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Survey of the incidence of post-stun behavioural reflexes in electrically stunned broilers in commercial conditions and the relationship of their incidence with the applied water-bath electrical parameters

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Abstract

Despite recent technological advances, stunning efficiency of broilers in commercial plants is still questionable and a major public concern. The aim of this survey was to assess the electric water-bath parameters used in poultry processing plants, establish practical ways for the assessment of stunning efficiency in these conditions, link their incidence to the applied current and propose methodology for the online evaluation of the effectiveness of electrical water-bath stunning. A total of 5,378 broilers were assessed randomly for signs of post-stun recovery at 4, 8, 13 and 25 s post-water-bath exit (WBE) through corneal reflex, rhythmic breathing, head shake and escape behaviour on three processing lines. The electric water-bath parameters used did not meet the recommended current and frequencies for effective stunning. No rhythmic breathing was detected with any of the birds at 4 s post water-bath exit, while this reflex returned by 8 s in some birds. Broilers that had their necks cut had a significantly lower incidence of breathing compared to those that missed the neck cutter. The electric water-bath stunners that were tested did not abolish the corneal reflex where 38.4% of birds tested positive, at 4 s post WBE. Pulsed DC-stunned birds that missed the knife had a significantly higher incidence of positive corneal reflexes than those that had their necks cut. Head shake was present on 26.7% of the birds when assessed at 25–30 s post WBE. The trends in incidence of behavioural reflexes with time post WBE may be used for the assessment of stunning efficiency, particularly on birds that miss the knife, but none of the assessed indicators could unequivocally demonstrate unconsciousness.

Keywords: *animal welfare, behavioural, broilers, reflex, stunning, water bath*

Introduction

The methods of stunning and slaughter have featured often in the public domain and have frequently come under scrutiny with the main concerns identified as poor stunning or birds regaining consciousness before death and feeling pain at slaughter (Fletcher 1999). In poultry processing plants, electronarcosis is the method that has been developed and electric water-bath stunning is the technique that is most widely used (Bilgili 1992; Raj 1998; Raj *et al* 2006a). However, recent research under laboratory conditions has found that multi-bird water-bath stunning does not result in consistent and effective stunning (Handle *et al* 2010). The increased consumption of poultry meat has led to an increase in the number of birds slaughtered and poor stunning or regular quick post-stun recovery will affect a number of individual birds, triggering the need for the development of reliable indicators of post-stun recovery that can be used in commercial broiler slaughterhouses.

From an animal welfare point of view, the assessment of an effective stun depends on the availability of reliable criteria for the evaluation of the state of the bird's central nervous system (CNS), where the presence of an epileptic seizure by analogy to human physiology is considered to be an indicator of unconsciousness (Gregory 1986). There are currently no direct methods for the assessment of the animal's mental state, including consciousness, that can be applied practically in the processing plant, and while the quest for identifying suitable physiological indicators and their link with an effective water-bath stun began in the late seventies (Kuenzel & Walther 1978; Scott 1978; Heath 1984; Gregory 1986), the current criteria used still gives variable results (Gregory 1989; Handle *et al* 2010; Prinz *et al* 2010a).

While the electroencephalographic (EEG) recordings and the somatosensory-evoked potentials (SEPs) are scientific tools for the assessment and understanding of the brain's activity, they are only applicable in laboratory conditions. In the everyday slaughterhouse environment, the effectiveness of stunning can only be assessed visually through observation of the animal's behavioural reflexes and physical state which have been the subject of a number of research papers (Table 1).

Table 1 Behavioural reflexes used as indicators of recovery in electrically stunned birds by source and reflex.

Author	A	B	C	D	E	F	G	H	I	J	K	L	M
Richards and Sykes (1964)				X									
Wormuth *et al* (1981)					X								
Heath 1984		X					X	X	X				
Schutt-Abraham *et al* (1987)***	X				X	X							X
Gregory and Wotton (1990a)	X	X	X										
Gregory and Wotton (1991)		X	X	X									
Gregory and Wotton (1992)		X	X	X									
Rawles *et al* (1995)		X	X										
Wilkins *et al* (1998)	X	X	X	X									X
Lambooij *et al* (1999)*					X								
Mouchoniere *et al* (1999)**		X			X	X							
Wilkins *et al* (1999)	X	X	X										
Wotton and Wilkins (1999)	X	X	X	X									
Wotton and Sparrey (2002)*	X				X								
Raj and O'Callaghan (2004)				X	X					X			
Raj *et al* (2006a)	X			X	X					X	X	X	
Raj *et al* (2006b)	X			X	X					X	X	X	
Raj *et al* (2006c)	X			X	X					X	X	X	
Handle *et al* (2009)				X	X								
Prinz (2009)	X				X	X				X			
Prinz *et al* (2010a)	X				X	X				X			
Prinz *et al* (2010b)	X				X	X				X			

A: Rhythmic breathing; B: Neck tension; C: Maintaining posture; D: Comb pinch; E: Corneal reflex; F: Wing flapping; G: Open fixed eyes; H: Tucked-in wings; I: Extended rigid legs; J: Spontaneous eye blinking; K: Head shake; L: Swallowing reflex; M: Muscle tone.
* Ostrich; ** Turkeys; *** Ducks, guinea fowl, laying hens and geese.

