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

Animal Welfare 2006, 15: 7-18
ISSN 0962-7286

The effects of amount and frequency of alternating current used in water bath stunning and of slaughter methods on electroencephalograms in broilers

ABM Raj*, M O'Callaghan and TG Knowles

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 172 individual broilers for 1 s with a constant root mean square (RMS) current of 100, 150 or 200 mA, delivered using a variable voltage/constant current stunner with 200, 400, 600, 800, 1000, 1200 or 1400 Hz sine wave alternating current (AC), followed by slaughter using a unilateral or ventral neck-cutting procedure, was evaluated. A binary logistic regression of broilers showing epileptiform activity or not following stunning showed that both the electrical frequency (Hz) and RMS current (mA) had a significant effect on the probability of the electroencephalogram (EEG) manifestation. The univariate analysis of variance showed that the time to the onset of less than 10% of the pre-stun relative power contents was significantly affected only by the interaction between electrical frequency and slaughter method. A similar analysis of variance of the time to reach less than 10% of the pre-stun total power content showed slaughter method, RMS current, the slaughter method/frequency interaction and the RMS current/frequency interaction to be either significant or approaching significance. Based on these results it is recommended that effective water bath stunning of broilers with a minimum constant current of 100, 150 and 200 mA could be achieved with electrical frequencies of up to 200, 600 and 800 Hz, respectively. In addition, It is likely that electrical frequencies of above 800 Hz would have required a minimum current of greater than 200 mA to induce epileptiform activity in the EEGs of broilers.

Keywords: *animal welfare, chicken, EEG, electrical stunning, frequency, slaughter*

Introduction

Humane slaughter regulations stipulate that electrical stunning must induce an immediate loss of consciousness and sensibility that lasts until death supervenes through slaughter (European Community 1993; Anon 1995). The waveform and frequency of the electric current used, the amount of current applied to individual chickens, the number of chickens in the water bath stunner at any one time, and the number of blood vessels severed in the neck at the time of slaughter, vary widely under commercial processing conditions. The duration of stunning also varies according to the length of the water bath used. The choice of these variables appears to be a matter of convenience, economics or ergonomics of poultry processing rather than on the basis of sound scientific principles.

The series of experiments presented here (this paper; Raj *et al* 2006a, pp 19-24, this issue; Raj *et al* 2006b, pp 25-30, this issue) were designed to ascertain, under laboratory conditions, the effect of some of these variables, such that the results could be used to rationalise stunning and slaughter parameters and protect bird welfare. The evaluation of electroencephalograms (EEGs), rather than the induction of seizures, helped to determine the effects of electrical stunning and slaughter parameters (Raj 2003). In the past, two EEG criteria have been used to determine effective electrical stunning: the occurrence of an epileptiform EEG and the abolition of somatosensory evoked potentials (SEPs) in the brain (see Schuett-Abraham 1999). Previous investigations have shown that the SEPs are abolished when the electrical stunning induced epileptiform activity leads to a quiescent EEG, that is, a reduction in the total power in the 2–30 Hz EEG frequency band to 10% or below the pre-stun levels (Raj & O'Callaghan 2004a,b). Therefore, the occurrences of epileptiform EEG followed by a reduction in the EEG total power content to 10% or below the pre-stun levels are considered to be reliable indicators of unconsciousness and insensibility under electrical stunning and slaughter conditions.

The results of a previous investigation, involving constant current head-only electrical stunning of broilers with three root mean square (RMS) current levels (mA) and three frequencies of a sine wave alternating current (AC), showed that the depth and duration of unconsciousness in broiler chickens was determined by the amount and frequency of the stunning current (Raj & O'Callaghan 2004a). In that study, minimum currents of 100, 150 and 200 mA were recommended, while using 50, 400 and 1500 Hz respectively, to achieve effective electrical stunning of broilers. In another study, the effectiveness of water bath electrical stunning of layer hens with a constant RMS current of 100 mA per bird delivered for 3 s using 100, 200, 400, 800 or 1500 Hz sine

Box 1

Equation 1

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

Equation 2

$$\frac{\text{post-stun power}}{\text{pre-stun power}} \times 100$$

Equation 3

$$ln\left(\frac{P}{1-P}\right) = -0.039(0.769) + 0.022(0.005) \times \text{current} -0.004(0.001) \times \text{frequency}$$

Equation 4

$$ln\left(\frac{P}{1-P}\right) = -0.688(0.886) + 0.025(0.006) \times \text{current} -0.005(0.001) \times \text{frequency}$$

wave AC was investigated (Raj & O'Callaghan 2004b). The results of this study suggested that water bath electrical stunning of chickens with a RMS current of 100 mA per bird delivered using 100 or 200 Hz was adequate, whereas a RMS current of 150 or 200 mA would be required while using 400 or 1500 Hz sine wave AC respectively, to ensure bird welfare at slaughter, provided that the two common carotid arteries and two external jugular veins in the neck were severed at slaughter. However, the cumulative effect of electrical frequency, amount of current and neck-cutting procedures has not been studied in detail. In this regard, the neck-cutting procedure (blood vessels cut) could be critical to achieving humane death within the bleed-out time, which is approximately 90 s under commercial broiler processing conditions. Otherwise, birds could regain consciousness during bleeding and/or enter the scald tanks alive.

This study determined the interactions between three constant RMS currents, seven sine wave AC frequencies and two neck-cutting procedures, on the efficacy of stunning and slaughter of broilers.

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, 172 six to seven week old commercial broilers, weighing on average 1.9 ± 0.27 kg (± standard deviation), were sedated with an intramuscular injection of 100 mg of ketamine hydrochloride (Ketaset: Willows Francis Veterinary, Crawley, UK) and anaesthetised with an intravenous injection of, on average, 25.3 ± 6.94 mg kg^{-1} body weight of 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 the following modifications. The EEG recording electrodes (1 mm diameter each) were implanted on the dura via holes drilled in the skull. The negative electrode was implanted on the right hemisphere, 9 mm rostrally to the bregma (the junction of the sagittal and coronal sutures) and 2 mm laterally to the sagittal suture. The positive electrode was implanted on the right hemisphere, 4 mm laterally to the bregma and 2 mm rostrally to the coronal suture. A common electrode was implanted on the left hemisphere, equidistant from the positive and negative electrodes, and an earth electrode was implanted 2 mm laterally to the common electrode. The somatosensory stimulating electrodes were implanted in the left wing: the negative electrode was looped around the medianoulnar nerve at the distal end of humerus and the positive electrode was looped around the median nerve 10 mm distally to the negative electrode.

