- 2 incidence of cardiac arrest in broilers stunned and slaughtered for the halal market.
- 3

8

10

11

12

- 4 S.B. Wotton¹, X. Zhang¹, J. McKinstry¹, A. Velarde² and T. G. Knowles¹.
- ⁵ ¹University of Bristol, School of Veterinary Sciences, Langford, Bristol BS40 5DU, UK.
- ² Animal Welfare Subprogram, Institut de Recerca i Tecnologia Agroalimentaries, Veïnat de Sies s/n, E17121
 Monells, (Girona) Spain
- 9 Corresponding author: steve.wotton@bris.ac.uk
 - Abbreviated Title: Water bath stunning on ECG

13 Summary

Multi-bird water bath stunning is the only method permitted for stunning broilers in halal plants in the UK. The electrocardiogram (ECG) was measured in broilers that were stunned electrically in a commercial water bath using four of the frequency/current parameters permitted under EU Regulation (1099/2009) plus a control treatment (pulsed DC waveform). The results showed that there were no differences between the treatments in that all the birds displayed a rhythmic heart beat following stunning and before slaughter.

An opportunity to observe recovery in a small number of broilers (nine) in a Spanish processing plant suggested that AC at 1000 Hz applied at 200 mA per bird would also meet both the legislative requirements and the needs of the halal market, in that all birds recovered from the stun.

24

Keywords: poultry, halal slaughter, water bath stunning, EU Regulation, frequency,
 current

27

28 Introduction

- 29 With an increasing population of Muslims in the UK, 2.4 million in 2009 (Kerbaj, 2009), the
- 30 halal food market has received progressively more attention. The Muslim population have PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.255v1 | CC-BY 4.0 Open Access | received: 24 Feb 2014, published: 24 Feb 2014

strict dietary requirements, based on the Qur'an (Riaz & Chaudry, 2003) and met through specific halal markets. In terms of meat consumption, Muslims are prohibited from eating pork, so beef and poultry are the preferred animal products (Havinga, 2010). In addition, Muslims are also prohibited from eating the meat from animals that are not alive at slaughter. Therefore, they either slaughter animals without stunning or slaughter animals with stunning, using a stunning method that does not result in the death of the animal. For birds, the only stunning method that is accepted by Muslims in the UK is electrical water bath stunning (Nakyinsige et al., 2013).

Previous research with chickens (Gregory and Wotton, 1987) has shown that cardiac muscle is particularly sensitive to electrical stimulation, but only at low frequencies i.e. 50 Hz. The magnitude of the applied current can also contribute to the incidence of cardiac arrest (known as stun to kill, when deliberate) and that the minimum required alternating current (AC) of 100 mA per bird at a low frequency such as 50 Hz will result in the death of about 80 - 90% birds (Gregory and Wotton, 1988, 1990). Wilkins, et al., (1998) showed that the use of frequencies progressively greater than 50 Hz was associated with a failure to induce a cardiac arrest. Therefore, where birds are processed for the Halal market, the use of high frequency AC will ensure that stunned birds are not killed in the water bath stunner but are alive when their necks are cut.

49 Consequently, those with a concern for animal welfare want to encourage methods that will enable the production of halal poultry meat from birds that are stunned prior to slaughter. 50 51 However, there is a compromise because water bath stunning per se has the potential to create welfare issues (Wotton and Wilkins, 2004), specifically variations in bird resistance will 52 53 result in variations in the current received by individual birds. In addition, further research into the electrical parameters required for effective water bath stunning has raised anomalies. 54 55 This is because processing plants that produce halal meat have preferred historically to use a very low current combined with high frequency pulsed DC to stun the birds, which they 56 believe will guarantee that the birds will not incur ventricular fibrillation during stunning 57 (Gregory et al., 1991). Raj, et al. (2006a,b,c) looked at whether increased frequencies 58 combined with different waveforms would result in effective stunning. Their research showed 59 that the minimum current to effectively stun increased with increasing frequency for AC and 60 that pulsed DC was not effective unless applied at very much higher current levels. They 61 suggested that at lower current levels, when applied as pulsed DC, birds could be electrically 62

63 immobilized and not stunned. The minimum currents proposed by Raj, et al. (2006a,b,c,) that PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.255v1 | CC-BY 4.0 Open Access | received: 24 Feb 2014, published: 24 Feb 2014 would produce effective water bath stunning were confirmed by Prinz (2009) and
incorporated into EC Regulation (1099/2009) as reproduced in table 1.