In laboratory conditions, Raj *et al* (2006b) confirmed that all 172 broilers stunned in a water bath with AC at various frequencies and currents experienced apnoea (absence of spontaneous breathing) immediately after stunning, irrespectively of whether or not an epileptic EEG was induced, with 95 out of the 172 birds showing breathing at 20 s from the end of stun. Post-stun rhythmic breathing was reported to return at 11.3–13.7 s by Wilkins *et al* (1998) with no marked differences in the time when broilers were stunned with different waveform-frequency combinations. However, this reflex is considered to be an unreliable indicator of unconsciousness by some authors (Wenzlawowich & von Holleben 2001; Raj *et al* 2006b) as the design of the electric water bath results in current flow through the entire body causing muscle tetanus and initial apnoea regardless of the bird's state of consciousness.

The corneal reflex is used widely in commercial slaughterhouses to assess stunning, because this reflex, controlled by eye muscles, would be absent during a generalised epileptiform insult, at least during the period of tetanus, induced by the stunning current. However, a negative response has also been proposed as an indication of approaching brain death, rather than a conscious brain, with its presence certainly being an indicator of a functional CNS (Gregory 1989; Wenzlawowicz & von Holleben 2001). While a positive corneal reflex was found in birds that did not subsequently recover post stunning (Prinz 2009), Raj *et al* (2006b) found 86 out of the 172 broilers stunned with three different amounts of current (100, 150 or 200 mA) delivered using seven different alternating current frequencies (200, 400, 600, 800, 1,000, 1,200 or 1,400 Hz) with positive corneal reflexes at 20 s post water-bath exit and 5 s after the neck cut. These results were also confirmed by the findings of Prinz *et al* (2010a) who found the corneal reflex present

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in 30% of the birds stunned with 60–150 mA AC with frequencies of 70–1,500 Hz at 20 s post stun.

Spontaneous eye blinking has been found to be clearly suppressed by electrical stunning, but as with the corneal reflex, it occurred in a number (15%) of apparently well-stunned broilers (minimum current 100 mA AC, 70 Hz [Prinz 2009]) and in 51% of the broilers at 20 s post stun (100–200 mA, AC, at a frequency of 200–1,400 Hz) by Raj *et al* (2006b). Spontaneous eye blinking was also found to be present at 40 s post stun (after the initial period of muscular tetanus) in AC-stunned broilers (60–150 mA at a frequency of 70–1,500 Hz), which did not subsequently recover and it has been suggested to be possibly a result of eyelid muscular fibrillation at the approach of brain death, and therefore a poor indicator of consciousness (Prinz *et al* 2010a). In the same study, the differentiation factor between eyelid convulsions as a result of the stunning current and the spontaneous eye blinking, was the frequency of eyelid closure. Regular periodic blinking is considered as the spontaneous eye blinking, whereas high frequency blinking is associated with eyelid muscular fibrillation.

Electrical-stunning-induced generalised epileptiform activity in the brain is always associated with a period of tetanus in all the muscles, including eye muscles, as mentioned previously. It was thought that after the termination of the epileptiform activity, the muscles recovered from the tetanus state and regained normal muscle tone or tension. Based on this, the time to return of neck tension has been described as another method for the assessment of recovery of consciousness by Gregory and Wotton (1990a), who showed the effect of stunning current on this reflex, where birds resumed neck tension at 44 s post stun when stunned with 44 mA and 105 s post stun when stunned with 105 mA at 50 Hz AC. No effect of stunning treatment on the time to the resumption of neck tension was, however, reported by Wilkins *et al* (1998) for different waveforms and frequencies.

The use of comb pinch as a method for the assessment of post-stun recovery of consciousness was first described by Richards and Sykes (1964). Subsequently, Gregory and Wotton (1990b) found only 12% of the birds demonstrated a positive reaction, despite the fact that 70% were already able to balance on their feet at that time. Comb pinch as an indicator of post-stun recovery was also used by Raj *et al* (2006b) where 33% of the birds stunned with AC at different frequencies and currents showed a positive comb pinch reflex at 20 s post stun.

The ability to stand and balance as a behavioural reflex has been used in several studies as conscious birds would be expected to maintain posture and balance when placed on their feet (Gregory & Wotton 1990a, 1991, 1992; Rawles *et al* 1995; Wilkins *et al* 1998).

