The Old School, Brewhouse Hill, Wheathampstead,
Hertfordshire AL4 8AN, UK

Animal Welfare 2006, 15: 25-30
ISSN 0962-7286

The effects of pulse width of a direct current used in water bath stunning and of slaughter methods on spontaneous electroencephalograms in broilers

ABM Raj*, M O'Callaghan and SI Hughes

School of Clinical Veterinary Science, University of Bristol, Langford BS40 5DU, UK
* Contact for correspondence and requests for reprints: M.Raj@bristol.ac.uk

Abstract

The effect of the pulse width of a direct current (DC) on the effectiveness of electrical water bath stunning, and slaughter, was evaluated in broilers (n = 29). Broilers were individually stunned in a water bath for 1 s with a constant peak current of 400 mA of 200 Hz DC delivered using a variable voltage/constant current stunner. The pulse width of the 200 Hz DC was set at 0.5, 1.5 or 2.5 ms (10, 30 or 50% of 5 ms current cycle). The results showed that pulse width had a significant effect on the incidence of epileptiform activity in the electroencephalograms (EEGs). A pulse width of 10% of the current cycle was less effective than pulse widths of 30 and 50% of the current cycle; there was no significant difference between a pulse width of 30 and 50%. The results of a univariate analysis showed that ventral neck-cutting resulted in a significantly shorter time to the onset of less than 10% of the pre-stun power contents in the 13–30 Hz and 2–30 Hz EEG frequency bands when compared with unilateral neck-cutting. It is concluded that a pulse width of 30 or 50% of the current cycle of 200 Hz DC, delivering 400 mA peak current, was better than using a pulse width of 10% of the current cycle.

Keywords: *animal welfare, chicken, EEG, electrical stunning, pulse width, slaughter*

Introduction

Humane slaughter regulations require that electrical stunning must induce an immediate loss of consciousness and sensibility that lasts until the onset of death via blood loss at slaughter (European Community 1993; Anon 1995). Electrical water bath stunning is the most commonly used stunning method for broilers; however, the electrical waveforms, frequencies and current levels used to stun broilers, and the blood vessels cut at slaughter vary widely (Ingling & Kuenzel 1978; Gregory & Wotton 1986; Bilgili 1992).

The results of our previous study, involving seven sine wave alternating current (AC) frequencies (200, 400, 600, 800, 1000, 1200 and 1400 Hz), and three constant current levels (100, 150 and 200 mA root mean square [RMS]) revealed that effective water bath stunning can be achieved in the majority of broilers with a minimum RMS current of 100, 150 or 200 mA, delivered using electrical frequencies of up to 200, 600 or 800 Hz, respectively (Raj *et al* 2006a, pp 7-18, this issue). The results also indicated that electrical frequencies of above 800 Hz were ineffective at the maximum of 200 mA RMS current tested in that study. The electrical frequencies of 200, 600 and 800 Hz sine wave AC have a period of 5.0, 1.67 and 1.25 ms, respectively; therefore, at the maximum of 200 mA RMS current, a period of 1.25 ms would appear to be the threshold level necessary to achieve effective water bath stunning (ie induce epileptiform activity in the electroencephalogram [EEG] within 1 s of the application of the current) in 90% of broilers. The results also indicated that in effectively stunned broilers only a ventral neck-cutting procedure prevented a return to consciousness and sensibility during bleeding.

The results of another study, which examined the effectiveness of electrical water bath stunning of broilers with a constant average current of 100, 150 or 200 mA delivered using 200, 800 or 1400 Hz pulsed direct current (DC), with mark:space ratio of 1:1, indicated that the probability of achieving effective stunning in a high proportion (80%) of broilers was limited to 200 mA average current (400 mA peak) delivered using 200 Hz (Raj *et al* 2006b, pp 19-24, this issue). Electrical frequencies of 200, 800 and 1400 Hz pulsed DC have a period of 5.0, 1.25 and 0.71 ms respectively; therefore, at the maximum of 200 mA average current, a period of 5 ms would appear to be the threshold level necessary to achieve effective water bath stunning in 80% of broilers. As before, in effectively stunned broilers, a ventral neck-cutting procedure prevented a return to consciousness and sensibility during bleeding.