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 were hung on shackles and stunned by immersing them in the water bath up to the base of their wings. A constant RMS current of 100, 150 or 200 mA was delivered for 1 s using 200, 400, 600, 800, 1000, 1200 or 1400 Hz sine wave AC. The birds were then slaughtered manually, on average 9.0 ± 2.13 s after the end of stunning (Table 1), using a unilateral or ventral neck-cutting procedure. The unilateral neck-cutting procedure severed the right common carotid artery and external jugular vein, whereas the ventral neck-cutting procedure severed the common carotid arteries and jugular veins on both sides of the neck. The blood vessels severed at slaughter were verified by post mortem examination by ABM Raj.

The stunning voltage and current profiles of each bird were digitally recorded, using an 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 1.4 ± 0.37 V. The spontaneous EEG, including SEPs, for each broiler was digitally recorded for 2 min pre-stunning and for up to 2 min post-stunning.

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 Electric, 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

https://doi.org/10.1017/S0962728600029882 Published online by Cambridge University Press

Table 1 The number of broilers with epileptiform EEGs following stunning with the different combinations of currents and frequencies, and time to neck-cutting procedures (parentheses indicate the number of broilers in which the EEG manifestations occurring after stunning were unclear because of artefacts or the delayed return of signals). (SD = standard deviation.)

Treatment frequency (Hz)	Treatment current (RMS) (mA)	n	Epileptiform EEG	Time to neck-cutting (s) Mean	SD
200	100	9	8 (1)	10.2	3.03
	150	7	7	8.6	1.10
	200	8	7 (1)	9.5	3.67
400	100	9	6 (3)	8.9	1.11
	150	8	6 (1)	10.1	2.14
	200	8	7 (1)	8.1	1.59
600	100	9	3	10.0	3.42
	150	7	6	9.7	1.94
	200	8	7	8.7	0.96
800	100	6	0	8.6	1.00
	150	9	4 (1)	8.5	0.91
	200	8	7	9.0	2.50
1000	100	8	2	8.4	1.02
	150	8	0 (1)	8.8	1.39
	200	8	5 (1)	9.4	3.42
1200	100	11	1 (1)	8.2	1.24
	150	8	1 (1)	9.2	1.82
	200	8	3	8.6	2.24
1400	100	9	1 (1)	9.5	2.85
	150	8	0	9.4	1.44
	200	8	2	8.7	2.08

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 relay switch, to protect the amplifier, and the use of a high voltage (620 V peak) for stunning led to a loss of EEG signal for a brief period immediately following stunning; the average time to the return of the EEG signal was 3.8 ± 3.11 s post-stunning. Furthermore, manual neck-cutting resulted in a brief loss of signal in some birds because of artefacts; in these birds an uninterrupted EEG was obtained on average 6.0 ± 5.62 s after the end of the stun.

Normally, conscious birds demonstrate protective reflexes and responses to certain stimuli and, as with most anaesthetics, stunning and slaughter treatments would be expected to either suppress or abolish them. Therefore, 172 broilers were observed and tested 5 s after neck-cutting (within 20 s post-stunning) for spontaneous breathing and blinking, 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 the presence of, consciousness and sensibility in birds and could therefore be used to evaluate or monitor the efficacy of stunning and slaughter under commercial conditions.

The EEG traces of the 172 broilers were subjectively evaluated to determine the presence or absence of epileptiform activity (Table 1). The occurrence of high amplitude spikes (of 3–8 Hz) in the EEG was regarded as epileptiform activity and was considered to be an indicator of effective electrical stunning. Owing to the delayed return of unambiguous EEG signals, it was not possible to determine this unequivocally in 13 out of the 172 broilers; these were distributed across the electrical stunning treatments. The changes occurring in the EEG were quantitatively analysed off-line using Fast Fourier Transformations (FFT) to determine the depth and duration of unconsciousness and insensibility induced with the electrical stunning parameters, the efficiency of slaughter procedures in terms of preventing a return to consciousness and sensibility, and inducing rapid death in effectively stunned chickens.

The digitised EEG signals of 158 broilers (EEG signals of 14 broilers were lost due to electrical or electronic failures) 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. The FFT was performed on epochs using seamless Hanning window and amplitude correction, and the energy spectra (V^2) were derived as power contents on each epoch. During the FFT, six pre-stun epochs were averaged to obtain a single pre-stun energy spectrum for each broiler. Energy spectrums were 'evaluated' using a built-in option in the software and the energy spectra of two EEG frequency bands, 2–30 Hz and

https://doi.org/10.1017/S0962728600029882 Published online by Cambridge University Press

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

Treatment frequency (Hz)	Treatment current (RMS) (mA)	Actual frequency (Hz)		Actual voltage (RMS) (V)		Actual current (RMS) (mA)		n
		Mean	SD	Mean	SD	Mean	SD	
200	100	207.8	6.11	167.5	11.64	101.0	2.45	6
	150	202.7	1.86	213.7	30.00	151.7	2.58	6
	200	204.4	2.93	257.1	36.59	199.1	2.42	8
400	100	401.9	1.81	167.5	17.74	99.0	2.33	8
	150	403.3	2.06	206.0	28.90	150.3	2.69	7
	200	403.6	4.00	279.1	27.69	198.4	4.57	8
600	100	604.9	2.80	144.4	13.10	99.0	1.63	7
	150	605.0	3.96	230.3	43.03	148.0	2.04	7
	200	602.1	1.89	285.3	37.50	198.8	6.41	8
800	100	802.5	1.52	157.8	23.65	97.0	2.10	6
	150	804.0	3.16	209.0	52.37	148.0	1.92	7
	200	805.6	2.64	276.9	10.48	199.6	5.88	7
1000	100	1004.6	3.10	142.1	14.84	96.6	1.27	7
	150	1003.7	3.93	226.8	35.12	145.8	3.06	6
	200	1004.6	3.57	272.6	31.24	198.8	4.33	8
1200	100	1204.5	4.28	144.1	19.68	96.5	3.54	10
	150	1210.6	8.70	213.6	18.88	143.0	8.10	7
	200	1200.2	6.52	266.0	24.23	195.2	6.04	8
1400	100	1403.8	2.05	134.1	24.14	96.0	1.87	9
	150	1401.5	1.93	214.3	36.10	143.6	5.73	8
	200	1402.7	2.23	274.9	48.28	193.6	5.68	8