The complete loss of muscle tone or total relaxation of skeletal muscles post stun has been used as a physical indicator of cardiac ventricular fibrillation which eliminates the chances of post-stun recovery by Wilkins *et al* (1998), who found that the majority of the birds stunned with 105 mA, 50 Hz sinewave AC developed a cardiac arrest and showed relaxed muscle tone.

Head shake and swallowing as an indicator of recovery of consciousness was used by Raj *et al* (2006b), who found 78 out of 172 birds shook their heads during bleeding and reported that, in general, the incidence of these reflexes increased when the electrical-stunning frequency was increased from 200 to 1,400 Hz AC.

Wing flapping (clonic seizures) was described to be present in head-only stunned broilers before they become tonic by Gregory and Wotton (1990a) and Prinz (2009) who concluded that wing flapping could not be indicative of successful stunning, which was more associated with post-stun convulsions after the birds had left the water bath. More than 50% of the birds that achieved successful stunning were found to exhibit this reflex by Prinz (2009). This clearly indicates that the amount of current required to induce seizures is less than that required to achieve loss of consciousness during water-bath stunning.

The current literature suggests that there are a number of behavioural indicators that have been considered in the assessment of the stunning efficiency, and while some of them have been associated with an epileptic EEG, none have been proven as an unequivocal guarantor of an unconscious brain.

The objectives of this study were to establish the indicators that can be used practically in the assessment of stunning efficiency in processing plants, to link their incidence to the applied current and neck cut and propose methodology for the online evaluation of the effectiveness of electrical water-bath stunning.

Materials and methods

Source of information and sample size

The data collection was conducted on three different slaughter lines (A, B and C) in two slaughter plants in the UK during 2010. This was designed to capture data from lines utilising different neck-cutting techniques, killing broilers at different weight ranges and using different stunning currents, frequencies and waveforms.

The birds were commercial broilers of mixed sex, at 35–40 days of age and with a weight range between 1.7 and 2.3 kg. The broilers were delivered to the slaughter plant in modules, which were then placed on the automatic system to present drawers of birds to the operatives for manual shackling. Birds were conveyed to a water-bath stunner, stunned and neck cut using the normal plant procedure. Slaughter lines A and B used pulsed, direct currents (pulsed DC) and line C used sine-wave alternating current (AC) in their water-bath stunners (Table 2). Neck cutting (7 s post WBE for line A and C and 5 s post WBE for line B) was achieved mechanically by a rotating blade, severing both carotid arteries, both jugular veins and on some birds the spinal cord (ventral neck cut) for lines A and B, and severing one carotid artery and one jugular vein (unilateral neck cut) for line C. All three

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Table 2 Stunning protocols for the three lines.

	Line A	Line B	Line C
No birds in the water bath	24	37	25
Current type	DC	DC	AC
Duty cycle*	30%	30%	–
Frequency (Hz)	550	550	400
Voltage (V)	75	69	143
Current (Amp)	1.4	2.9	2.2–2.9
Estimated average current per bird (mA)	56	80	102
Dwell time in the water bath (s)	14	10	13
Slaughter line speed (birds per min)	184	189	144
Control panel ammeter (A)	1.3–1.5	2.7–3.1	2.5–2.8

* AC voltage and current are expressed as RMS, while pulsed DC current and voltage are expressed as average values.

slaughter lines had a back-up slaughterman ensuring birds that missed the neck cutter had their appropriate blood vessels severed manually within 20–35 s after exiting the water-bath stunner (referred to as post WBE).

The assessment for post-stun recovery was completed by checking the birds for reflexes and recording the numbers that tested positive for each reflex at the given time post stun and expressed as a proportion or percentage of the total number of birds tested. Due to the speed of the processing line or the layout of the processing equipment, eg protective guards, not all indicators and times of assessment reported in the literature through laboratory experiments, could be applied in a commercial situation. The type of neck-cutting procedure applied has a practical implication on the assessment of some behavioural reflexes, where head shake, swallowing or breathing will not yield significant data on birds that are decapitated or with severed spinal cords (line A and B). In this survey, behavioural reflexes were assessed directly on the processing line and no account was taken for birds that may have developed ventricular fibrillation (cardiac arrest) during the stunning process. Only one reflex per bird was assessed. The data collected from line A on the corneal reflex was limited in this study due to inaccessibility of the processing line at 4 and 8 s post WBE.

A total of 5,378 (1,407 on line A, 2,401 on line B and 1,570 on line C) broilers were assessed randomly for signs of post-stun recovery or poor stunning at 4, 8 13 and 25 s post WBE by corneal reflex, rhythmic breathing, head shake and escape behaviour.

