very high but, nevertheless, occasionally birds (0.027% LINCO system and 0.003% Stork PMT system) were able to regain consciousness. Examples of evaluation of behaviour during induction are presented in this paper and animal welfare aspects are compared. Controlled-atmosphere stunning systems for broilers using less than 40% CO_2 until animals are unconscious, show obvious advantages compared to electrical water-bath stunning, for example, the avoidance of shackling and achieving high stunning effectiveness.

Keywords: *animal welfare, broiler, CO_2 stunning, CAS, key parameters, monitoring points*

Introduction

Scientific results concerning welfare during gas stunning of poultry need to be transferred into slaughterhouse conditions. Even if a stunning method is scientifically approved, the system developed for putting this method into practice has to be evaluated with regard to animal welfare. Hence, the evaluation of welfare of a gas-stunning system under practical conditions involves the following issues: (i) good welfare during pre-stunning handling and supply to the system to avoid injuries, reduce excitement and for a gentle induction of the stunning process; (ii) scientifically based induction conditions and corresponding clinical appearance, which can be verified under practical conditions; broilers must have lost consciousness before they enter high CO_2 concentrations (> 40%, see Regulation [EC] No 1099/2009 Annex I, Chapter I). As loss of consciousness is not instantaneous, the induction phase must be gentle and must not include aversive effects; (iii) sufficient depth of stunning to assure that, in combination with a given stun-stick interval and quality of neck-cutting, no animal regains consciousness before dying; and (iv) suitable process control and monitoring of relevant welfare parameters including definitions of key parameters and monitoring points to enable easy checks by the plant staff and competent authority.

The authors have been involved in providing scientific expertise to German competent authorities and to European manufacturers/industry, to assist with the decision whether, and under what conditions, systems for gas stunning of poultry could be installed according to the German Animal Welfare legislation. In Germany, currently, CO_2 stunning is permitted for turkeys, whereas for broilers, until EC regulation 1099/2009 comes into force, only a temporary permission for testing is possible.

The controlled-atmosphere stunning (CAS) systems for poultry described in this paper according to Council Regulation (EC) No 1099/2009 of 24 September 2009 on the protection of animals at the time of killing (Annex I, Chapter I, Methods, Table 3, Gas methods) can be subsumed under Line 2: "Carbon dioxide in two phases", which is described as "Successive exposure of conscious animals to a gas mixture containing up to 40% of carbon

Table 1 Details on system and methodology.

Category	Stork PMT 2 phase gas-stunning system	LINCO progressive gas-stunning system
Investigation period, Country	8 days in January, March, May & July 2004, Germany	9 days in July, September & November 2009, Italy
Liveweight	1.5–2.4 kg	1.6–4.1 kg
Transport system	Stork PMT Containers	LINCO Maxiload Containers
Unloading of containers	By fork lift	By fork lift
Transport time	1–2 h	1–3 h (single cases up to 6 h)
Lairage time	2–4 h	2–3 h (single cases up to 6 h)
Birds supply to the gas atmospheres	Containers are automatically emptied, birds pass through the stunning system on moving belts	Crates are automatically depiled from the container, birds in their crates pass through the stunning system
Gas supply and regulation	Two constant pre-mixed atmospheres supplied/extracted by tubes; two regulation/recording points, alarm system	Supply from evenly distributed nozzles at the bottom of the pit from a source of 100% CO_2; one regulation point, three recording points
CO_2 concentrations	Phase 1: 40% CO_2/30% O_2/30%N_2 Phase 2: 80% CO_2 in air	Increasing CO_2% in the air up to 50–65%, 40% CO_2 is reached after 110/250/210 s (small/medium/heavy birds)
Dwell times	1 min $_{\text{(Phase 1)}}$, 2 min $_{\text{(Phase 2)}}$	275-440 s depending on weight
Stun-stick interval	Mean: 31–45 s (range: 19–90 s)	Mean: 102 s (range: 36–179 s)
Investigations of behaviour during induction	Through the windows at defined time-points of exposure	By camera fixed in the crates while passing through the gas
Number of birds in the evaluation of stunning effectiveness	107,500 broilers from 42 flocks	90,000 broilers from 43 flocks

dioxide, followed when animals have lost consciousness, by a higher concentration of carbon dioxide". Key parameters required are carbon dioxide concentration, duration of exposure, quality of the gas and temperature of the gas. The maximum stun-to-stick/kill interval is not mentioned as a key parameter assuming that the stunning method excludes the possibility of re-awakening.