66

67

68

69

70

71

72

73

74

75

76

77

 Table 1. AC electrical requirements for water bath stunning equipment

 (average values per animal)

Frequency (Hz)	Chickens	Turkeys	Ducks and geese	Quails
< 200 Hz	100 mA	250 mA	130 mA	45 mA
From 200 to 400 Hz	150 mA	400 mA	Not permitted	Not permitted
From 400 to 1500 Hz	200 mA	400 mA	Not permitted	Not permitted

The new EC Regulation (1099/2009) does offer processors a choice from three ranges of current and frequency combinations to stun chickens before slaughter. However, there is some concern voiced from the Muslim Authorities that these parameters may result in the death of a proportion of birds prior to slaughter. Indeed, there is little published information on whether these parameters would induce ventricular fibrillation and hence result in the death of the bird in the water bath stunner. The research described below was designed to makes an important contribution to the field of pre-slaughter stunning of birds for the halal market by quantifying the effect of these new parameters in terms of the incidence of induced ventricular fibrillation.

78

79 Materials and methods

The trial was conducted over a total of three days in a commercial halal plant with the approval of the University of Bristol Ethical Review Group (UIN/13/037). The broilers were supplied from five different farms. A total of 759 broilers were studied, randomly selected from 17 separate transport loads. The experimental design ensured that birds were evenly distributed across the treatment groups (Table 2) such that treatments 1 to 3 and treatments 4 & 5 each contained a similar number of broilers from each load.

86 The birds were weighed on arrival at the processing plant before loading onto the primary

processing line. The average bird live weight was 1.83 kg for treatments 1 to 3 and was

PeerJ PrePrints

similar for the different loads used. The birds used for treatments 4 & 5 had an average live
weight of 1.90 kg.

90

No.	No. of birds	RMS Current (mA/bird)	Frequency (Hz)	Waveform	Origin
1	152	45	500	Pulsed DC	'Control' waveform - favoured for halal
2	152	102	200	Sinusoidal AC	
3	152	146	400	Sinusoidal AC	EC Regulation
4	151	199	600	Square wave AC	(1099/2009)
5	152	199	1500	Square wave AC	

91 Table 2. The five treatment groups that were tested for their effect on bird heart function.

The electrical parameters used for all five treatments were applied for 5.7 s (dwell time) in a 94 95 commercial water bath stunner regulated using a factory calibrated Poultry Stun Monitor (AGL Consultancy Ltd.) in conjunction with a factory calibrated "Fluke" Scopemeter (Fluke 96 97 UK Ltd.). Following stunning and slaughter, individual birds were removed from the processing line and placed on a single shackle mounted on an A-frame to enable ECG 98 recordings to be made. Fine needle electrodes were inserted intrathoracically in each bird >1599 s after both carotid arteries were severed manually and the signal was amplified using a pre-100 amp (Gould Bioelectric, UK). The signal was subsequently passed through a noise removal 101 102 system (Humbug 50/60 noise removal system) to eliminate the background noise and digitally recorded at a sampling rate of 1,000 samples per second using a Vision Data 103 Acquisition System (LDS Test & Measurement Ltd.). Each trace was analysed in real time 104 for the presence of a repeating QRS complex (Figure 1). 105

106

107 The results were statistically analysed and are presented as the percentage of birds that did

not recover together with a 95 per cent confidence interval for the percentage calculated using

109 Wilson's method (Altman et al, 2000).

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.255v1 | CC-BY 4.0 Open Access | received: 24 Feb 2014, published: 24 Feb 2014

92

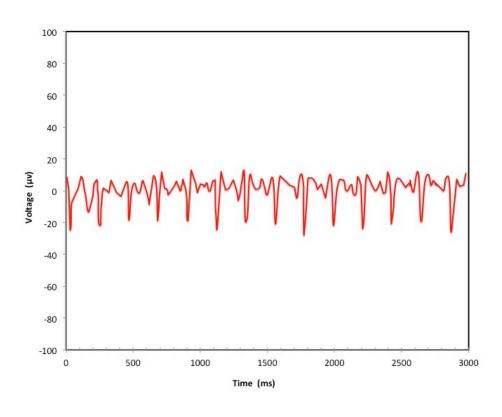
93

PeerJ PrePrints

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.255v1 | CC-BY 4.0 Open Access | received: 24 Feb 2014, published: 24 Feb 2014

Figure 1. Post stun ECG of a broiler that was stunned using a 100 mA at 200 Hz, AC 112 waveform.