Assessments of birds stunned but not neck cut (birds that missed the blade) were also conducted and the results recorded separately from those birds that had their necks cut. The results from these assessments were used for comparison with the same of birds that had their necks cut to establish the effect of neck cutting on the incidence of reflexes and post-stun recovery.

Birds that emerged unstunned from the electric water bath expressed typical escape behaviour, with wing flapping, vocalising, pulling themselves up on the shackles with flexed legs and heads either pointing upwards or sideways that resulted in them failing to go through the neck cutter.

All assessments were carried out on inverted birds that were hanging from the shackles and no birds were removed from the slaughter line at any time.

Stunning protocols

The number of birds in the water bath and the stun duration for the three lines are shown in Table 2. The applied stunning currents, voltages, frequencies and waveforms in the water baths on the three slaughter lines were measured with a factory-calibrated Scopemeter (Floke Corporation, Everett, WA, USA).

Physical reflexes assessment

Rhythmic breathing was determined by observing movement in the vent area of the birds while they were conveyed past the observer as described by Schutt-Abraham *et al* (1987), Gregory and Wotton (1990a) and Wilkins *et al* (1999).

Corneal reflex was assessed by holding the bird's head with one hand and touching the cornea with a finger of the other hand as described by Wotton and Sparrey (2002) and Raj and O'Callaghan (2004), which provokes a blinking reaction in a conscious bird.

Head shake was observed visually as described by Raj *et al* (2006a), in this study at 25–30 s post water-bath exit on the birds of line C with the unilateral neck cut.

Escape behaviour expressed as vigorous low frequency wing flapping combined with vocalisation, and birds pulling themselves up on the shackles, with heads pointing either up or sideways, as distinguished from high frequency muscle tremors or twitches, was assessed visually at 13 s post WBE.

Spontaneous eye blinking was assessed as described by Prinz *et al* (2010a) where the differentiation criteria between eyelid convulsions as a result of the stunning current and the spontaneous eye blinking, was the frequency of eyelid closure. Regular periodic blinking is considered as spontaneous eye blinking, whereas high frequency blinking is associated with eyelid muscular fibrillation.

Statistical analysis

An '*F*-test' was used to compare variances for each one of the tested reflexes, at different times post stun, or between two slaughter lines. For any '*F*-value' higher than 0.05 the sample means were evaluated using a two-tailed *t*-test (equal variances). Alternatively, a two-tailed *t*-test for unequal variances was used as required. The null hypothesis was that there were no real differences between the samples and therefore the hypothesised mean difference when comparing sample means was accepted as zero.

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Table 3 Incidence of birds showing signs of rhythmic breathing and corneal reflex at different times post electric-water-bath exit (%).

	Line A				Line B				Line C			
Time post WBE	**Positive**	**SD**	**CV**	**SEM**	**Positive**	**SD**	**CV**	**SEM**	**Positive**	**SD**	**CV**	**SEM**
Rhythmic breathing												
4 s	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0
8 s NC	5.3%	± 0.6	11.5%	0.000	3.1%	± 1.7	56.5%	0.13	1.7%	± 0.5	29.6%	0.001
13 s NC	12.0%	± 1.7	13.9%	0.002	7.1%	± 1.1	15.5%	0.17	26.6%	± 13.3	49.8%	0.010
8 s MK	36.1%	± 14.9	41.4%	0.021	22.9%	± 8.5	37.0%	0.95	41.9%	± 16.1	224.4%	0.014
13 s MK	77.5%	± 6.8	8.8%	0.014	58.0%	± 8.2	14.2%	1.10	43.1%	± 5.8	13.5%	0.008
Corneal reflex												
4 s	n/a	–	–	–	n/a	–	–	–	38.4%	± 3.9	10.0%	0.003
8 s NC	n/a	–	–	–	30.7%	± 8.6	28.0%	0.003	33.0%	± 0.9	2.8%	0.001
13 s NC	6.8%	± 0.4	6.4%	0.000	5.6%	± 1.3	22.7%	0.001	45.0%	± 3.9	8.6%	0.002
8 s MK	n/a	–	–	–	66.3%	± 18.5	28.0%	0.025	54.7%	± 7.7	14.1%	0.011
13 s MK	93.8%	± 4.3	4.5%	0009	91.1%	± 10.5	11.6%	0.006	68.5%	± 20.1	29.4%	0.028

NC: Neck cut; MK: Missed knife; SD: Standard deviation; CV: Coefficient of variance; SEM: Standard error of the mean; WBE: Water-bath exit.

Table 4 Effect of neck cutting on the incidence of birds showing signs of breathing and tested positive for corneal reflex on all three lines at 8 and 13 s post electric-water-bath exit.