13–30 Hz, were extracted. The power contents in these EEG frequency bands were calculated as the area under the curve using the Impression software (Gould Nicolet Technologies, Loughton, Essex, UK). The power contents were then exported to a spreadsheet (Microsoft Excel) in which the relative changes occurring during the post-stun period were calculated for individual birds (see Box 1, Equation 2). The times to the onset of reduction in the power contents of the 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 the 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 the 2–30 Hz power contents in three consecutive 1 s epochs to less than 10% of the pre-stun level is indicative of the onset of isoelectric EEGs (Raj & O'Callaghan 2004a,b). The actual electrical frequencies and RMS 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. Thirteen broilers out of 172, in which the EEG manifestation occurring immediately following stunning was unclear, were entered as 'non-epileptiform EEG', giving the benefit of doubt in favour of improved bird welfare. Analyses carried out including (n = 172) and excluding (n = 159) these birds resulted in similar models. 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, and the interval between the end of stunning and neck-cutting, are presented in Table 1. All the broilers experienced seizures accompanied with apnoea immediately after stunning. A binary logistic regression of broilers showing epileptiform activity, or not, following stunning treatments revealed that both the electrical stunning frequency (Hz) and RMS current (mA) had a statistically significant effect on the probability of the EEG manifestation (*Wald* = 42.4, df = 1, $P < 0.001$; *Wald* = 16.4, df = 1, $P < 0.001$, respectively), the model (see Box 1, Equation 3) correctly classifying 82% of birds. 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.

Exclusion from the analysis of the thirteen broilers, in which the EEG manifestations occurring immediately after the stun were unclear, did not dramatically alter the relationship of the RMS current and frequency as determinants of the epileptiform EEG (see Box 1, Equation 4).

https://doi.org/10.1017/S0962728600029882 Published online by Cambridge University Press

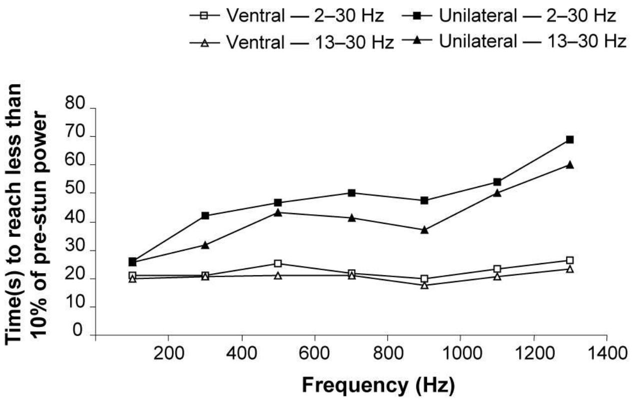
The probability of an epileptiform EEG occurring following stunning decreased as frequency was increased at each of the RMS current levels tested. Table 1 shows the proportion of birds manifesting epileptiform EEG at each combination of frequency and current tested in this study. The probability of inducing epileptiform activity, and therefore of effective stunning, with a given current was limited to a range of electrical frequencies.

The univariate analysis of variance showed that the time to the onset of less than 10% of the pre-stun power content in the 13–30 Hz band was not significantly affected by the amount of RMS current ($F = 2.60$, df = 1, $P = 0.11$). There was no significant interaction between the RMS current and slaughter method ($F = 2.24$, df = 1, $P = 0.14$), or between the RMS current and electrical frequency ($F = 1.11$, df = 1, $P = 0.29$). However, there was a significant interaction between the electrical frequency and slaughter method ($F = 9.17$, df = 1, $P < 0.003$). 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 13–30 Hz band did not vary significantly between the electrical frequencies (Figure 1). By contrast, when a unilateral neck-cutting procedure was performed, the time to the onset of less than 10% of the pre-stun power in the 13–30 Hz band increased almost linearly as the electrical stunning frequency was increased (Figure 1).

The interaction between electrical stunning frequency and slaughter method (Figure 2) becomes clear in the trends observed in the time to the onset of less than 10% of the pre-stun power in the 13–30 Hz EEG frequency band: (a) it was similar in broilers that had unilateral or ventral neck-cutting following stunning with 200 or 400 Hz; (b) in broilers stunned with 600, 800, 1000 or 1200 Hz ventral neck-cutting led to a rapid loss of power as seen in birds stunned with 200 or 400 Hz, whereas unilateral neck-cutting took considerably longer; and (c) broilers stunned with 1400 Hz took the longest time to reach 10% or less of the pre-stun power in the 13–30 Hz EEG frequency band, even after ventral neck-cutting. In addition, the incidence of epileptiform EEG could have contributed to this effect as there were distinct differences in the time to the onset of 10% or less of the pre-stun power in the 13–30 Hz EEG frequency band between the broilers with epileptiform EEG following stunning and those without. When a ventral neck-cutting procedure was performed, the average time to reach 10% or less of the pre-stun power in the 13–30 Hz EEG frequency band was very similar in broilers with epileptiform EEG (mean = 21.0 ± 4.67 s) to those without epileptiform EEG (mean = 20.4 ± 7.32 s). By contrast, when a unilateral neck-cutting was performed, the average time to reach 10% or less of the pre-stun power in this EEG frequency band was shorter in broilers with the epileptiform EEG (mean = 38.6 ± 23.40 s) than those without epileptiform EEG (mean = 50.5 ± 22.69 s).