Materials and methods

The two systems were investigated at two different slaughter plants using the same protocol, however some adaption had to be made due to different features of the systems (eg in behavioural measurements). During several slaughter days in different seasons loading density, transport and lairage times, behaviour and risk for injuries during transport of the containers/crates towards the system, at container unloading and on the conveyor belts, loading density on the conveyor belts, behaviour during exposure to the gas, stun-stick interval, stunning effectiveness, gas concentrations and dwell times were recorded. Some of these details are given in Table 1.

LINCO progressive stunning system

An automatic de-piling device places the uncovered crates onto a conveyor belt, which is covered with a metal rail to ensure that birds cannot escape. The number of containers de-piled per hour is adjusted with regard to slaughter speed according to the average weight of the animals and the average number of animals in the containers. The stunning system is placed in a pit with a depth of approximately 5 m. The crates are lowered step-wise in piles. After having reached a depth according to the preset maximum CO_2 concentration (50–65%), the crates move to a second pile and are raised up again twice as rapidly as the speed of descent downwards (according to the programmed speed settings). The stunned birds are transported within their crates to the hoisting area on a conveyor belt. There, the birds are shackled and cut by an automatic blade. CO_2 is injected (from a source of 100% CO_2) at the bottom of the pit from evenly distributed nozzles below bird level and without impact on the unstunned birds in the upper levels. CO_2 concentration is controlled by using one reading point. For recording purposes, there are three measuring points at different heights (100 cm from the deepest point of the pit, 100 cm beyond the exit and at variable height attached to the belt between the downward and upward crate column). Within one setting, the CO_2 concentration is kept within a range which varies for the different measuring points between 3.6 and 7.6%, the higher point showing greater fluctuations than at the deeper one. An alarm system had not yet been installed. If necessary, rapid supply of CO_2 is available. Illumination is possible by opening a metal cover from the top of the system to enable the system to be checked. Visibility of the animals during the stunning process is limited due to the way the system operates (the crates are stacked). The animals can be seen at the lowest point of the system on the conveyor and it is also possible to see them when the crates move upwards again, as the space between the single crates is then greater than when on their way down.

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Stork PMT 2 phase stunning system

The containers were tilted stepwise (20 and 60°) in a closed, dark cubicle, accessible in case of emergency via a side door, the birds sliding over a system of distributing sheets from the container levels onto a soft belt. The number of containers emptied per hour is adjusted according to the birds' weight and slaughter speed. Containers are checked for remaining birds by an infra-red device, which triggers an alarm if any bird remains within a container. The belt speed is adjusted depending on number of birds per container, to ensure there is enough space for the birds to find their place on the belt. The birds are transported on two level belts, which can be accessed if necessary via doors from the side and from the top. The latter can be used for live animal inspection. The belt slopes downward at 15° and the birds pass through a curtain onto a level belt in the gas atmosphere, where they have about 10% more space than in the transport crates. The stunning tunnel has a clear height of 35 cm above belts and from the entrance to the exit curtain is closed. It is ventilated by two constantly pre-mixed gas atmospheres, supplied and extracted by an external tube system and controlled by measuring and regulation devices. The first atmosphere is humidified to ease induction. A visual and acoustic alarm, followed by automatic stop of all belts is triggered if the gas concentration deviates from the set points for more than 20 s (phase I > ± 4% CO_2/O_2% // phase II > –4%/ + 20% CO_2). The exit belt delivers the stunned birds into a shackling carrousel, from where they are transported towards the automatic knife. Limited viewing of the tunnel was provided by three windows in each in the first phase and second phase sections, through which the birds could be observed for 2 s, respectively (10–13, 30–33, 55 s after entering Phase I).

Gas concentrations

CO_2 and O_2 concentrations were measured continuously using a PBI Dansensor A/S, Ringsted Denmark (CheckMate II O_2 (ZrOx)/CO2, Ser Nr 76081104), in combination with a pump (Type PM13421-NMP30, Fa Neuberger, Freiburg, Germany) connected to a hose (diameter 4 mm) fixed within the pit at defined heights between the piles of crates, in the closed tunnel at defined distances from the tunnel entrance, at fixed measuring points in each phase and directly on the moving belt to measure at birds' head level while they are exposed to Phase I. The interval between measurements was 5 s. Additionally, a mobile gas measuring system was passed through each system (for LINCO: mobile device [Pragma, Italy]), measuring interval 5 s, from which records from the display were taped on video and transferred into an Excel® sheet; for Stork PMT: CheckPoint (PBI-Dansensor), measuring interval 22 s. The gas-measuring systems were placed between the birds on the belt or in the crates used at the same time to check the behaviour during transit (see below).