	NC	**MK**	***P*-value**	**SED**	**NC**	**MK**	***P*-value**	**SED**
Birds breathing 8 s post WBE					*Birds breathing 13 s post WBE*			
Line A	5.3%	36.1%	$P < 0.05$	0.02	12.0%	77.4%	$P < 0.01$	0.014
Line B	3.1%	22.9%	$P < 0.05$	0.02	7.1%	58.0%	$P < 0.01$	0.011
Line C	1.7%	41.9%	$P < 0.05$	0.02	26.6%	43.1%	ns	0.013
Corneal reflex 8 s post WBE					*Corneal reflex 13 s post WBE*			
Line A	n/a	–	–	–	6.8%	93.8%	$P < 0.001$	0.009
Line B	30.7%	66.3%	$P < 0.01$	0.025	5.6%	91.1%	$P < 0.001$	0.006
Line C	33.0%	54.7%	$P < 0.05$	0.011	45.0%	68.5%	ns	0.028

NC: Neck cut; MK: Missed knife; SED: Standard error of difference; WBE: Water-bath exit; ns: $P > 0.05$.

Results

Stunning protocols and neck cutting

A number of birds were found to miss the neck cutter, with the lowest incidence observed on Line A (1.4%), followed by Line C (3.0%) and Line B, where 5.3% of the birds missed the neck cutter. The birds on line B were found to have a greater variation in live weight, with both very small and very large birds being processed at the same time. The main reason for birds missing the neck cutter was identified as their size. Small birds that did not reach the water in the electric water bath emerged from the water bath unstunned. Consequently, their heads were 'held up' and they were not picked up by the guide rails of the neck cutters.

Neck cutting on lines A and B produced consistent severance of both carotid arteries and both jugular veins, but did not produce consistent severance of the spinal cord, with 20% of the birds on line A and 16.2% of the birds on line B retaining intact spinal cords after passing through the neck cutter. Neck cutting was carried out at 7, 5 and 7 s post WBE for lines A, B and C, respectively.

Rhythmic breathing

No birds demonstrated signs of rhythmic breathing at 4 s post WBE in any of the three slaughter lines (Table 3).

The number of birds which returned to rhythmic breathing at 8 s post WBE on line A (5.3%) was found to be significantly higher ($P < 0.001$) than the same for the birds on line

Table 5 Effect of time on the incidence of birds showing signs of breathing and that tested positive for corneal reflex on all three lines between 4 and 8 s, and 8 and 13 s post electric-water-bath exit.

	4 s	8 s	*P*-value	8 s	13 s	*P*-value
Birds showing signs of breathing post WBE						
Line A NC	0.0%	5.3%	$P < 0.001$	5.3%	12.0%	$P < 0.01$
Line A MK	0.0%	36.1%	$P < 0.001$	36.1%	77.4%	$P < 0.05$
Line B NC	0.0%	3.1%	$P < 0.001$	3.1%	7.1%	$P < 0.05$
Line B MK	0.0%	22.9%	$P < 0.001$	22.9%	58.0%	$P < 0.01$
Line C NC	0.0%	1.7%	$P < 0.001$	1.7%	26.6%	$P < 0.05$
Line C MK	0.0%	41.9%	$P < 0.001$	41.9%	43.1%	ns
Birds testing positive for corneal reflex post WBE						
Line B NC	n/a	–	–	30.7%	5.6%	$P < 0.001$
Line B MK	n/a	–	–	66.3%	91.9%	$P < 0.05$
Line C NC	38.4%	33.0%	ns	33.0%	45.0%	$P < 0.01$
Line C MK	38.4%	54.7%	$P < 0.05$	54.7%	68.5%	ns

NC: Neck cut; MK: Missed knife; WBE: Water-bath exit; ns: $P > 0.05$.

C (1.7%), for those birds that had their neck cut. No significant differences were found in the incidence of rhythmic breathing for birds on line B and line C at both 8 and 13 s post WBE, on both neck cut and birds that missed the blade.

Neck cutting was found to have a negative effect (reduction) on the number of birds breathing rhythmically. Birds that had their necks cut generally showed a lower incidence of breathing compared to those that had missed the knife (Table 4).

Time was found to positively influence (increasing effect) the occurrence of birds breathing rhythmically after the exit of the electric water bath (Table 5). While no birds showed signs of breathing at 4 s post WBE on line A, there was a significant ($P < 0.001$) proportion (5.3 and 36.1%) of birds breathing at 8 s post WBE, of those that had their necks cut or missed the knife, respectively. The effect of time was found to be reduced, with smaller and less significant differences between birds breathing at 8 and 13 s post WBE in comparison to the results for birds breathing at 4 and 8 s post WBE on all lines.

Corneal reflex

The incidence of a positive corneal reflex varied from 5.6% at 13 s post WBE on broilers that had their neck cut on line B to 93.8% on birds that had missed the neck cutter at 13 s post WBE on line A (Table 3).