A similar analysis of variance of the time to reach less than 10% of the pre-stun total power content showed slaughter method ($F = 4.06$, df = 1, $P < 0.046$), RMS current ($F = 6.0$, df = 1, $P < 0.015$), the slaughter method/frequency interaction ($F = 8.55$, df = 1, $P < 0.004$) and the RMS current/frequency interaction ($F = 3.62$, df = 1, $P < 0.059$) to be statistically significant, or approaching significance (at $P = 0.05$). When a unilateral neck-cutting procedure was performed, the average time to the onset of less than 10% of the pre-stun total power was 50.4 ± 25.60 s; this was reduced to 22.7 ± 6.68 s by using a ventral neck-cutting procedure. This effect was unlikely to be due to the small differences in the time interval between the end of stunning and the neck-cutting procedure (mean = 8.9 ± 2.12 s; 9.1 ± 2.17 s, for unilateral and ventral neck-cutting procedures respectively). The average time to the onset of less than 10% of the pre-stun total power was similar in broilers that were stunned with 100 or 150 mA (mean = 38.1 ± 26.3 s; 38.7 ± 22.3 s, respectively) and these times were longer than was recorded for broilers stunned with 200 mA (mean = 33.2 ± 21.0 s). The average time to the onset of less than 10% of the pre-stun total power following a ventral neck-cutting procedure did not differ significantly with electrical stunning frequency, ranging from 19.8 s to 26.6 s, but increased almost linearly with an increase in electrical stunning frequency following a unilateral neck-cutting procedure (Figure 1).

Figure 1



The effect of the interaction between electrical stunning current frequencies (Hz) and slaughter methods on the average time to the onset of less than 10% of the pre-stun power contents in EEGs.

The interaction between electrical stunning frequency and slaughter method becomes apparent in the trends observed in the time to reach 10% or less of the total power (Figure 3). Ventral or unilateral neck-cutting of broilers that had been stunned with 200 Hz led to a rapid loss of total power. In contrast, unilateral neck-cutting of broilers that had been stunned with 400, 600, 800, 1000, 1200 or 1400 Hz took substantially longer to a loss of total power than with the ventral neck-cutting procedure. However, although ventral neck-cutting resulted in a loss of total power in these broilers, there was a tendency to return to above 10% of pre-stun levels during bleeding. The total power content in the EEG of broilers that were stunned with 1200 or 1400 Hz and had ventral neck-cuttings returned to

https://doi.org/10.1017/S0962728600029882 Published online by Cambridge University Press

Table 3 The effect of electrical stunning variables used on the number of broilers with positive reflexes and responses during bleeding; responses and reflexes were tested 5 s after neck-cutting.

Frequency (Hz)	Current (mA)	n	Breathing	Blinking	Palpebral reflex present	Swallowing and/or head shaking	Response to comb pinch
200	100	9	2	2	2	1	1
	150	7	0	0	0	0	0
	200	8	1	1	1	1	1
400	100	9	5	5	4	3	1
	150	8	6	4	4	4	3
	200	8	1	1	1	1	0
600	100	9	5	5	5	5	2
	150	7	3	3	3	3	2
	200	8	4	4	4	4	3
800	100	6	4	4	4	4	3
	150	9	6	5	5	5	4
	200	8	3	3	3	3	2
1000	100	8	8	8	8	5	4
	150	8	5	5	4	4	4
	200	8	4	4	4	4	3
1200	100	11	7	6	6	5	5
	150	8	6	6	6	5	3
	200	8	4	4	4	4	3
1400	100	9	9	7	7	7	5
	150	8	7	6	6	5	3
	200	8	5	5	5	5	5
Unilateral neck–cutting	–	86	72	72	72	70	54
Ventral neck–cutting	–	86	23	16	14	8	2
Total	–	172	95	88	86	78	56

pre-stun levels at some point during bleeding before decreasing again because of the effect of bleeding.

It is also possible that the incidence of epileptiform EEG contributed to this effect as there were certain differences in the time to the onset of 10% or less of the pre-stun total EEG power between the broilers with epileptiform EEG following stunning and those without epileptiform EEG. When a ventral neck-cutting procedure was performed, the average time to reach 10% or less of the pre-stun power in the 2–30 Hz EEG frequency band was very similar in broilers with epileptiform EEG (mean = 22.2 ± 5.00 s) to those without epileptiform EEG (mean = 23.1 ± 7.79 s). In contrast, when a unilateral neck-cutting procedure was performed, the average time to reach 10% or less of the pre-stun EEG total power was shorter in broilers with the epileptiform EEG (mean = 45.1 ± 26.38 s) than those without the epileptiform EEG (mean = 58.1 ± 23.44 s).

It is worth noting that the power content in the 2–30 Hz EEG frequency band failed to reach less than 10% of the pre-stun levels even after 108 s post-stun in some broilers that were stunned with electrical frequencies of 600 Hz or more and had the unilateral neck-cutting procedure. The apparent effect of the interaction between the frequency and RMS current, although not statistically significant, was probably because increasing the RMS current with 200 and 400 Hz reduced the average time to a loss of total power, whereas increasing the RMS current with higher frequencies had no significant effect on the time to loss of total power, especially with 1400 Hz.