Together, the results of these studies indicated that water bath electrical stunning of broilers with sine wave AC is more effective than stunning with pulsed DC. This conclusion is based on the fact that both the 200 Hz sine wave AC and the pulsed DC have a period of 5 ms, yet the probability of achieving effective stunning was higher with the sine wave AC (> 90%) than with the pulsed DC (80%). The

https://doi.org/10.1017/S0962728600029900 Published online by Cambridge University Press

Box 1

$$\text{period} = \frac{1000 \text{ ms}}{\text{chosen electrical frequency}}$$

results also imply that the threshold level of the period of an electric current necessary to achieve effective stunning differs between sine wave AC and pulsed DC. The peak voltage and peak current necessary to achieve effective stunning with 200 Hz pulsed DC, with a mark:space ratio of 1:1, was calculated to be 2.3 and 2.8 times, respectively, the voltage and current necessary with 200 Hz sine wave AC. The inefficiency of a pulsed DC is probably because the current flows in a positive direction only, whereas an AC flows gradually in positive and negative directions. In addition, sine wave AC has a relatively slower rate of voltage change and longer excursion distance than a pulsed DC (Ingling & Kuenzel 1978). *In vitro* studies involving brain slices have also revealed that sinusoidal electric fields are more effective than DC fields in affecting neuronal function. In this sense, AC fields are effective regardless of whether the electrical field is parallel or perpendicular to the cell axis, whereas DC fields are only effective when the electrical field is parallel to the neuronal axis (Bawin *et al* 1984; Bawin *et al* 1986). In general, biphasic current waveforms were found to be more effective than anodic or cathodic pulses (McIntyre & Grill 2002).

However, a pulsed DC is commonly used to stun broilers under commercial conditions, and the pulse widths of DC are invariably reduced. It has been suggested that the pulse width, duty cycle or mark:space ratio of a pulsed DC would also determine the effectiveness of stunning, but this has never been investigated using neurophysiological tools or a constant current stunner (see Ingling & Kuenzel 1978). It is thought that one method of testing the effect of pulse width would be to select a peak current of 400 mA (200 mA average), of a 200 Hz pulsed DC that was found to be effective in inducing epileptiform EEGs in a high proportion (80%) of broilers, and deliver it using different pulse widths (Raj *et al* 2006b, pp 19-24, this issue). However, the effect of different neck-cutting methods of broilers stunned with different pulse widths of DC also needs to be investigated, so that a neck-cutting method, which prevents a return to consciousness and sensibility during bleeding, can be recommended.

This study was designed to determine the effect of water bath electrical stunning of broiler chickens with three pulse widths of a 200 Hz pulsed DC, delivering a peak current of 400 mA, and of slaughter using either a unilateral or ventral neck-cutting procedure. The changes occurring in the EEGs were quantitatively analysed using Fast Fourier Transformations (FFT) to determine the depth and duration of unconsciousness and insensibility induced by the stunning, and the efficiency of slaughter procedures in terms of preventing a return to consciousness and sensibility, and inducing rapid death.

Materials and methods

This study was carried out with the approval of an Ethical Review Process and under the Animals (Scientific Procedures) Act 1986. In total, 29 six to seven week old commercial broilers, weighing on average 1.7 ± 0.17 kg (± standard deviation), were sedated with an intramuscular injection of 100 mg of ketamine hydrochloride (Ketaset: Willows Francis Veterinary, Crawley, UK) per bird, and anaesthetised with an intravenous injection of, on average, 20.9 ± 4.72 mg kg^{-1} body weight pentobarbitone sodium (Sagatal: Rhone Merieux, Georgia, USA). The anaesthetised broilers were implanted with EEG recording and somatosensory stimulating electrodes as described by Gregory and Wotton (1989) with modifications (see Raj *et al* 2006a, pp 7-18, this issue). The EEG recording electrodes were switched to an open circuit, using a relay switch, during the application of a stunning current so that the impact of the electrodes on current flow through the brain would be negligible.

After overnight recovery from the surgical procedure (15 h minimum; birds were able to feed, drink and also able to run around the resting pen as observed during catching), the broilers were individually stunned in a water bath stunner for 1 s using a variable voltage/constant current stunner (Sparrey *et al* 1993). The frequency of the stunning current was set by adjusting the period of electric current (see Box 1); the constant current was set using a dummy load of 1033 Ω. The pulse width was set in the stunner using an in-built facility. Individual broilers were hung on shackles and stunned by immersing them into the water bath up to the base of their wings for 1 s with a constant peak current of 400mA delivered using 200Hz pulsed direct DC (unipolar square wave). The period of 200Hz current is 5 ms and the peak current was delivered with three pulse widths, 10, 30 or 50% of the period, which corresponds to 0.5, 1.5 or 2.5 ms.