Behaviour studies during the induction of stunning

These were performed differently for each system, continuous video analysis (LINCO) and description of behaviour through windows at certain time points of the process (Stork PMT). In the LINCO system one crate was equipped with a video camera and an infra-red light source. The recording equipment took half of the space of the crate and was separated from the birds by a Perspex panel. The other half of the crate was filled with birds at the routine stocking density. Behaviour was analysed according to the time from entering the gas atmosphere. From a total of 48 video films, the behaviour of 77 birds from 35 different flocks could be analysed continuously. The displayed behavioural signs were classified in a similar way to that used by Barton Gade *et al* (2001) and Webster and Fletcher (2001) after entry into the stunning system: onset of swallowing, onset of deep breathing through an open beak, onset of deep breathing with neck stretched upwards and duration of this neck posture, onset of head shaking and number of head-shaking repetitions, jumping (lifting both feet from the belt, possible sign of a flight reaction), time of loss of ability to stand, time of final eye closure, time of complete loss of neck tension and posture, onset of convulsions. Complete loss of neck tension and posture was defined as "animals not able any more to control the movements of the neck", which is lying either on the ground, on the back or on a nearby bird without any muscle tension. It was also checked whether all birds visible on the video had lost posture before they entered high concentration of more than 40% CO_2 to verify whether they were unconscious then.

In the Stork PMT system, the behaviour of the broilers was monitored through the windows by direct observation (17 flocks for 5 min each window) and a 'window standard' including the behavioural signs analysed for the LINCO system was described. In addition, from a video record of 105 min of window 1 (10–13 s after entrance into Phase I) among 833 clearly visible birds it was possible to analyse whether or not they showed breathing with an open beak. Frequency of single jumps, if observed during the 2 s window observation time, was also counted. Furthermore, from the videos of window 3 (approx 140,000 broilers from 37 flocks) it was possible to check, whether all animals had lost posture before entering the second phase of 80% CO_2.

Stunning effectiveness

Stunning effectiveness was monitored for the LINCO system in 89,850 birds from 43 flocks (29,950 at each position) and for the Stork PMT system in 107,500 birds from 42 flocks. The birds were observed for a period of 5 min just before they reached the automatic blade (position 1), after the manual back up cutting area (position 2) and before entering the scalder (position 3). Evaluation of stunning effectiveness was carried out according to Table 2. Before scalding birds were checked for any movement.

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Table 2 Monitoring stunning effectiveness before/after the automatic blade.

Evaluation	Risk of regaining consciousness	AWAKE
Just before killer/cutting	Regular breathing + Positive corneal reflex	Flapping (+ rightening) + Regular breathing (+ vocalisation) + Positive corneal reflex
Immediately after killer/cutting	Regular breathing	Regular breathing + Rightening (flapping*) (+ vocalisation)

* As unco-ordinated flapping after neck cut could be due to a broken neck (through the automatic blade), it was not included in the evaluation.

Results

Supply of birds to the system

Excitement of the birds at supply or irregular supply can compromise welfare during stunning. Overloading of containers may lead to irregular supply, piling up of broilers on the conveyor belts or delay before cutting. The same effect occurs if belt speed or transport speed of crates is not adjusted properly. Inadequate stunning may result if birds can hide their heads under others and breathe the air trapped between their feathers. Other risk factors may be weather conditions during transport and lairage, or a high noise level during lairage. For both systems these problems were kept to a minimum. The main difference of supply between the two systems is whether the animals stay in their crates (LINCO) or whether they are tipped out of the containers (Stork PMT).

When containers were tilted for emptying, excitement of the birds before stunning increased. Birds showed this by wing flapping and vocalisation during the first minute (average 34 s, maximum 60 s, n = 73 containers with 130 to 180 broilers per container) after having slipped out of the container until each animal had found its position. The same behaviour patterns were seen when the broilers were moved between the different conveyor belts.

When birds were transported in the crates, excitement of the birds increased, if the movement of the crates was not smooth enough but quite bumpy. Fitful movements lead to more agitation of the broilers in the crates. Transport of uncovered crates caused injuries in single cases if birds were able to poke their heads or wings out of the crates. For the LINCO system these risk positions have been eliminated in the course of the investigation.

