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

Animal Welfare 2006, 15: 19-24
ISSN 0962-7286

The effects of amount and frequency of pulsed 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 effectiveness of electrical water bath stunning of broilers (n = 96) for 1 s with a constant average current of 100, 150 or 200 mA delivered using a variable voltage/constant current stunner with 200, 800 or 1400 Hz pulsed direct current (DC), with a mark:space ratio of 1:1, followed by slaughter using a unilateral or ventral neck cutting procedure, was evaluated. The results of a binary logistic model showed that both the electrical frequency and average current had significant effects on the probability of inducing epileptiform electroencephalogram (EEG) and therefore, of effective stunning. The results of univariate analysis showed that only slaughter method had highly significant effects on the power contents in the 13–30 Hz and 2–30 Hz EEG frequency bands. Based on these results, it is recommended that a minimum of 200 mA average (400 mA peak) current per bird should be delivered using 200 Hz pulsed DC, with a mark:space ratio of 1:1, to achieve effective water bath stunning in 80% of broilers. Frequencies of above 200 Hz pulsed DC would presumably require average currents of greater than 200 mA. Electrical water bath stunning of broilers with 200 mA average current of 200 Hz resulted in cardiac arrest in six out of eight broilers that showed epileptiform activity. Two other broilers that had cardiac arrest showed no epileptiform EEGs indicative of effective stunning. Owing to the prevalence of cardiac arrest in conscious broilers, the use of pulsed DC for water bath stunning of broilers could be questioned on ethical and bird welfare grounds.

Keywords: *animal welfare, chicken, EEG, electrical stunning, frequency, 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). Pulsed direct current (DC) is widely used to stun animals, including poultry. Ingling and Kuenzel (1978) speculated that the effectiveness of stunning with pulsed DC and sine wave alternating current (AC) could be entirely different due to the rapidity of the voltage change and the excursion from the peak voltage; sine wave AC has a relatively slower rate of voltage change and longer excursion distance than pulsed DC. However, the effectiveness of different waveforms of electrical stunning current has not been evaluated in poultry.

The present study was carried out to determine the effect of water bath electrical stunning of broiler chickens with three constant average current levels delivered using three different frequencies of pulsed DC, followed by either a unilateral or ventral neck-cutting procedure. The changes occurring in the electroencephalogram (EEG) 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 the 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, 96 six to seven week old commercial broilers, weighing on average 1.9 ± 0.31 kg (± standard deviation), were sedated with an intramuscular injection of ketamine hydrochloride (100 mg per bird) (Ketaset: Willows Francis Veterinary, Crawley, UK) and anaesthetised with an intravenous injection of, on average, 22.9 ± 4.09 mg kg^{-1} body weight pentobarbitone sodium (Sagatal: Rhône Mérieux, Georgia, USA). The anaesthetised broilers were implanted with EEG recording and somatosensory stimulating electrodes as described previously by Raj *et al* (2006a; pp 7-18, this issue).

After overnight recovery from the surgical procedure (15 h minimum), 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, Equation 1); the constant current was set using a dummy load of 1033 Ω. The duration of stunning was set to 1 s using the built-in timer on the stunner. Individual broilers

https://doi.org/10.1017/S0962728600029894 Published online by Cambridge University Press

Table 1 The number of broilers with epileptiform EEG following stunning with the different treatments used in this study (SD = standard deviation).

Treatment frequency (Hz)	Treatment current (mA)	Number of broilers (n)	Epileptic EEG (n)	Fibrillated at stun (n)	Time to neck cut (s) Mean	SD
200	100	8	3	0	8.2	0.62
	150	5	1	4	8.2	0.58
	200	10	8	5	7.7	0.93
800	100	8	2	0	8.0	0.75
	150	10	2	0	8.6	0.67
	200	9	5	0	8.0	0.82
1400	100	8	0	0	8.2	0.57
	150	8	2	0	8.0	0.18
	200	8	3	0	8.6	0.60

Box 1

Equation 1

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

Equation 2

$$ln\left(\frac{P}{1-P}\right) = -2.646(1.162) + 0.1925(0.00687) \times \text{current} - 0.00128(0.00058) \times \text{frequency}$$

were hung on shackles and stunned by immersing them in the water bath up to the base of their wings. Broilers were stunned for 1 s with a constant average current of 100, 150 or 200 mA, delivered using 200, 800 or 1400 Hz pulsed DC (unipolar square wave). Because the mark:space ratio of the pulsed DC was kept at 1:1 for the three frequencies, the corresponding peak currents would be twice that of the average current, that is 200, 300 and 400 mA respectively. The stunning voltage and current profiles of each bird were digitally recorded using an Elditest Differential Voltage Probe (RS Electronics, Corby, Northamptonshire, UK) and a 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 ± 0.31 V The spontaneous EEG, including SEPs, was recorded for at least 1 min pre-stunning and for up to 2 min post-stunning. Neck-cutting was performed manually on average 8.2 ± 0.70 s from the end of stunning (Table 1), using 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.