The stunning voltage and current profiles of each bird were digitally recorded, using Elditest Differential Voltage Probe (RS Electronics, Corby, Northamptonshire, UK) and Fast Response Current Probe (RS Electronics, Corby, Northamptonshire, UK), on to a Vision Data Acquisition System (Gould Nicolet Technologies, Loughton, Essex, UK; ISO9001:2000 compliant). Somatosensory evoked potentials (SEPs) were induced with 1.3 ± 0.24 V. The spontaneous EEG, including SEPs, was recorded for at least 1 min pre-stunning, and for up to 2 min post-stunning. The pre-stun EEGs were used to test that the signals appeared normal and without artefacts. neck-cutting was performed manually, on average 7.9 ± 0.69 s from the end of stunning (Table 1), using either a unilateral or a ventral neck-cutting procedure. The unilateral neck-cutting procedure severed one common carotid artery and one external jugular vein on the right, whereas the ventral neck-cutting procedure severed the two common carotids and two external jugular veins. The number of blood vessels cut during slaughter was verified by post mortem examination by ABM Raj.

https://doi.org/10.1017/S0962728600029900 Published online by Cambridge University Press

Table 1 The number of broilers with epileptiform EEG following stunning and the time to neck-cutting procedures (SD = standard deviation).

Treatment frequency (Hz) (Period = 5 ms)	Treatment current (peak mA)	Pulse width (ms) (% duty cycle)	Total number of birds (n)	Epileptic EEG (n)	Fibrillated at stun (n)	Time to neck-cutting (s) Mean	SD
		0.5 (10%	8	1	1	8.1	0.66
200	400	1.5 (30%)	11	8	5	7.6	0.44
		2.5 (50%)	10	8	6	7.7	0.93

Table 2 Actual electrical frequencies (Hz) and average currents (mA) applied to broilers that were used in quantitative evaluation of EEGs (SD = standard deviation).

Treatment frequency (Hz)	Treatment current (peak mA)	Pulse width (ms) % duty cycle)	Total number of birds (n)	Frequency (Hz) mean	SD	Peak current (mA) mean	SD	Average voltage (V) mean	SD	Average current (mA) mean	SD
200	400	0.5 (10%)	7	200	1.41	384	36.28	58	5.82	41	3.63
		1.5 (30%)	6	200	0.47	391	33.82	177	14.40	119	8.17
		2.5 (50%)	4	200	0.45	403	13.88	275	22.37	198	8.59

The EEG signals were passed through a relay switch, built in this laboratory that protected the pre-amp during the application of high voltages, and were then amplified using a pre-amp (10 000×, 0.5–100 Hz filter band; Gould Bioelectric, Gould Nicolet Technologies, Loughton, Essex, UK). The amplified EEG signals were passed through a noise removal system (Humbug 50/60 noise removal system: Gould Bioelectric, Gould Nicolet Technologies, Loughton, Essex, UK) to eliminate background noise, and then digitally recorded at a sampling rate of 20 000 s^{-1} using a Vision Data Acquisition System (Gould Nicolet Technologies, Loughton, Essex, UK). This extremely high sampling rate was necessary to record more precisely the voltage and current profiles, especially for the high frequency stunning currents. The use of high voltage (620 V peak) for stunning and a relay switch to protect the amplifier lead to the loss of EEG signals; the average time to the return of EEG signal was 5.5 ± 4.46 s, and EEGs without artefacts induced by neck-cutting were obtained, on average, from 7.7 ± 5.8 s post-stun.

Twenty-nine broilers were observed and tested within 5 s of neck-cutting for spontaneous breathing and blinking, a response to touching of the eye with a fingertip (inducing the palpebral reflex), head shaking in response to blood entering the nostrils, a swallowing reflex in response to accumulating blood in the neck wound, and a response to comb pinching using the index finger and thumb. It was thought that the presence of some of these reflexes would indicate an imminent return to, or presence of, consciousness and sensibility in birds, and, therefore, could be used to evaluate or monitor the efficiency of stunning and slaughter under commercial conditions.