Behaviour during induction of stunning

The sequence of behaviours of broilers found during analysis of the continuous video in the LINCO system (slowly increasing CO_2 concentrations) can be described as follows. Before entering the stunning system most of the birds were sitting. Several seconds before the entrance some of the birds stood up because of jerky crate movements. A few seconds after entering the CO_2 system all birds started to 'swallow', and directly afterwards or sometimes simultaneously to show 'deep breathing with an open beak'. Deep breathing was followed by 'head shaking' and 'deep breathing with the neck stretched upwards'. Some birds stood up while head shaking. For the heavier birds it was hard to stand up, as they did not have enough headroom. 'Jumping' was only observed in small birds, as for medium and big birds jumping was impossible as a result of the height of the boxes. Four of the 29 small birds (13.8%) showed jumping once, one of them twice. Subsequently the animals 'lost their ability to stand', and could be seen stumbling and sitting down resulting in an unstable sitting position. And then they finally 'closed the eyelids', before they stopped 'deep breathing with neck stretched upwards'. Subsequently, broilers lost control of neck movement. Their heads started to fall either in front or back, depending on body position. This ended in 'complete loss of neck tension and posture' and birds were lying quietly and breathing regularly before they started to convulse. In total, 29 small birds, 24 medium birds and 24 heavy birds were observed during their progress through the system. Due to movements within the crates not all birds could be observed for all behavioural patterns. Time patterns are given in Table 3.

In a ventilated 40% CO_2/30% O_2/30%N_2 atmosphere (Stork PMT), the behaviour, observed at times while passing the windows can be described as follows. At the first window, which means 10–13 s after entering the anaesthetic mixture, most of the broilers were sitting, some were 'breathing with open beak' and few reacted with 'head shaking'. In some cases wing shaking was also seen. The percentage showing 'deep breathing with open beak and upwards stretched neck' varied between flocks (an average of 28% [18–34%] showed this at the moment they passed window 1). Eyes were open and no wing flapping occurred. Single birds jumped (average 9% per flock [2–16%] jumped at the moment they passed window 1). This did not depend on size as the birds had enough head room. Observations at incidental belt stops (short stops if slaughter line stopped, which prolonged observation time through window) revealed that control of neck movement, ie the head falling either in front or back occurred shortly after passing the first window (15–25 s after entering Phase I). Loss of posture

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Table 3 Time of behaviour patterns of broilers in the LINCO system (slowly increasing CO_2%- concentrations).

Behaviour	Small birds (1.6–2.0 kg)	Medium birds (2.6–3.4 kg)	Heavy birds (3.5–4.1 kg)
Onset of swallowing* (average [min-max])	n = 17 7.7 s (2–15 s)	n = 15 9.8 s (3–21 s)	n = 13 10.7 s (4–24 s)
Onset of deep breathing through an open beak* (average [min-max])	n = 20 10.8 s (2–22 s)	n = 18 13.3 s (5–23 s)	n = 16 13.4 s (7–24 s)
Onset of head shaking* (average [min-max])	n = 23 15.0 s (6–28 s)	n = 23 19.6 s (2–32 s)	n = 19 21.9 s (5–40 s)
Number of head-shaking repetitions (average [min-max])	n = 20 7.5 (3–17)	n = 21 8.4 (3–15)	n = 19 7.7 (2–14)
Onset of deep breathing with upwards stretched neck and open beak* (average [min-max])	n = 25 16.1 s (5–31 s)	n = 21 20.6 s (14–32 s)	n = 20 26.1 s (12–52 s)
End of deep breathing with neck stretched up* (average [min-max])	n = 25 55.4 s (26–90 s)	n = 19 83.1 s (59–114 s)	n = 18 78.2 s (47–145 s)
Time of loss of ability to stand* (average [min-max])	n = 18 42.4 s (18–78 s)	n = 15 68.1 s (35–84 s)	n = 14 62.6 s (29–88 s)
Time of final eye closure* (average [min-max])	n = 13 48.2 s (27–65 s)	n = 11 68.1 s (37–137 s)	n = 12 70.8 s (47–95 s)
Time of complete loss of neck tension and posture* (average [min-max])	n = 16 81.9 s (62–112 s)	n = 12 124.5 s (97–156 s)	n = 9 123.4 s (93–165 s)
Onset of convulsions* (average [min-max])	n = 29 118.5 s (57–162 s)	n = 24 173.1 s (70–257 s)	n = 21 156.7 s (84–329 s)