With time (from 8 s post WBE to 13 s post WBE), the number of birds that displayed a positive corneal reflex on line B reduced for those birds that had their necks cut (from 30.7 to 5.6%) and increased for those birds that missed the knife (from 66.3 to 91.1%), indicating a pronounced effect of the slaughter over the DC stunning. There was no clear trend for an increase or reduction of the frequency for any of the birds that tested positive for this reflex on line C.

No significant differences were found in the incidence of the corneal reflex at 8 s post WBE between birds on line B and line C that had missed the knife or birds that had their necks cut. Within the group of birds that had their necks cut, there was a significantly ($P < 0.001$) higher proportion of birds testing positive at 13 s post WBE for a corneal reflex on line C (45.0%) compared to the same time for birds on line A (6.8%). The group of birds that missed the knife on line B had a significantly ($P < 0.01$) higher prevalence of positive corneal reflex (66.3%) at 8 s post WBE compared to the same in the group that had their necks cut (30.7%) (Table 4). This trend was exaggerated at 13 s post WBE, where the birds on line A and B showed significantly higher ($P < 0.001$) incidence of the corneal reflex for the group that missed the knife (93.8 and 91.1%, respectively) compared to the birds that had their necks cut (6.8 and 5.6%, respectively for line A and B). No significant differences (no effect of the neck cut) were found in the proportion of the birds that tested positive for corneal reflex on line C at 13 s post WBE.

With time, there was a small significant ($P < 0.05$) increase of the number of birds that tested positive for corneal reflex between 4 and 8 s post WBE (from 38.4 to 54.7%) in those birds that missed the knife on line C (Table 5). The birds that had their necks cut on line B had a reduced incidence (from 30.7 to 5.6%) of a positive corneal reflex between 8 and 13 s post WBE, where time significantly ($P < 0.001$) influenced this trend.

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Head shake

More than a quarter (26.7%) of the assessed birds showed signs of head shaking between 25 and 30 s post WBE.

Escape behaviour

Escape behaviour was exhibited typically only by birds that had missed the water in the electric water bath and emerged completely unstunned. However, not all birds that had missed the stunner, showed this behaviour, with some of the unstunned birds hanging calmly on the shackles. Generally, more birds expressed this behaviour shortly after the WBE where 1.0, 4.8 and 2.9% of the birds on line A, B and C, respectively, showed this reflex at 8 s post WBE and 0.7, 4.7 and 2.0% of the birds showed this behaviour on the same lines (A, B and C, respectively) at 13 s post WBE.

Spontaneous eye blinking

An attempt to assess and record spontaneous eye blinking was made, which showed a variable frequency of blinking that randomly fell either in or out of the time interval in which the reflex was assessed. Due to the randomness of the initial results on a moving slaughter line, no further assessments of this reflex were made.

Discussion

Stunning protocols

This study revealed large variations in the calculated average current per bird between the three slaughter lines, which has previously been reported by Raj and O'Callaghan (2004), Raj *et al* (2006a,b,c) and Handle *et al* (2009), to be common in poultry processing plants within Europe. While the applied current on line C was close to the recommended (in the UK) current of 105 mA per bird at 50 Hz, AC (Gregory & Wotton 1990b), the calculated average pulsed DC current for birds on line A and B was almost a quarter of that (200 mA per bird) recommended by Raj *et al* (2006a) for an effective stun with a DC frequency of ≤ 200 Hz with 1:1 mark:space or 50% duty cycle, suggesting that those lines had birds that were inadequately stunned or electrically immobilised at worst. From an animal welfare point of view, an average current of 56 mA per bird represents considerable concern, particularly for the female broilers, which have a higher resistance (Rawles *et al* 1995; Prinz 2009) and therefore would receive a substantially lower current than the calculated average current per bird for the multi-bird water-bath stunner.

Higher stunning frequencies also produce a lower incidence of broken bones and breast muscle haemorrhages (Raj *et al* 2001), which are likely to be the drivers behind the frequencies chosen (400–550 Hz) on the processing lines in this study.

Neck cutting and birds missing the neck cutter

A comparison of the three lines showed that there was a variable number of birds that missed the neck cutter after the WBE, and while there are no set times for the severance of the blood vessels after stunning, the WASK (1995) regulation requires bleeding to be complete, rapid and profuse and to be completed before the animals have regained consciousness. In this respect, any delays in the neck-cutting procedure will have a negative impact on bird welfare.