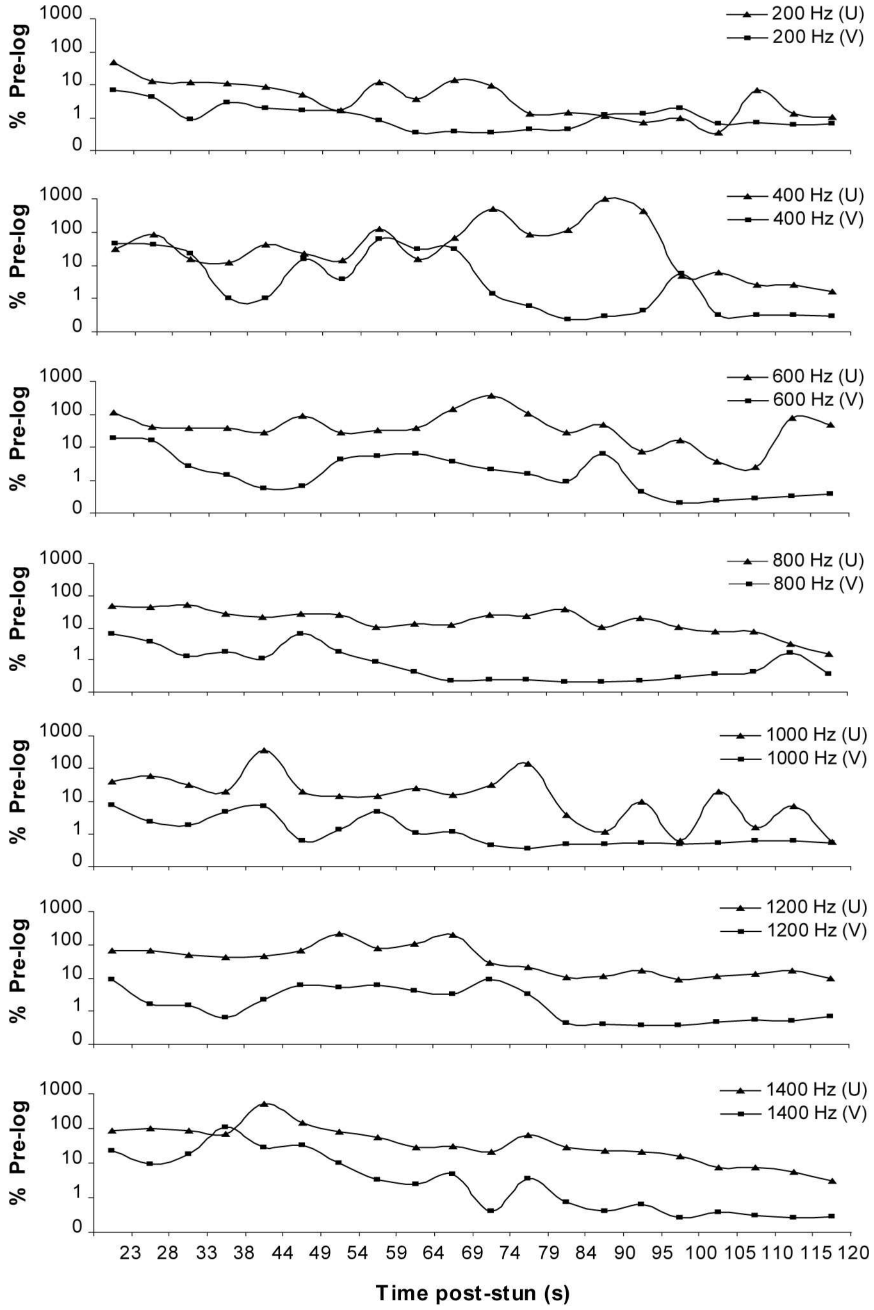
Evaluation of the responses and reflexes during bleeding supported the findings from the analysis of the epileptiform EEGs. It can be seen in Table 3 that: (a) increasing the frequency of electrical stunning at a chosen RMS current level resulted in an increasing number of birds with positive responses and reflexes; (b) increasing the amount of RMS current with a chosen frequency decreased the number of birds with positive responses and reflexes; and (c) the unilateral neck-cutting procedure resulted in the majority of birds having positive responses and reflexes, whereas the use of the ventral neck-cutting procedure reduced the number of birds with positive responses and reflexes. The relationship between the marked trends in the changes in the variables in Table 3 and the changes in epileptiform EEG are well established and so no formal statistical analysis is presented for the data in Table 3.

The presence of swallowing, head shaking and a response to comb pinching signified a return to consciousness in these broilers. At 100 mA of RMS current, 40 birds across the seven frequency treatments were breathing during bleeding; of these 40 birds, 27 had unilateral neck-cutting (the remaining 13 birds had ventral neck-cuttings) and of these 20 responded to comb pinching. At 150 mA of RMS current, 33 birds across the seven frequency treatments were breathing during bleeding; of these 33 birds, 24 had

https://doi.org/10.1017/S0962728600029882 Published online by Cambridge University Press

Figure 2

The effect of electrical stunning frequencies (Hz) and slaughter methods on the relative power contents (% pre-stun, logarithmic) in the 13–30 Hz EEG frequency band for unilateral (U) and ventral (V) neck-cuttings procedures.



unilateral neck-cutting (the remaining 9 birds had ventral neck-cuttings) and of these 19 responded to comb pinching. At 200 mA of RMS current, 22 birds across the seven frequency treatments were breathing during bleeding, and of these 22 birds all except one bird, which was stunned with 1400 Hz, had unilateral neck-cuttings, and of these 17 responded to comb pinching.

Discussion

Overall, the results of this study indicated that the electrical water bath stunning of broilers with 200 Hz sine wave AC was more effective than at higher frequencies, and that the threshold current necessary to induce epileptiform EEG in a high proportion (90%) of broilers increased as the electrical frequency increased from 200 to 800 Hz. In this regard, effective water bath stunning of broilers with a minimum current of 100, 150 and 200 mA could be achieved with electrical frequencies of up to 200, 600 and 800 Hz, respectively. It is possible to suggest that electrical frequencies above 800 Hz would have required a minimum current greater than 200 mA to induce epileptiform activity in the EEGs of the majority of broilers.

Unfortunately, the scientific literature concerning constant current water bath stunning of broilers involving quantitative EEG analysis is scarce. Nevertheless, the relationship between the amount and frequency of current found in this study is in agreement with the previous findings of head-only electrical stunning of broilers, and water bath electrical stunning of end of lay hens (Raj & O'Callaghan 2004a,b respectively). Although different criteria were used to ascertain the efficacy of electrical stunning under different conditions, other reports involving turkeys (Mouchoniere *et al* 1999) and rainbow trout (Robb *et al* 2002) also found that, at a given current level, the efficacy of stunning decreased when the electrical frequency was increased. We propose that the effect of a stunning current depends upon the 'period' of electric current used (see Box 1, Equation 1) and it decreases markedly when the 'period' is below the threshold limit necessary to induce a sustained period of depolarisation of the neurones (Raj & O'Callaghan 2004a), which should occur in large groups of neurones involving both the cerebral hemispheres to develop into generalised epilepsy. This effect could be attributed to the rapidity of the voltage change and the excursion from the peak voltage (Ingling & Kuenzel 1978). In this regard, 200 Hz sine wave AC has a relatively slower rate of voltage change and longer excursion distance than the higher frequencies tested in this study. The results of the present study provide evidence to support this interpretation. The induction of epileptiform activity, attributable to the stunning of broilers with any constant RMS current, be it 100, 150 or 200 mA, was found to be limited to a range of electrical frequencies and the probability of inducing epileptiform EEG increased with increasing amount of current delivered with the higher frequencies (600 and 800 Hz). In addition, the proportion of broilers showing epileptiform activity following stunning with 200 Hz, delivering a constant RMS current of 100 or 200 mA, involving 168 and 257 V RMS, respectively, was similar. Therefore, the generally held belief that higher stunning currents suppress epilepsy is questionable and it could perhaps happen when the voltage (or current) is sufficiently high to induce sudden neuronal death.