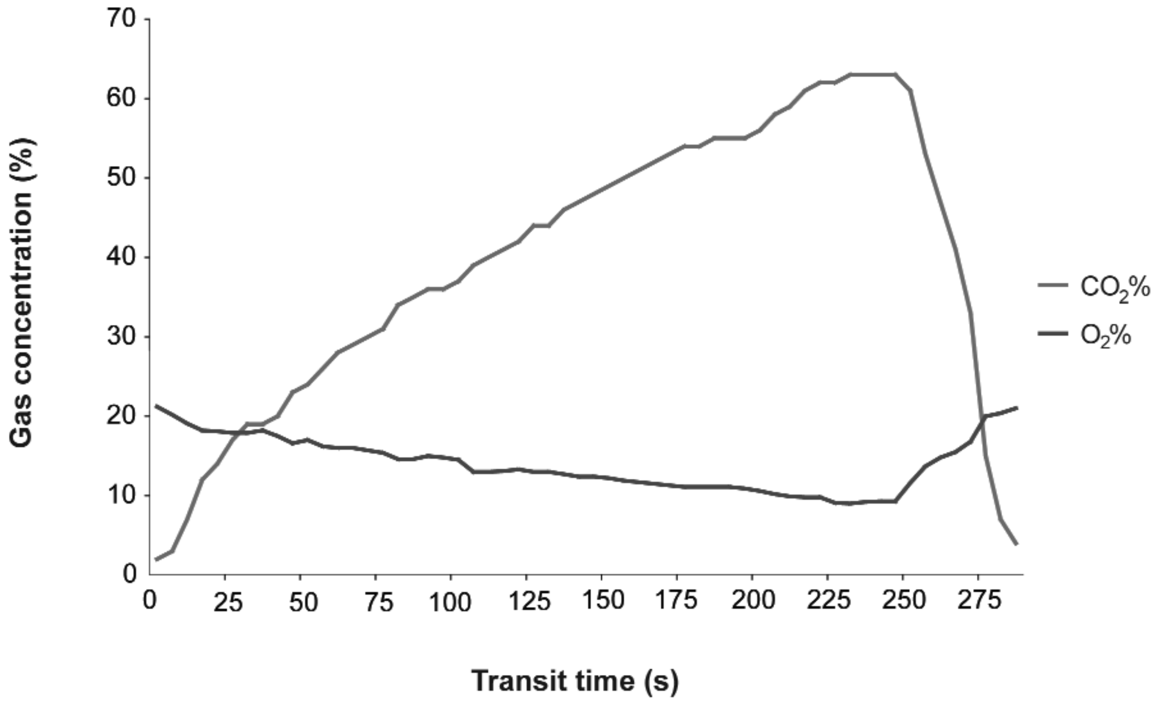
* Seconds after entering gas atmosphere.

Table 4 Stunning effectiveness before/after the automatic blade and before scalding.

Evaluation	Effectively stunned	Risk of regaining consciousness	AWAKE
LINCO (n = 89, 950)			
Just before killer/cutting	99.987%	0.000%	0.013%
Immediately after killer/cutting	99.966%	0.007%	0.027%
Before scalding	99.983%	0.000%	0.017%
Stork PMT (n = 107,500)			
Just before killer/cutting	99.961%	0.039%	0.000%
Immediately after killer/cutting	99.856%	0.141%	0.003%
Before scalding	100.00%	0.000%	0.000%

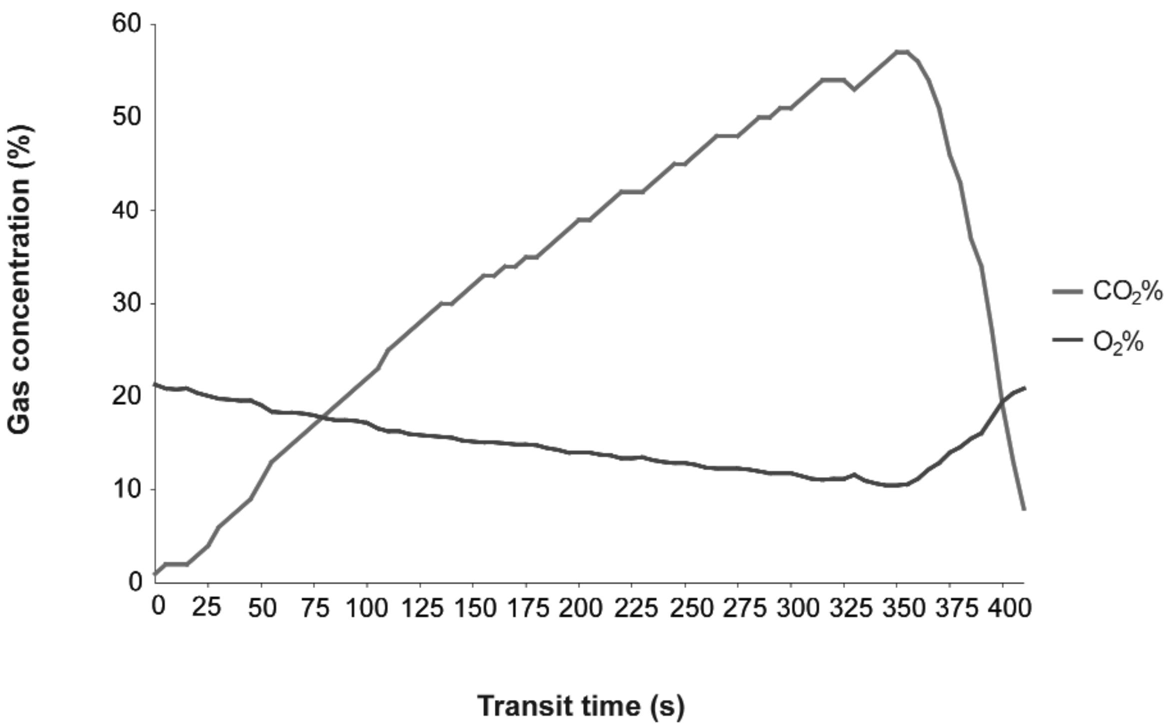
happened thereafter 18–35 s after entering Phase I. Remarkably, there were differences of several seconds between broilers of the same flock. Heavy animals lost posture later than light animals (< 2 kg). At the second window, 30–33 s after entering the gas, nearly all broiler heads had fallen either to the front or the back. Only in the heaviest flocks single animals with their heads up were observed. Part of the flock had already lost posture. Eyes were either open or closed, the beak was closed or still opening and closing, single birds showed convulsions. At the third window, 55 s after entering the gas atmosphere, all broilers had lost posture and neck tension, their feet often pointing upwards, eyes generally closed, the beak also mostly closed only for single birds still opening and closing, very few birds showed convulsions. In Phase II (fourth and fifth window, ie just after entering 80% CO_2 and 47 s later birds were lying, their feet stretched upwards, breathing was seen only by belly movements, eyes and beak were closed, convulsions could be seen only very rarely.