A ventral neck cut has been reported to cause a quicker loss of blood as well as a faster reduction in brain activity (loss of power to less than 10% of the pre-stun EEG in the 13–30 Hz frequency band) by Raj *et al* (2006a), which would suggest a quicker loss of consciousness with a subsequent reduction in reflex activity. These findings were in line with results in the current study, where the birds with a ventral neck cut were found to have a significantly ($P < 0.001$) lower incidence (6.8%) of positive corneal reflexes compared to those birds with a unilateral neck cut (45%) at 13 s post WBE. The above findings indicated a stronger effect of neck cutting over the effect of the DC stunning on the incidence of the corneal reflex, which would generally indicate poor stunning.

Rhythmic breathing

The results of the present study, where none of the stunned birds on both AC and DC lines showed signs of breathing at 4 s post water-bath exit, concur with the previous reports (Raj *et al* 2006a, b) and can be suggested to be a result of the generalised post-stun tetanus rather than lack of consciousness.

On line A and B, where the birds were stunned with a frequency of 550 Hz and with 56 and 80 mA, respectively, pulsed DC, a large proportion (77.4 and 58.0%, respectively) of the birds that had missed the neck cutter showed signs of breathing at 13 s post WBE. This confirms the findings of Prinz *et al* (2010b), who reported that all chickens stunned with 80 mA pulsed DC of greater than 400 Hz, resumed breathing within the first 40 s post stun. The incidence of birds that tested positive for breathing on line C were similar to those reported by Prinz (2009) when assessing breathing after stunning with similar water-bath parameters (100 mA AC with frequency of 400 Hz) to those of line C. They were, however, lower (26.6%) than the 55.5% reported by Raj *et al* (2006b) for AC-stunned birds with 100 mA at a frequency of 400 Hz, which is possibly due to the fact that they were assessed at a later time (20 s) post WBE. While there was no difference in the incidence of breathing at 8 s post WBE between birds on line A and B, at 13 s post WBE the difference became significant with 77.4% of the birds on line A testing positive for breathing as opposed to 58.0% on line B. It is unclear as to what may have caused this disparity, as the stunning current has been reported to have no effect on the time to recovery of rhythmic breathing (Wilkins *et al* 1999). This lack of effect may be caused by the muscle tetanus and apnoea induced by the electric current flowing through the body irrespective of whether or not the birds were well stunned as suggested by Raj *et al* (2006b). The number of birds displaying breathing at 8 s post WBE on line A (5.3%) was found to be significantly higher ($P < 0.001$) than those for the birds on line C (1.7%). Similarly, the difference in the presence of breathing for those birds that missed the knife on lines A and C at 13 s post WBE was significant, whereas birds on line C had significantly lower ($P < 0.01$) incidence of breathing compared to the birds on line A.

Corneal reflex

The electric water-bath stunner did not manage to abolish completely the presence of a corneal reflex with the AC-stunned birds, with 38.4% of the birds testing positive at 4 s post WBE, which indicates an absence of generalised muscle tetanus post stunning on those birds. Furthermore, 33.0% of the birds on that line tested positive at 8 s, and 45.0% at 13 s post WBE. These results were in line with the findings reported by Raj *et al* (2006b) where 44.4% (4 out of 9 birds) of the AC-stunned birds (100 mA and frequency of 400 Hz) were found with a positive corneal reflex at 20 s post WBE. These incidences were, however, higher than those indicated by Prinz *et al* (2010a), who did not find any positive reflexes at ≤ 20 s post WBE, when any combination of current and frequency was used.

The incidence of a positive corneal reflex reduced with time on line B for those birds that had their necks cut (from 30.7 to 5.6%), and increased with time for those birds that missed the knife (from 66.3 to 91.1%), possibly indicating a recovery of consciousness of the birds that missed the knife. In contrast, there were no trends observed in the incidence of a corneal reflex in birds on line C for either those that missed the knife or had their necks cut. This may be attributed to the higher AC currents received on line C, which were closer to the current recommended by Gregory and Wotton (1990b). From a neurological point of view, neck cutting, including spinal cord severance, should not influence the presence of this reflex as it is facilitated by the 5th cranial (afferent) and 7th cranial (efferent) nerves, and indicates brain function at sub-cortical level (Scagliotti 1999). However, the group of birds that missed the knife on line B in this survey had a significantly (P < 0.01 and P < 0.001) higher prevalence of a positive corneal reflex at 8 and 13 s post WBE, respectively, compared to those that had their necks cut. This is most likely caused entirely by the interruption of the blood supply to the brain confirmed by the exaggerated difference at 13 s post WBE between the groups with cut and intact necks, as was suggested by Raj *et al* (2006a). Generally, the effect of time on the incidence of a positive corneal reflex on line C was either not significant or showed a slight increase (between 8 and 13 s), indicating that with time and up to 13 s post WBE, a higher proportion of the birds displayed this reflex. This would fit a normal physiological trend to post-stun recovery (if bleeding does not prevent it) post-electric stunning, as indicated by Wilkins *et al* (1998), and could not be a conclusive indicator of stunning efficiency. Within the birds that had their necks cut on line B, there was a significant reduction in the incidence of a corneal reflex with time. Both of these indicate that the observed differences were in fact caused by neck cutting rather than the time alone.