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 which also controlled the stun duration, 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 Electric, Gould Nicolet Technologies, Loughton, Essex, UK) to eliminate background noise and were 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 in order to record more precisely the voltage and current profiles, especially for the high frequency stunning currents. The use of a high voltage (620 V peak) for stunning and a relay switch to protect the amplifier led to the temporary loss of EEG signals; the average time to a return of the EEG signal was 3.5 ± 2.32 s.

Seventy-four broilers were observed and tested 5 s after 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.

Of the 96 broilers used in this study, 22 had EEG signals that were either masked by noise or lost; consequently these birds were not included in the subjective analysis of the EEGs. The EEG traces of the remaining 74 broilers were subjectively evaluated to determine the presence or absence of epileptiform activity (Table 1). The appearance of epileptiform activity in the EEG was considered to be an indicator of effective electrical stunning. Additionally, nine broilers suffered cardiac arrest during stunning with 150 mA (n = 4) or 200 mA (n = 5) average current delivered using 200 Hz, as determined from the dilated pupils and relaxed carcass. The digitised EEG signals of 65 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 the FFT, as described previously (Raj *et al* 2006a, pp 7-18, this

https://doi.org/10.1017/S0962728600029894 Published online by Cambridge University Press

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

Treatment frequency (Hz)	Treatment current (mA)	Average frequency (Hz) Mean	SD	Average voltage (V) Mean	SD	Average current (mA) Mean	SD	Number of broilers (n)
200	100	203	0.46	150	25.97	100	1.89	8
	150	203	0.45	230	42.05	153	2.88	4
	200	203	0.05	275	22.37	198	8.59	4
800	100	813	2.07	163	20.18	105	0.89	8
	150	813	2.07	208	24.12	154	3.74	8
	200	813	4.56	294	17.1	205	5.04	9
1400	100	1401	0.64	142	21.36	105	1.58	8
	150	1401	2.85	225	44.03	145	4.96	8
	200	1401	9.87	266	24.69	205	9.87	8

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. A reduction in the 13–30 Hz power contents in three consecutive 1 s epochs to less than 10% of the pre-stun level was considered to be indicative of unequivocal loss of sensibility. A reduction in the 2–30 Hz power contents in three consecutive 1 s epochs to less than 10% of the pre-stun level is indicative of 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) 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 a binary logistic model (eg Hosmer & Lemeshow 2000), in which current level and frequency were treated as continuous variables. The time to the onset of reduction in power contents 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 are presented in Table 1. All the broilers used in this study experienced seizures accompanied with apnoea immediately after stunning. A binary logistic regression of broilers showing epileptiform activity, or not, following stunning treatments showed that both the electrical stunning frequency (Hz) and average current (mA) had significant effects on the probability of epileptic EEG manifestation (*Wald* = 4.8, df = 1, $P < 0.05$; *Wald* = 7.8, df = 1, $P < 0.01$, respectively). The model (see Box 1, Equation 2) correctly classified 74% of birds. The model predicted 88% of non-epileptiform and 50% of epileptiform birds correctly; this was probably attributable to the fact that stunning broilers with 100 or 150 mA average current with 800 or 1400 Hz respectively, was below the threshold current necessary to induce epileptiform activity in a considerable proportion of broilers. In the model, *ln* is the natural logarithm and '*P*' is the probability of epileptiform EEG; the standard error of the parameter estimates are shown within brackets.

The probability of the occurrence of epileptiform EEG decreased as frequency increased at each of the average current levels tested (Table 1). The probability of inducing epileptiform activity, and therefore of effective stunning, in a high proportion (80%) of broilers was limited to 200 mA average current delivered using 200 Hz pulsed DC. It is likely that electrical frequencies of above 200 Hz pulsed DC would have required average currents of greater than 200 mA to induce epileptiform activity in the EEGs of a similar proportion of broilers.