Figure 1



Gas concentrations CO_2/O_2 during transit of small chickens (LINCO).

Figure 2



Gas concentrations CO_2/O_2 during transit of heavy chickens (LINCO).

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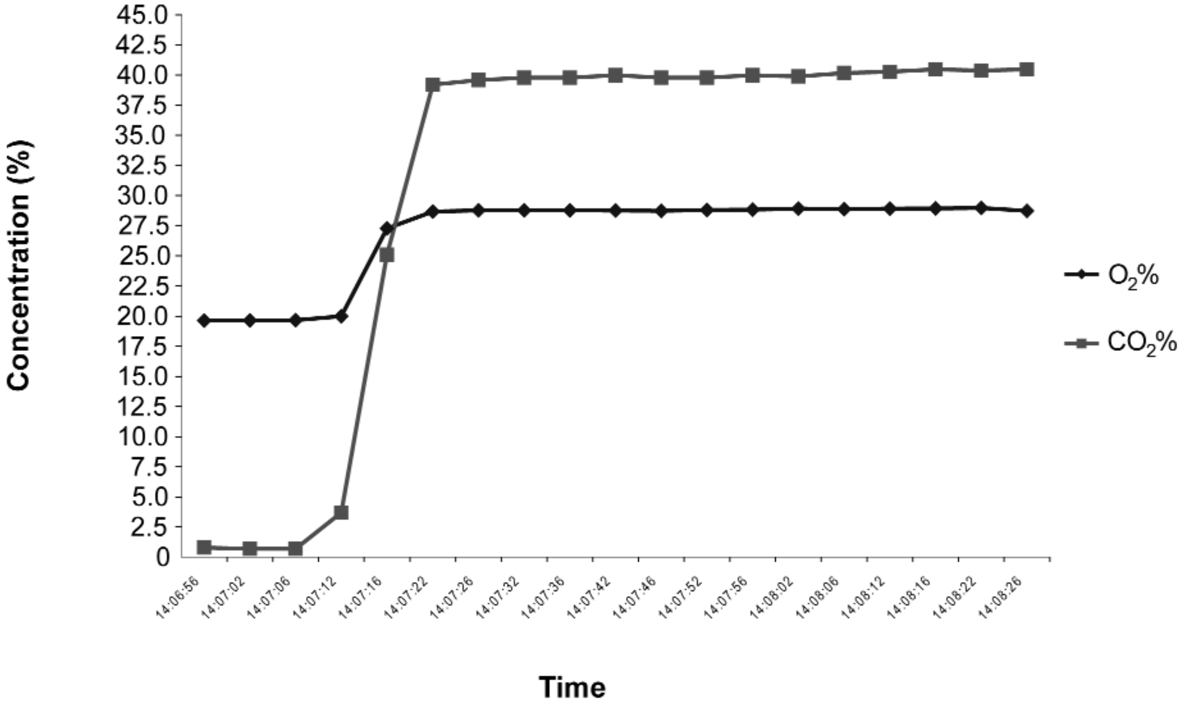
Figure 3



Gas concentrations CO_2/O_2 while entering Phase I, measuring pipes travelling through the system at animal height (Stork PMT).