The results of this study showed that the incidence of epileptiform EEG increased as the stunning current was increased, especially with 400, 600 and 800 Hz, suggesting a biological relationship between the incidence of epileptiform activity and the amount of stunning current, rather than the epileptiform activity being random. When the epileptiform activity, occurring as a result of effective electrical stunning with currents appropriate to the frequencies, led to quiescent or profoundly suppressed EEGs, as determined from the power contents in the EEG frequency bands (Figure 2, Figure 3), positive responses and reflexes were absent during bleeding (Table 3). In contrast, when a profoundly suppressed EEG failed to follow the epileptiform activity, broilers that were inadequately stunned recovered soon after the termination of epileptiform activity or during bleeding.

Clearly, the results of this study indicate that the epileptiform activity induced with 200 Hz sine wave AC led to a sustained period of quiescent EEG, whereas stunning with higher frequencies, especially 800 Hz or more, failed to induce quiescent EEGs. We previously suggested that this effect could be attributed to the electrical stunning frequency-dependent nature of neurotransmitter release mechanisms in the brain (Raj & O'Callaghan 2004a,b).

A concerning finding of our studies is that head-only or water bath electrical stunning resulted in all broilers experiencing seizures accompanied by apnoea, irrespective of whether or not an epileptiform EEG was induced. These physical signs have been used to monitor the effectiveness of water bath electrical stunning, and the time to the return of spontaneous breathing has been used as an indicator of the duration of unconsciousness (Gregory & Wotton 1990; Wilkins *et al* 1999). Although the time to the return of spontaneous breathing appears to be correlated with the time to return of EEG activity resembling consciousness and sensibility following effective head-only electrical stunning, at least in sheep (Velarde *et al* 2002), the time to the return of spontaneous breathing may not be a good indicator of the return of consciousness under water bath electrical stunning conditions. This is because, under the water bath electrical stunning conditions, the current would flow through the whole body and this would result in tetanus and apnoea in all the chickens irrespective of whether or not they have been stunned. Therefore, it is not surprising that previous investigations did not find significant differences in the time to the return of spontaneous breathing following electrical water bath stunning of poultry with various waveforms, frequencies and current levels (Wilkins *et al* 1999).

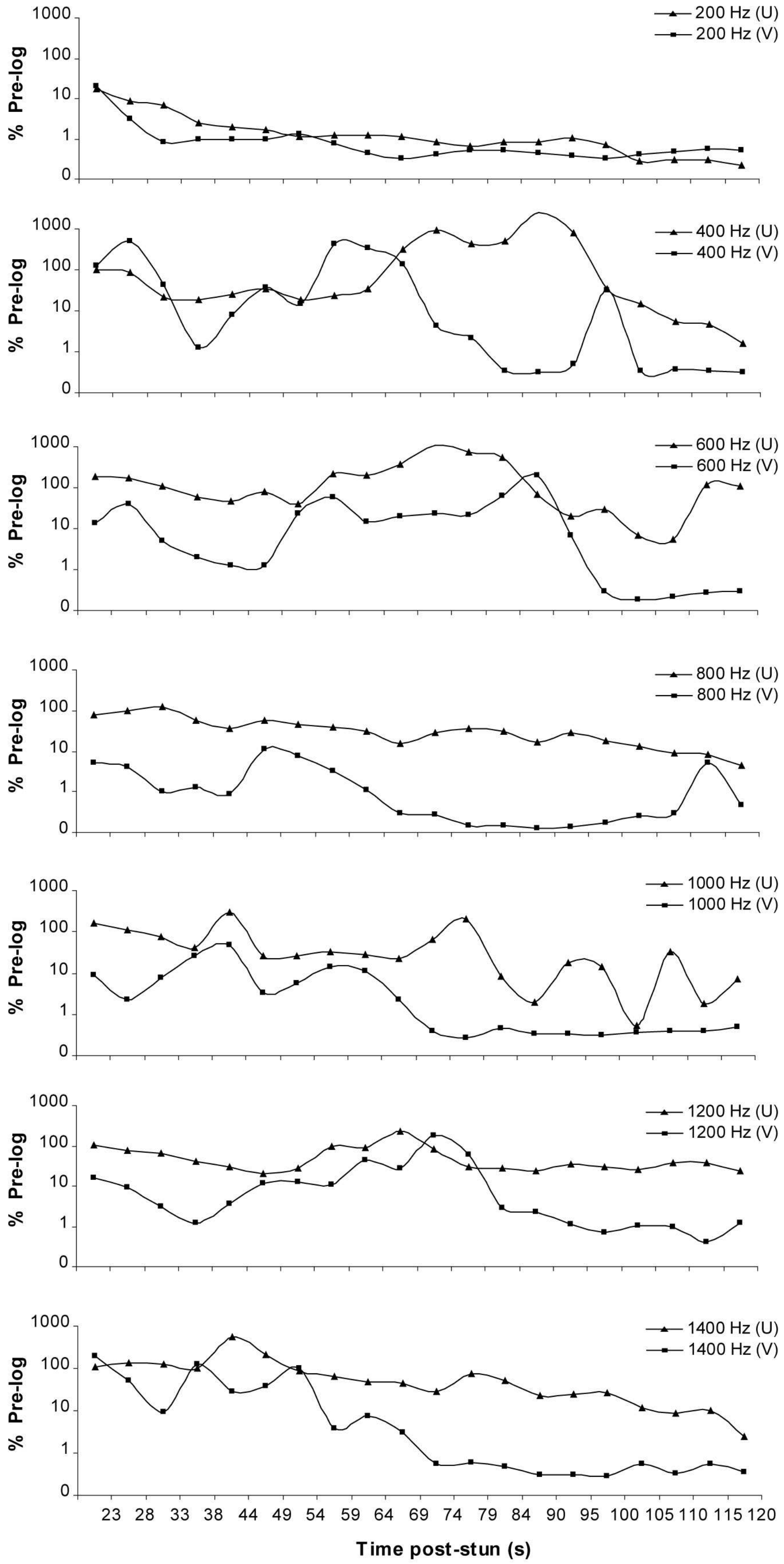
We previously suggested that, in the absence of epileptiform EEG and a sustained period of profoundly suppressed EEG following the epileptiform activity, it would be difficult to assume unconsciousness and insensibility in chickens and therefore, the avoidance of pain and suffering during bleeding (Raj & O'Callaghan 2004a,b). The results of this study indicate that in spite of the occurrence of epileptiform activity, and the prompt and accurate severance of two common carotid arteries and two external jugular veins, many broilers stunned with a frequency of greater than 800 Hz regained consciousness and sensibility, as indicated by the return of power in the EEG frequency bands (Figure 2) and by broilers showing positive responses and reflexes during bleeding (Table 3). This is due to the fact that a profoundly suppressed EEG did not follow epileptiform activity in these broilers.