The univariate analysis showed no statistically significant interactions between any of the variables tested in this study. It also showed that neither the frequency of current nor the average current had a statistically significant effect on the time to onset of less than 10% of the pre-stun power content in the 13–30 Hz frequency band ($F = 1.63$, df = 2, $P = 0.21$; $F = 0.63$, df = 2, $P = 0.54$, respectively). However, slaughter method had a highly significant effect on the time to loss of power in this frequency band ($F = 101.04$, df = 1, $P < 0.001$). When the ventral neck-cutting procedure was performed, the average time to the onset of less than 10% of the pre-stun power in the 13–30 Hz EEG frequency band was significantly shorter than that required with the unilateral neck-cutting procedure (mean = 20.4 ± 11.96 s; 48.7 ± 21.90 s, respectively; Figure 1).

A similar analysis of variance of the time to reach less than 10% of the pre-stun total power content (2–30 Hz) showed that only slaughter method had a significant effect ($F = 104.68$, df = 1, $P < 0.001$). When ventral neck-cutting was performed, the average time to the onset of less than 10% of the pre-stun power in 2–30 Hz EEG frequency band was significantly shorter than that required with unilateral neck-cutting (mean = 21.5 ± 12.73 s; 53.2 ± 23.24 s, respectively; Figure 1).

Table 3 The number of broilers with positive reflexes and responses during bleeding; responses and reflexes were tested within 20 s of the end of stunning and 5 s of neck-cutting.

Frequency (Hz)	Current (mA)	Number of broilers (n)	Breathing	Blinking	Palpebral reflex present	Swallowing and/or head shaking	Response to comb pinch
200	100	8	4	5	5	4	2
	150	5	3	3	3	3	2
	200	10	2	2	2	2	2
800	100	8	4	4	4	4	2
	150	10	6	6	6	6	4
	200	9	5	5	6	5	3
1400	100	8	4	4	4	4	1
	150	8	4	4	4	4	4
	200	8	4	4	4	4	2
Unilateral neck-cut	–	39	35	35	35	35	22
Ventral neck-cut	–	35	1	2	2	1	0
Total	–	74	36	37	37	36	22

Evaluation of the responses and reflexes during bleeding (Table 3) indicated that: (a) increasing the frequency of electrical stunning from 200 to 800 Hz almost doubled the number of birds with positive responses and reflexes and, in comparison with the 800 Hz, the incidence was very similar in broilers stunned with 1400 Hz; (b) increasing the amount of average current had no significant effect on the incidence of birds with positive responses and reflexes; and (c) unilateral neck-cutting resulted in the majority of birds with positive responses and reflexes, whereas ventral neck-cutting substantially reduced this incidence. Evidently, regardless of the effectiveness of electrical stunning frequencies and current levels, ventral neck-cutting overwhelmingly reduced the incidence of responses and reflexes during bleeding in comparison with unilateral neck-cutting.

Discussion

Although the accuracy of prediction by the statistical model of epileptiform EEG was low, the results of this study indicated that, at the current levels tested, electrical water bath stunning of broilers with 200 Hz pulsed DC is more effective than electrical stunning at higher frequencies. At 200 Hz an average current of 200 mA is required to induce epileptiform EEGs in a high proportion (80%) of broilers. Electrical water bath stunning of broilers with higher frequencies of a pulsed DC would require average currents of greater than 200 mA. In previous studies (Raj & O'Callaghan 2004a,b), we proposed that the effect of a stunning current depends upon the period (see Box 1, Equation 1) of electric current used to stun the chickens; the results of this present study also support this hypothesis. Furthermore, the relationship between the amount and frequency of current found in this study is in agreement with our previous reports involving constant current electrical water bath stunning of broilers with sine wave AC (Raj *et al* 2006a, pp 7-18, this issue).