Head shake

In AC-stunned broilers at a frequency of 400 Hz and a current of 100 mA, Raj *et al* (2006b) reported 3/9 birds (30%) to have tested positive for this reflex. This is similar to the findings of this study where 26.7% of the assessed birds on line C (102 mA AC with 400 Hz) tested positive for head shake at 25–30 s post WBE.

Escape behaviour

Escape behaviour was seen in birds that emerged unstunned, from the electric water bath. Any escape behaviour seen after neck cutting will be influenced inevitably and suppressed by the extent of blood loss and gives an inaccurate indication of the stunning efficiency. While the incidence of escape behaviour can be used as an indicator of poor bird welfare in the processing plant as birds do not reach the live water in the electric water bath, it should not be used as indicators of stun efficiency or post-stun recovery, as not all unstunned birds expressed this behaviour.

Spontaneous eye blinking

This reflex was identified in a number of publications as a indicator of ineffective stunning in broilers stunned with different currents, waveforms and frequencies under laboratory conditions (Raj *et al* 2006a,b; Prinz 2009; Prinz *et al* 2010a). On a moving processing line the window of opportunity to assess a reflex is in the region of 2–3 s and due to the randomness of this reflex as well as the low frequency of birds displaying spontaneous blinking, it was not considered to be a practical method for the assessment of stun efficiency in processing plant conditions.

In general, on lines A and B, the slaughter method had a more pronounced impact than the stunning method, which is in agreement with the finding for pulsed DC by Raj *et al* (2006c), while the current findings on line C concurred with those Raj *et al* (2006b) for AC-stunned birds.

Conclusion

The current survey showed that electric-water-bath parameters used for broiler stunning in the UK vary substantially between different processing lines and do not provide the recommended amount of average current for each bird ensuring effective stunning. Consequently, large proportions of the broilers exhibit a range of behavioural reflexes post stunning, suggesting inadequate stunning. In the processing plants, birds stunned with pulsed DC at low current have higher incidence of behavioural reflexes compared to those birds stunned with AC at a higher current. Birds that had ventral neck cut had a lower incidence of spontaneous and evoked reflexes compared to those broilers that had the unilateral neck cut. While there are back-up slaughtermen ensuring manual neck cut on birds that have missed the mechanical neck cutter, the operation is often performed late after electric WBE and poorly stunned broilers have time to progress towards recovery. Late manual neck cut also reduces the time between neck cut and the scalding tank entry. All those require tighter regulation on the electric-water-bath parameters (current and wave form) used in poultry processing plants, as well as the type of neck cut and the time lapse between water-bath exit and back-up slaughterman to minimise the number of birds recovering post stunning and improve welfare.

In this study, only the assessment of rhythmic breathing, corneal reflex and head shake showed to be suitable for use as indicators of stunning efficiency at certain times, post stun or post neck cut due to the practicality of their assessment. Not all unstunned birds demonstrated escape behaviour, and therefore this indicator could not provide information objectively on stunning efficiency.

Water-bath stunning does not lead to complete abolition of the corneal reflex, but produces complete abolition of the rhythmic breathing within the first 8 s post WBE. Therefore, assessment of rhythmic breathing as criteria of effective stunning can only practically provide meaningful information on stunning efficiency, when assessed after 8 s post WBE.

Assessment by any method within the processing plant, aimed solely at stunning efficiency, should be carried out on stunned birds that have missed the neck cutter, to mitigate the effect of neck cutting on the loss of reflexes. Stunning effectiveness may be assessed by establishing the effect of neck cutting and blood loss through comparison of the incidence of behavioural reflexes (corneal reflex and rhythmic breathing) on birds that missed the neck cutter and those birds that had their necks cut, where a significant reduction in the number of birds with positive reflexes after neck cutting will indicate a large effect of neck cutting itself and therefore poor stunning. This method, however, only shows overall trends and does not take into account the individual bird's consciousness during slaughter and therefore its welfare.

Bird size variability is the main factor causing birds to miss the electric water bath and the neck-cutting equipment, which needs to be addressed by adequate operational procedures in the poultry processing plants.

The general recommendations for further research will arise from the fact that in both laboratory conditions and in practice, the incidence of positive behavioural reflexes post stunning is highly variable in terms of their manifestation with poor correlation between their incidence and an epileptic EEG recording. Possible further studies on the physiology and neuronal activity of the avian brain under electric current application may, with alternative methods and techniques, help to understand the reasons behind inconsistent induction of epileptiform activity and their link to behavioural reflexes.

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