The effects of the interaction between electrical stunning frequency and slaughter method on the time to reach 10% or less of the pre-stun power contents in the EEG frequency bands suggests that high frequencies (800 Hz or more) either failed to effectively stun broilers, as indicated by the lack of epileptiform activity in the majority of broilers, or failed to induce a sustained period of quiescent EEG, as indicated by the absence of a loss of power contents in the EEG frequency bands. Consequently, the unilateral neck-cutting procedure, applied on average within 10 s of stunning, could not induce a level of brain ischemia that is deemed necessary to prevent a return to consciousness and sensibility during bleeding. An interesting finding of this

https://doi.org/10.1017/S0962728600029882 Published online by Cambridge University Press

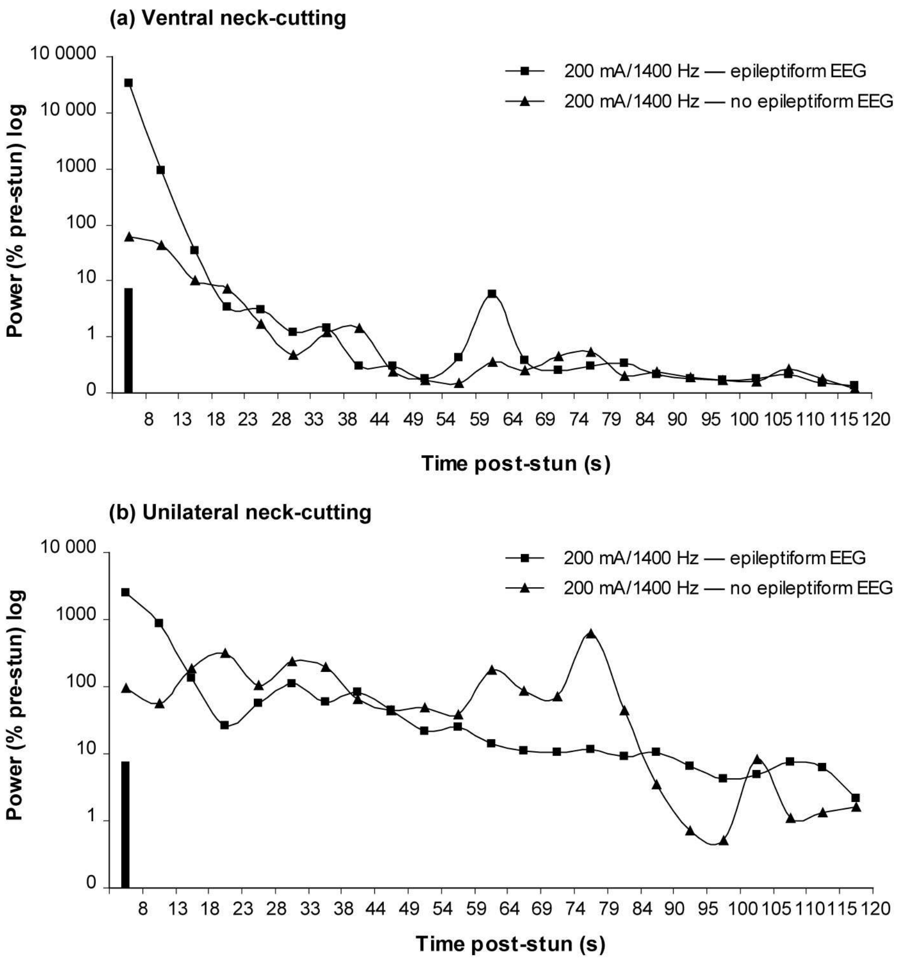
Figure 3

The effect of electrical stunning frequencies (Hz) and slaughter methods on the EEG total power contents (% pre-stun, logarithmic) in broilers; unilateral (U) and ventral (V) neck-cuttings procedures.



https://doi.org/10.1017/S0962728600029882 Published online by Cambridge University Press

Figure 4



Typical examples of the effect of epileptiform activity in the EEG and of slaughter methods on the power contents in the 13–30 Hz EEG frequency band of broilers during bleeding. The vertical bar at 8 s post-stun indicates neck-cutting.

study is that the overall time to the onset of 10% or less of the pre-stun power contents in the EEG frequency bands were, on average, 12 s shorter in broilers that showed epileptiform activity than in those without the epileptiform EEG. This difference is considerably higher in broilers subjected to a unilateral neck-cutting procedure. An example of this is presented in Figure 4 using data from broilers that were stunned with 200 mA of 1400 Hz and subjected to either (a) ventral or (b) unilateral neck-cutting procedure. In addition, all the responses and reflexes tested in this study were present in these broilers, which suggests that their presence signifies a return to consciousness and therefore could be used as monitoring points under commercial poultry processing conditions.