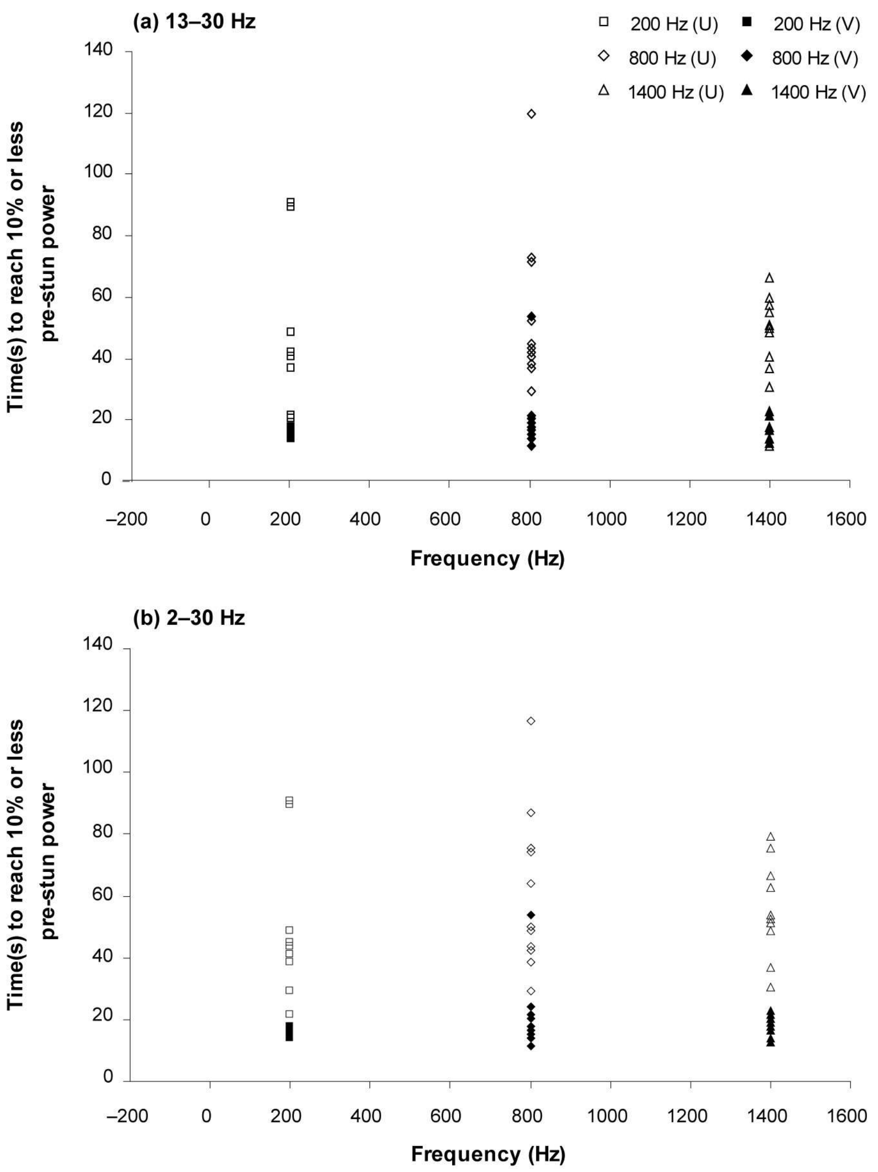
The results of the present study and those of our previous study (Raj *et al* 2006a, pp 7-18, this issue), both involving electrical water bath stunning of broilers with constant currents, indicate that the waveform of current determines the effectiveness of electrical water bath stunning in chickens. In this regard, sine wave AC appears to be more effective than pulsed DC. For example, while using a 200 Hz sine wave AC, a constant RMS current of 100 mA (peak = 100 × 1.414 = 141 mA; see Ingling & Kuenzel 1978 for calculation) was found to be adequate to induce epileptiform EEGs in the majority (90%) of broilers and, subsequently, a sustained period of quiescent EEGs (reduced power contents in the EEG frequency bands to less than 10% of the pre-stun levels) (Raj *et al* 2006a, pp 7-18, this issue). The results of the present study showed that, while using a 200 Hz pulsed DC with a mark:space ratio of 1:1, an average current of 200 mA (400 mA peak) would be necessary to induce epilepsy in a similar proportion (80%) of broilers. This suggests that at 200 Hz, current delivered with a sine wave AC is more effective at disrupting the membrane potentials of neurones and inducing epilepsy in the chicken brain than pulsed DC. In addition, while using a variable voltage/constant current stunner, the RMS voltage required to deliver a RMS current of 100 mA was found to be 168 V; in the present study, the average voltage required to deliver an average current of 200 mA was 275 V. The peak voltages necessary to deliver these current levels were calculated to be 238 V AC (168 RMS V × 1.414) and 550 V DC (275 average V × 2) respectively (see Ingling & Kuenzel 1978 for details). Evidently, the peak voltage and current necessary to achieve effective stunning with 200 Hz pulsed DC is 2.3 and 2.8 times, respectively, the voltage and current necessary with 200 Hz sine wave AC.