Loss of consciousness before entering a high CO_2 concentration

In the LINCO system, all of the 77 broilers observed on the videos lost clinical signs of consciousness before a level of 40% CO_2 was reached, which was verified by simultaneous gas measurements. Also, for the Stork PMT system, analysis of video-recordings from the third window, revealed no animal showing signs of consciousness before entering to the second phase.

Stunning effectiveness

Stunning effectiveness was very high in both systems.

For the LINCO system in total, the stunning efficiency was 99.979% (small birds: 99.969%/medium birds 99.997%/heavy birds 99.964%). The birds that woke up were mainly smaller birds within their weight group. Birds awake before scalding had been overseen by the person performing the manual cutting in case of failure of automatic cutting.

For the Stork PMT system, immediately prior to cutting no animal showed signs of regaining consciousness. After neck cutting 0.003% were classified as awake (one bird out of 36,072). A reduction of the CO_2 concentration in the second phase (75 instead of 80%) increased the percentage of birds awake directly after neck cutting to 0.06% (n = 11,690). These low CO_2 concentrations were rectified by the gas control unit of the system. Stunning effectiveness seemed to depend on sufficient time in lairage as the effectiveness decreased if lairage time was 1.5 h or less (in these cases, risk of regaining consciousness or being awake directly after neck cutting was 0.68 and 0.03%, respectively [n = 10,200]). Again, smaller birds woke up more frequently than heavier birds.

Process monitoring and control

For the LINCO system, independently measured CO_2 and O_2 concentrations were comparable to recordings of the control system. The gas concentrations (CO_2/O_2) at different levels of the stunning system varied due to the different settings. Within one setting the CO_2 concentrations at the reading points were kept in an acceptable range, which seemed to be typical for the system, with the higher reading point generally showing higher fluctuations than the deeper. Measurements at one level (100 cm below entrance level) showed, that concentrations of CO_2 and O_2 were kept constantly within a small range. It is characteristic of the LINCO system that the CO_2 concentration increases very slowly. The settings are fixed in the manufacturer's instructions for the different weight classes of the broiler. For small birds higher concentrations are used and a shorter transit time than for medium and big birds (examples of drive-through measurements are given in Figures 1 and 2).

There was no alarm system to indicate low or excessively high CO_2 levels. These are legally required and necessary to detect deficiencies, either in case the CO_2 concentration exceeds 40% at a higher point in the pit than foreseen (so that the birds risk suffering because of reaching aversive

CO_2 concentrations before they are unconscious), or in case CO_2 level decreases and the birds risk recovering. The measuring interval should correspond to the set step time to create a reliable alarm system. Visibility of the animals during the stunning process is limited due to the design of the system (the crates are stacked) and some modifications would be necessary to meet the respective requirements of the German authorities.

For the Stork PMT system, the gas control system leads to uniform distribution of the gas mixtures in both phases. Birds quickly reach defined atmospheres with limited variations (± 1 to 2%; see Figure 3). Independently measured CO_2 and O_2 concentrations were similar to the recordings of the control system, except in one case where calibration had not been performed correctly. The alarm system was functioning and operating according to the settings. When the alarm was triggered, the supply of birds to the system stopped as planned. Transport of the birds towards the gas atmosphere could be monitored through the system's side and top doors. During the induction phase there was only very limited visibility, which was criticised by the German authorities and this has been rectified for systems installed since then, with the number of windows for monitoring the induction phase being doubled.

Discussion

Field studies and practical experience cover a huge number of animals and thus supplement laboratory studies with regard to determination of requirements for the optimum use of new stunning systems. Stunning methods can be defined scientifically, but industry develops different systems to put a method into practice. The quality and variation of key parameters will depend on the implementation of the method. Therefore, reliable monitoring points including alarm settings have to be defined specifically for each system. Authorities responsible for approval and surveillance need access to the necessary information on the national and international level. Manufacturers have to provide this information to the slaughter industry.

The supply of birds to a system includes risk factors for animal welfare associated both with tipping the birds out of a container on to belts, and with transporting the birds in crates. The latter can only be advantageous if performed smoothly, avoiding uneven crate movements and risk of injury. The viewing of birds through windows can be easier to implement for transport of birds on belts, whereas transport in crates may require more sophisticated monitoring systems (eg cameras).

Viewing access is necessary for plant staff and the competent authority, because the behaviour of the birds during induction is critical with regards to animal welfare. Checks must be possible whether behavioural indicators show evidence of reduced welfare. Moreover, loss of consciousness must be verified before birds enter high concentrations of CO_2 (> 40%). Windows for inspection can be covered and the cover only opened for checks, to prevent birds being disturbed by the incoming light.