The EEG traces of 29 broilers were subjectively evaluated to determine the occurrence or absence of epileptiform activity (Table 1). Manifestation of epileptiform activity in the EEG was considered to be an indicator of effective electrical stunning. Birds that fibrillated at stun were excluded from the FFT analysis (n = 12, Table 1). The digitised EEG signals of the remaining 17 broilers were extracted at a sampling rate of 200 s^{-1}, and subjected to FFT using Impression software (Gould Nicolet Technologies, Loughton, Essex, UK). For each broiler, 60 pre-stun and 120 post-stun epochs, each of approximately 1 s, were separated and used to perform FFT, as described previously (Raj *et al* 2006a, pp 7-18, this issue). The time to the onset of reduction in the power contents of EEG frequency bands to less than 10% of the pre-stun levels in three consecutive epochs were then determined on the basis that: (a) reduction in 13–30 Hz power contents in three consecutive 1 s epochs to less than 10% of the pre-stun level is indicative of unequivocal loss of sensibility; and (b) reduction in 2–30 Hz power contents in three consecutive 1 s epochs to less than 10% of the pre-stun levels is indicative of the onset of isoelectric EEGs (Raj & O'Callaghan 2004a,b). The actual electrical frequencies and average currents used to stun these broilers were determined from the digital records (Impression software: Gould Nicolet Technologies, Loughton, Essex, UK) of voltage and current profiles, and are presented in Table 2.

The frequency of occurrence (or absence) of epileptiform EEGs in broilers following stunning treatments was subjected to statistical evaluation using Fisher's exact tests. The time to the onset of reduction in power content to less than 10% of the pre-stun levels in three consecutive 1 s epochs were subjected to univariate analysis of variance to investigate the effects of the stunning and slaughter treatments. Electrical stunning frequencies and current levels were entered in this analysis as covariates, and slaughter method was entered as a categorical variable.

Results

The number of broilers showing epileptiform activity in the EEG, the interval between the end of stunning and neck-cutting, and the number of broilers having cardiac arrest at

https://doi.org/10.1017/S0962728600029900 Published online by Cambridge University Press

Figure 1

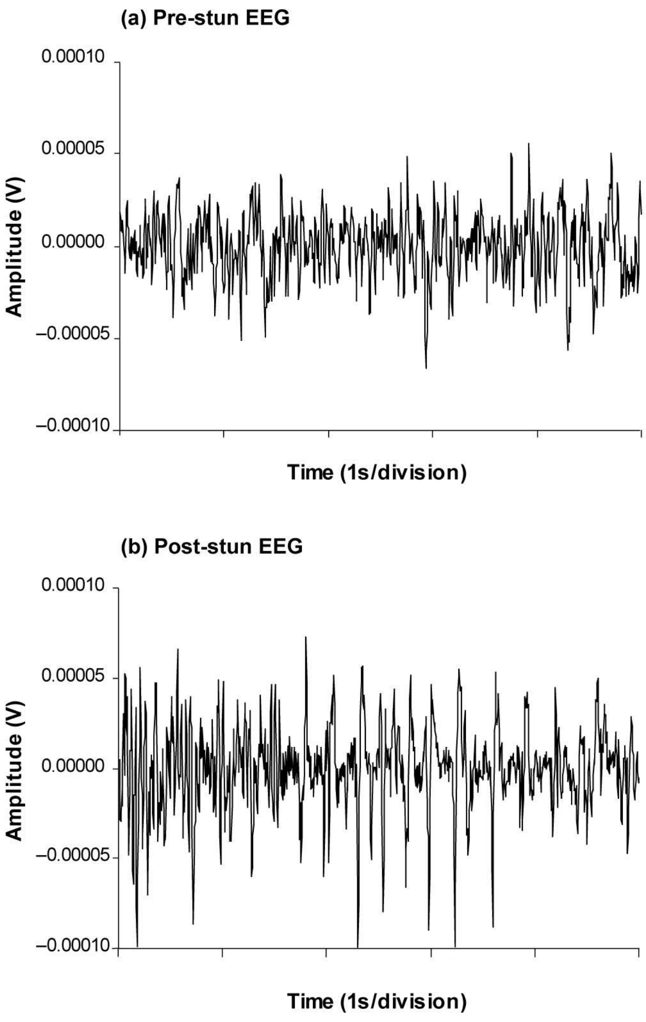


Illustration of epileptiform activity in the EEG (5 s epochs) of a broiler following electrical water bath stunning.