Bilgili (1992), while testing constant voltages with fixed electrical resistors, found that the voltage necessary to deliver a fixed amount of current at a given resistance was higher with a pulsed DC than with sine wave AC; it also increased with increasing electrical frequency. The inefficiency of a pulsed DC inducing epileptiform activity in chicken brains could be attributed to the fact that the current only flows in a positive direction, whereas the AC flows in both positive and negative directions.

https://doi.org/10.1017/S0962728600029894 Published online by Cambridge University Press

Figure 1

The effect of stunning frequency and slaughter methods on the time to onset of less than 10% of the pre-stun power contents in the two EEG frequency bands, **(a)** 13–30 Hz and **(b)** 2–30 Hz, for unilateral (U) and ventral (V) neck-cutting procedures.



The scientific literature concerning extracellular microstimulation of central neurones revealed that: (a) symmetrical biphasic stimuli activated fibres of passage, axon terminals and local cells around the electrode at similar thresholds; (b) a predominantly anodic (positive) pulse selectively activated local cells; (c) a predominantly cathodic (negative) pulse selectively activated fibres of passage; (d) in all the waveforms the threshold for activation of axon terminals was lower than the threshold for direct activation of cells; and (e) the output from stimulated cells, which affected other cells, was dependent on stimulation frequency (McIntyre & Grill 2002). This is also why in direct brain cell stimulation studies electrical fields perpendicular to the cell laminae are believed to be much more effective than those in parallel axes. This knowledge and understanding facilitated the deployment of selective stimulation of the central nervous system in the treatment of brain disorders in humans; however, it has yet to be transferred to electrical stunning situations.

The present study and others in this series (Raj *et al* 2006a, pp 7-18, this issue; Raj *et al* 2006b, pp 25-30, this issue) provide some insight into the efficacy of electrical stunning waveforms and frequencies in terms of inducing epileptiform activity indicative of immediate loss of consciousness in chickens and further studies are required in mammals.

Unfortunately, owing to the safety limit set in the constant current stunner used, it was not possible in this study to determine the minimum currents necessary to achieve effective stunning with 800 and 1400 Hz pulsed DC; however, the results showed that these frequencies failed to induce epileptiform activity in high proportion of broilers at 400 mA peak current. The results of this study also indicated that water bath stunning with 200 Hz pulsed DC induced cardiac arrest in five out of five, and six out of ten broilers that were stunned with average currents of 150 mA and 200 mA, respectively. Kuenzel and Wathers (1978) reported that stunning broilers with DC significantly decreased heart rate when compared with AC. Our previous study (Raj *et al* 2006a, pp 7-18, this issue) showed that a RMS current of 200 mA, delivered using 200 Hz sine wave AC, did not produce cardiac arrest. It is very likely that the high voltages necessary to deliver average currents of 150 and 200 mA with 200 Hz pulsed DC were detrimental to cardiac function (Table 2). However, in the present study, none of the broilers that suffered cardiac arrest following stunning with 150 mA average current, and only two out of the six broilers that suffered cardiac arrest following stunning with 200 mA average current, had epileptiform EEGs indicative of effective stunning. Owing to the induction of cardiac arrest in conscious broilers, the use of pulsed DC for water bath stunning of broilers could be questioned on ethical and bird welfare grounds.

Conclusions and animal welfare implications

A minimum of 200 mA average (400 mA peak) current per bird should be delivered using 200 Hz pulsed DC with a mark:space ratio of 1:1 to achieve effective water bath stunning in 80% of broilers. Frequencies of above 200 Hz pulsed DC would presumably require average currents of greater than 200 mA. In effectively stunned broilers, a ventral neck-cutting procedure, which severs the two common carotid arteries and two external jugular veins, would avoid a return to consciousness and sensibility during bleeding. However, electrical water bath stunning of broilers with these current parameters resulted in cardiac arrest in some conscious broilers, which would not be acceptable on either ethical or animal welfare grounds; therefore, the use of pulsed DC for water bath stunning should be actively discouraged.

Acknowledgements

This research project was carried out 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

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

Hosmer DW and Lemeshow S 2000 *Applied Logistic Regression*, 2nd Edition. John Wiley & Sons: Chichester, UK

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

Kuenzel WJ and Wathers JH 1978 Heart rate, blood pressure, respiration, and brain waves of broilers as affected by electrical stunning and bleed out. *Poultry Science 57*: 655-659

McIntyre CC and Grill WM 2002 Extracellular stimulation of central neurons: 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 stunning currents on the electroencephalogram and somatosensory evoked potentials in broilers. *Animal Welfare 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 pulse width of a direct current used in water bath stunning and of slaughter methods on spontaneous electroencephalograms in broilers. *Animal Welfare 15*: 25-30

Raj ABM, O'Callaghan M and Knowles T G 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

https://doi.org/10.1017/S0962728600029894 Published online by Cambridge University Press