The effect of CO_2 atmospheres on broiler welfare and the significance of various signs during the induction phase is still under discussion among scientists. The sequence of behaviour patterns observed here, after entrance into the stunning system until complete loss of neck tension and posture, corresponds with previous findings (Lambooij *et al* 1999; Barton Gade *et al* 2001; Webster & Fletcher 2001) although the time of appearance of a particular behavioural pattern varied due to differences in the rate of increase of CO_2 concentration over time. The significance of head shaking with regard to animal welfare is under discussion (Webster & Fletcher 2001). It seems likely that the response is related primarily to novel or alerting stimuli (Dunnington & Siegel 1986; McKeegan *et al* 2007). According to Barton Gade *et al* (2001), the observed behavioural patterns are considered to indicate a mild-to-medium aversion to the gas mixture. Webster and Fletcher (2004) considered the use of concentrations of up to 60% CO_2 as not aversive.

McKeegan *et al* (2006) studied the impact of gas mixtures containing different percentages of CO_2 (10, 25, 40 and 55%) on the behaviour of broiler chickens during the first 10 s of exposure. In 25% CO_2, the number of birds showing 'gasping/ heavy breathing' (4 out of 10 chickens) was higher than in 40 and 55% CO_2 (3 out of 10). In 40% CO_2, the birds began to withdraw from the gas, and in 55% CO_2 the reaction was described as 'marked withdrawal'. These results indicate that most broilers seem to tolerate concentrations up to 40% CO_2. Concentrations higher than 40 or 55% seem to cause pain or a higher aversiveness as they showed increased withdrawal. The addition of 30% oxygen to the carbon dioxide in nitrogen mix was associated with increased time spent feeding and reduced head shaking (McKeegan *et al* 2007).

In the present study, all of the observed broilers lost clinical signs of consciousness before a level of 40% CO_2 was reached. External signs for loss of consciousness are loss of neck tension and loss of posture as well as eye closure (Raj *et al* 1992).

Webster and Fletcher (2004) and McKeegan *et al* (2007) also mentioned the issue of convulsions causing pain or injuries to neighbouring birds not yet unconscious. This effect could not be totally excluded in the present study for both systems. However, more intense convulsions can be seen in atmospheres containing argon than in CO_2/O_2 atmospheres (Coenen *et al* 2000; Webster & Fletcher 2004). This effect may be managed by reducing density on belts or in crates but also by modifying the rate of increase in CO_2 concentration, a subject about which further research is needed.

Both systems show very high stunning effectiveness (99.97% LINCO system and 99.86% Stork PMT system) but no irreversible stunning. Nevertheless, even though stunning effectiveness is relatively high, occasionally broilers are able to regain consciousness. This problem increases with insufficient bleeding. This shows the importance of an effective routine back-up despatch procedure by responsible and trained staff (back-up cutting in case of no cut or missed cut or back-up stunning in case of re-

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awakening). The classification of CO_2 stunning of poultry as an irreversible stunning method (see regulation EC 1099/2009) does not take into account that the risk of re-awakening cannot be totally excluded. Practical experience and investigations including high numbers of animals show that for a small percentage of birds the method will function as a simple stunning method. With regard to the actual settings of the Stork system in most of the German plants, which include lower oxygen concentration in the first phase and also lower CO_2 concentrations in the first and second phases (Phase 1: 1 min 28% CO_2/ 22% O_2, Phase 2: 2 min 70% CO_2 in air), stunning effectiveness may be slightly lower than for the settings used in 2004. Thus, definition and monitoring of a stun-stick interval as a key parameter will be necessary for both systems.

Daily routine surveillance of animal welfare at stunning includes monitoring of animal-based parameters, such as behaviour during stunning and stunning effectiveness as well as monitoring of the key technical parameters, for which in the LINCO system alarm thresholds, were missing in the present investigation. For each weight class the settings of CO_2 concentration and dwell times (step times) have to be defined specifically to the conditions in the plant including the depth of the pit, the level in the pit where 40% CO_2 is reached, the time until this CO_2 concentration is reached, the total dwell time, and the stun-stick interval.

As an overall conclusion, the investigation of two CO_2 gas-stunning systems under practical conditions in Germany and Italy — using less than 40% CO_2 until animals are unconscious followed by higher CO_2 concentrations to ensure a lasting stunning effect — revealed obvious advantages compared to electrical water-bath stunning, for example, avoiding shackling and achieving high stunning effectiveness. However, the interpretation of some behavioural signs during the induction phase still remain under discussion. The complex situation of every single installation requires a plant-specific approval with regard to animal welfare including necessary training of competent authority and personnel.

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