Figure 2

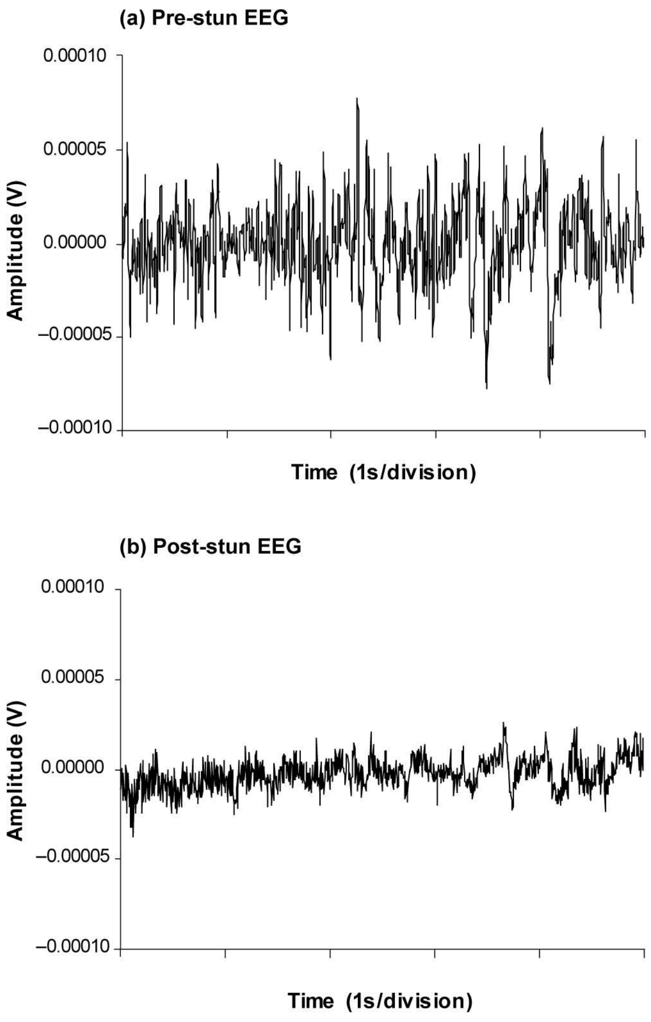


Illustration of the absence of epileptiform activity in the EEG (5 s epochs) of a broiler following electrical water bath stunning.

stunning are presented in Table 1. One out of 8 broilers stunned with 10% pulse width, 5 out of 11 broilers stunned with 30% pulse width, and 6 out of 10 broilers stunned with 50% pulse width suffered cardiac arrest during stunning. One out of 5 broilers stunned with 30% pulse width and 2 out of 6 broilers stunned with 50% pulse width that suffered cardiac arrest had no epileptiform EEGs, which is indicative of effective stunning.

All the broilers used in this study experienced seizures accompanied with apnoea immediately after stunning. The Fisher's exact test on the number of broilers showing epileptiform activity following stunning treatments showed that pulse width had a significant effect on the occurrence of epileptic EEG ($\chi^2 = 9.8$, df = 2, $P < 0.008$). Further analyses using Fisher's exact tests (2 × 2 tables, 2-sided) showed statistically significant differences between the 10 and 30% pulse widths ($\chi^2 = 6.7$, df = 1, $P < 0.02$) and 10 and 50% pulse widths ($\chi^2 = 8.1$, df = 1, $P = 0.015$); there was no significant difference between the 30 and 50% pulse widths.

Examples of EEG traces with and without the manifestation of epileptiform activity following stunning are presented in Figures 1 and 2, respectively.

The univariate analysis showed no statistically significant interactions between the pulse widths and neck-cutting methods tested in this study. It also showed that the pulse widths had no significant effects on the time to the onset of less than 10% of the pre-stun power contents in the 13–30 Hz and 2–30 Hz EEG frequency bands ($F = 0.29$, df = 2, $P = 0.75$; $F = 1.08$, df = 2, $P = 0.37$, respectively). Slaughter method (neck-cutting procedure) had a highly significant effect on the time to loss of power in both of these EEG frequency bands (13–30 Hz: $F = 26.82$, df = 1, $P = 0.001$; 2–30 Hz: $F = 18.48$, df = 1, $P < 0.001$). When a ventral neck-cutting procedure was performed, the average time to the onset of less than 10% of the pre-stun power in the EEG frequency bands was significantly shorter than that with a unilateral neck-cutting procedure (13–30 Hz: mean = 14.5 ± 1.92; 34.2 ± 11.59, respectively; 2–30 Hz: mean = 15.5 ± 2.07; 37.6 ± 14.33, respectively).