The results of this study showed that the ventral neck-cutting procedure, which severs all the major blood vessels, had an overwhelming affect on the time to the loss of relative and total power contents in the EEG frequency bands (mean = 21 ± 1.78 s; 23 ± 2.49 s, respectively). Broilers with epileptiform EEGs appeared to be unconscious and insensible during this period because the epileptiform activity persisted, although with decreasing amplitude during bleeding, and terminated in a quiescent EEG. In addition, the ventral neck-cutting of broilers that did not show epileptiform activity, and were therefore judged not to be effectively stunned, resulted in a similar time to loss of power in the EEG frequency bands. Typical examples of these scenarios are presented in Figure 4: four broilers were stunned with 200 mA of 1400 Hz, two had ventral neck-cuttings (Figure 4a) and two had unilateral neck-cuttings (Figure 4b) at a similar time post-stun (mean = 7.4 s; 7.5 s, respectively). None of the physical responses and reflexes was present in these broilers; however, the ventral neck-cutting procedure alone could not be justified or relied upon to induce death in inadequately stunned or immobilised broilers. Finally, when slaughter is performed without prior stunning, any manipulation of neck-cutting wounds during this period is likely to cause additional pain, distress and suffering in birds.

The ventral neck-cutting of broilers, stunned with a current appropriate to the electrical frequency necessary to induce immediate unconsciousness and insensibility, ensured a humane and rapid death. In contrast, when a unilateral neck-cutting procedure was performed, the relative and total power contents in the EEG frequency bands failed to reach 10% or less of the pre-stun levels in some birds even after 120 s; therefore, a unilateral neck-cutting procedure is unlikely to prevent a return to

https://doi.org/10.1017/S0962728600029882 Published online by Cambridge University Press

consciousness and sensibility in effectively stunned birds, induce rapid death in all the broilers and/or prevent live birds from entering scald tanks.

Gregory and Wotton (1986), using anaesthetised and mechanically ventilated chickens (layer hens), previously reported on the time to loss of spontaneous EEG activity following decapitation, induction of cardiac arrest and various commercially practised neck-cutting procedures. In that study, the time to reach 5% of the pre-slaughter integrated EEG activity was used as one of the criteria to determine the state of brain function in chickens. However, these times were suggested to be overestimates because of the effects of the anaesthetic used and the mechanical ventilation provided to the birds.

Evaluation of the responses and reflexes during bleeding provided useful monitoring points in the present study. It is known, for example, that spontaneous breathing returns after the termination of electrical stunning-induced seizures (Schuett-Abraham 1999); spontaneous blinking is present in conscious animals but diminished in Stage III (eyes closed in light to deep anaesthesia) and abolished in Stage IV of anaesthesia (overdose); and the swallowing reflex, which is normally stimulated by the presence of saliva or food in the pharynx, is lost at a medium depth of anaesthesia (Rural Area Veterinary Service 2004). Head shaking may occur in response to blood entering nostrils of conscious birds and a response to comb pinching (a painful stimulus) is clearly indicative of a return to consciousness and sensibility, although it is not always reliable in electrically stunned poultry (Richards & Sykes 1964; Schuett-Abraham 1999). The presence of responses and reflexes in broilers used in the present study, in association with the power contents in the EEG frequency bands, is a clear indication of a return to consciousness and sensibility during bleeding. For the monitoring of good animal welfare, birds should not exhibit these responses and reflexes at any time after stunning.

It is possible to suggest that the relationship between electrical stunning frequency, the amount of current and slaughter methods found in this study would also apply to other species of poultry; however, this needs to be confirmed. Further studies, in mammals, involving clearly defined neurophysiological criteria (eg quantitative EEG rather than behaviour or reflexes alone) and stunning with a wide range of electrical frequencies, current levels and commonly used slaughter methods are also warranted to elucidate the relationships, if any, between these variables and to set appropriate standards.

Conclusions and animal welfare implications

Effective water bath stunning of broilers can be achieved with a minimum constant current of 100, 150 or 200 mA, delivered with electrical frequencies of up to 200, 600 and 800 Hz respectively. However, it is likely that the use of electrical frequencies above 800 Hz would require a minimum current of greater than 200 mA to induce epileptiform activity in the EEGs of majority of broilers. In effectively stunned broilers, ventral neck-cutting is critical to avoid a return to consciousness and sensibility, to prevent pain and suffering during bleeding, and to prevent live birds from entering scald tanks. The amount of current necessary to induce immediate loss of consciousness and sensibility should be delivered using a constant source of current, rather than a constant source of voltage, and both the carotid arteries should be cut to achieve humane slaughter.

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 Stationery Office (HMSO): London, UK

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

Gregory NG and Wotton SB 1990 Effect of stunning on spontaneous physical activity and evoked activity in the brain. *British Poultry Science 31*: 215-220

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

Mouchoniere M, Le Pottier G and Fernandez X 1999 The effect of current frequency during waterbath stunning on the physical recovery and rate and extent of bleed out in turkeys. *Poultry Science 77*: 485-489

Raj ABM 2003 A critical appraisal of electrical stunning in chickens. *World's Poultry Science Journal 59*: 89-98

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 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 2006a 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 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

Richards SA and Sykes AH 1964 Observations on the electrical stunning and slaughter of poultry. *Veterinary Record 76*: 835-839

Robb DHF, O'Callaghan M, Lines JA and Kestin SC 2002 Electrical stunning of rainbow trout (*Oncorhynchus mykiss*): factors that affect stun duration. *Aquaculture 205*: 359-371

Rural Area Veterinary Service 2004 Monitoring the Anaesthesia Patient. www.ruralareavet.org/PDF/Anesthesia-Patient_Monitoring.pdf (accessed 7 November 2005)

Schuett-Abraham I 1999 Humane Stunning of Poultry — Part I: Electrical Stunning. *EC Seminar Animal Welfare* 30 pp. 24 August–2 September 1999. Dublin, Ireland. www.schuett-abraham.de/isa-poult-en.htm (accessed 7 November 2005)

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

Velarde A, Ruiz-de-Torre JL, Rosello C, Fabrega E, Diestre A and Manteca X 2002 Assessment of return to consciousness after electrical stunning in lambs. *Animal Welfare 11*: 333-341

Wilkins LJ, Wotton SB, Parkman ID, Kettlewell PJ and Griffiths P 1999 Constant current stunning effects on bird welfare and carcass quality. *Journal of Applied Poultry Research 8*: 465-471

https://doi.org/10.1017/S0962728600029882 Published online by Cambridge University Press