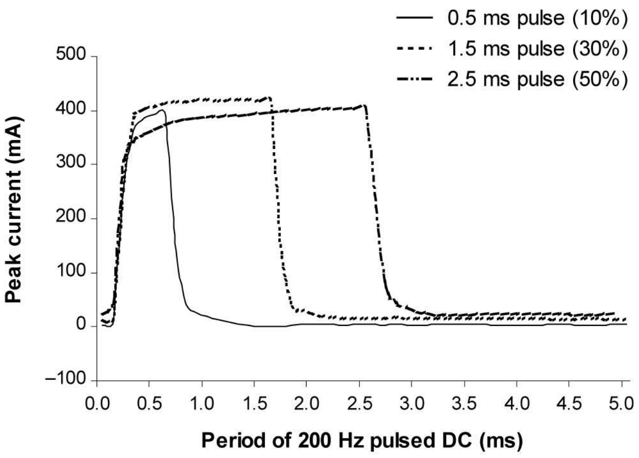
https://doi.org/10.1017/S0962728600029900 Published online by Cambridge University Press

Table 3 The number of broilers with positive reflexes and responses during bleeding. Responses and reflexes were tested within 20 s from the end of stunning (5 s of neck-cutting) for unilateral (U) and ventral (V) neck-cutting procedures.

Treatments pulse width (ms) (duty cycle) and slaughter method	n	Breathing	Blinking	Palpebral reflex present	Swallowing and/or head shaking	Response to comb pinching
0.5 (10%) U	4	3	3	3	3	3
0.5 (10%) V	4	2	2	2	0	0
1.5 (30%) U	6	3	3	3	2	0
1.5 (30%) V	5	0	0	0	0	0
2.5 (50%) U	5	2	2	2	2	2
2.5 (50%) V	5	0	0	0	0	0
Total	29	10	10	10	7	5

Evaluation of the responses and reflexes during bleeding (Table 3) indicated that (a) increasing the pulse width from 10 to 30 or 50% of the current cycle reduced the occurrence of these responses and reflexes being present during bleeding; (b) ventral neck-cutting of broilers stunned with the pulse width of 10% of the current cycle failed to eliminate the responses and reflexes during bleeding; and (c) unilateral neck-cutting of broilers that were stunned with the pulse widths of 30 or 50% of the current cycle resulted in a considerable proportion of birds with positive responses and reflexes, whereas ventral neck-cutting of these broilers eliminated the occurrence of the responses and reflexes during bleeding.

Figure 3



The stunning current profiles of three pulse widths of 200 Hz DC used in this study delivering a peak current of 400 mA.

Discussion

Overall, the results of this study indicated that a pulse width of 200 Hz DC, delivering a constant peak current of 400 mA, had a significant effect on the proportion of broilers showing epileptiform EEGs following stunning. In this regard, pulse widths of 10, 30 and 50% of the current cycle induced epileptiform activity in 13, 73 and 80% of broilers, respectively. The biological significance of this finding is that at 200 Hz electrical frequency, the threshold peak current necessary to induce epileptiform activity increases with decreasing pulse width. It would also mean that at 400 mA peak current a minimum of a 1.5 ms pulse width would be necessary to induce depolarisation in chicken brain neurons (Figure 3). *In vitro* studies involving electrical stimulation of neural tissue to evoke behavioural responses in conscious animals also found a significant effect of pulse width on the probability of evoking a behaviour controlled by the stimulated group(s) of neurones (see review by Tehovnik 1996).

The evaluation of responses and reflexes showed that they were present in a considerable proportion of broilers that were stunned with the pulse width of 10% of the current cycle and slaughtered using a ventral neck-cutting procedure; broilers that were stunned with the pulse width of 30 or 50% of current cycle and slaughtered using a unilateral neck-cutting procedure had equally high incidences of responses and reflexes during bleeding. The bird welfare implications of these results are: (a) a pulse width of 10% of current cycle of 200 Hz DC led to inadequate stunning and even a ventral neck-cutting procedure was unable to prevent a return to consciousness during bleeding; and (b) unilateral neck-cutting of broilers that were stunned with pulse widths of 30 and 50% of the current cycle of 200 Hz DC also resulted in a return to consciousness during bleeding. Furthermore, the induction of cardiac arrest in 20% of birds that did not manifest epileptiform EEG (indicative of effective stunning) following stunning with a pulse width of 30 or 50% current cycle is disturbing; these birds would not have been stunned adequately or, at worst, remained conscious during the induction of cardiac arrest. Owing to this, the use of pulsed DC, irrespective of the pulse width, raises serious bird welfare concerns.

Conclusions and animal welfare implications

It is concluded that using a pulse width of 30 or 50% of the current cycle of 200 Hz DC, delivering a peak current of 400 mA, was better than using a pulse width of 10% of the current cycle to stun broilers. Electrical water bath stunning of broilers with these parameters results in cardiac arrest in the majority of broilers. Owing to the induction of cardiac arrest in conscious broilers (without the epileptiform EEG), the widespread use of pulsed DC, irrespective of its pulse width, raises serious bird welfare concerns.

Acknowledgements

This research project was carried out mainly with funds from the Department of Environment, Food and Rural Affairs (Defra) of the UK (Research Project MH0118: *Neurophysiology of Electrical Stunning Broilers*).

References

Anon 1995 *The Welfare of Animals (Slaughter or Killing) Regulations*. Statutory Instrument 1995 No. 731. Her Majesty's Stationary Office (HMSO): London, UK

Bawin SMA, Sheppard AR, Mahoney MD, Abu-Assal M and Adey WR 1986 Comparison between the effects of extracellular direct and sinusoidal currents on excitability in hippocampal slices. *Brain Research 362*: 350-354

Bawin SMA, Sheppard AR, Mahoney MD and Adey WR 1984 Influences of sinusoidal electric fields on excitability in the rat hippocampal slice. *Brain Research 323*: 227-237

Bilgili SF 1992 Electrical stunning of broilers — basic concepts and carcass quality implications: a review. *Journal of Applied Poultry Science 1*: 135-146

European Community 1993 Directive 93/119/EC on the protection of animals at the time of slaughter or killing. *European Community Official Journal 340*: 21-34

Gregory NG and Wotton SB 1986 Effect of slaughter on the spontaneous and evoked activity of the brain. *British Poultry Science 27*: 195-205

Gregory NG and Wotton SB 1989 Effect of electrical stunning on somatosensory evoked potentials in chickens. *British Veterinary Journal 145*: 159-164

Ingling AL and Kuenzel WJ 1978 Electrical terminology, measurement and units associated with the stunning technique in poultry processing plants. *Poultry Science 57*: 127-133

McIntyre CC and Grill WM 2002 Extracellular stimulation of central neurones: influence of stimulus waveform and frequency on neuronal output. *Journal of Neurophysiology 88*: 1592-1604

Raj ABM and O'Callaghan M 2004a Effect of amount and frequency of head–only stunning currents on the electroencephalogram and somatosensory evoked potentials in broilers. *Animal Welfare Journal 13*: 159-170

Raj ABM and O'Callaghan M 2004b Effects of electrical water bath stunning current frequencies on the spontaneous electroencephalogram and somatosensory evoked potentials in hens. *British Poultry Science Journal 45*: 230-236

Raj ABM, O'Callaghan M and Hughes SI 2006b The effects of amount and frequency of pulsed direct current used in water bath stunning and of slaughter methods on spontaneous electroencephalograms in broilers. *Animal Welfare 15*: 19-24

Raj ABM, O'Callaghan M and Knowles TG 2006a The effects of amount and frequency of alternating current used in water bath stunning and of slaughter methods on electroencephalograms in broilers. *Animal Welfare 15*: 7-18

Sparrey JM, Kettlewell PJ, Paice MER and Whetlor WC 1993 Development of a constant current waterbath stunner for poultry processing. *Journal of Agricultural and Engineering Research 56*: 267-274

Tehovnik EJ 1996 Electrical stimulation of neural tissue to evoke behavioural responses. *Journal of Neuroscience Methods 65*: 1-17