113



Ceru PrePrints 114 115

116

Results 117

All of the 759 broilers studied demonstrated a repeatable cardiac rhythm following the 118 application of the electrical water bath stunning treatment. None of the birds suffered a 119 ventricular fibrillation, which would lead to a cardiac arrest. Wilson's method gives a 95% 120 confidence interval for this proportion (zero) within a single treatment as ranging from 0 to 121 2.5 per cent. Thus given the sample size of 152 it is unlikely that the true underlying 122 proportion that would have had a cardiac arrest would be greater than 2.5%. Given that all 123 the treatments resulted in zero per cent cardiac arrest it is possible to calculate a confidence 124 interval across the treatments used as a whole. However, we omit treatment 1 from this 125 calculation as it is now outside the law (EC Regulation 1099/2009). Thus, Wilson's method 126 gives a 95 per cent confidence interval of 0 to 0.6 per cent for the 0 per cent cardiac arrest 127 from the 607 birds within treatments 2 to 5. Hence, it is unlikely that an underlying figure for 128

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.255v1 | CC-BY 4.0 Open Access | received: 24 Feb 2014, published: 24 Feb 2014

birds showing cardiac arrest would ever be greater than 0.6%. Figure 1 show a typical ECG
recording of a broiler that was stunned by approximately 100 mA at 200 Hz, 131 Volts
alternating current (VAC) given an average dwell time of 5.7 s in a water bath that immersed
10 birds in the water bath at any one time. The ECG recordings demonstrate that the birds
did not develop a cardiac arrest through ventricular fibrillation as a result of the stunning
treatments applied.

The mean stunning current for treatments 1, 2, 3, 4 and 5 was approximately 45 mA, 102 mA and 146 mA, 199 mA and 199 mA, respectively (Table 2). In order to achieve the required current per bird, the voltage was manually adjusted. When selecting the mid-range current/frequency combination (treatment 3) to stun birds, the range of the voltage required to maintain 146 mA was 168 V to 200 V across the different birds from the different transport loads. The stunning dwell time within the water bath stunner varied slightly between treatments and loads.

During the experiment, the birds sampled were from 17 separate transport loads and these loads came from different farms. Although the number of birds sampled from each load, for each treatment was not identical, this should not have affected the results obtained.

146 **Discussion**

147 Muslims cannot eat the meat from animals that are not alive at the point of slaughter. Historically this requirement has been achieved by applying low current, pulsed DC at high 148 frequency and by rejection of any birds that were deemed to be already dead at neck cutting 149 by the halal slaughterman. Research has shown that pulsed DC and AC waveforms when 150 applied at low current levels may not produce consistent stunning (Raj, et al., 2006a,b,c; 151 Prinz, et al., 2010). The use of low average currents represents a welfare concern, 152 particularly for the female broilers, which have a higher resistance (Rawles, et al., 1995; 153 Prinz, 2009) and therefore would receive a substantially lower current than the calculated 154 average current per bird. Water bath stunning systems are at present constant voltage by 155 design; a constant voltage is applied between a 'live' water bath electrode and an earthed rail 156 that makes contact with the shackles. On average more than 10 birds are immersed in the 157 water bath and subjected to the stunning voltage at any one time. The major concern over the 158 159 calibration of water bath stunners for poultry is the potential large variation in impedance (i.e.

resistance to the flow of electrical current) between birds within the stunner. Major variation
in bird impedance can be caused by the interface between the bird's leg and the shackle.
Variations in the size of the legs of birds and the development of the leg-scales can produce
large variations in contact impedance (Wotton and Wilkins, 2004)

Higher stunning frequencies in the past have been shown to produce a lower incidence of broken bones and breast muscle haemorrhages (Raj et al., 2001), which are likely to be the drivers behind the frequencies chosen commercially. The use of higher frequencies also reduces the likelihood of birds suffering from a cardiac arrest.

The requirement of EC Regulation (1099/2009) is for the application of a range of current/frequency combinations, where the magnitude of the minimum currents (table 1) are significantly higher than those employed in the past. Concern has been expressed by some Halal Certification Bodies that these higher currents will result in the death of birds in the water bath. However, there can be no derogation for the use of those parameters applied previously for halal production as that would result in the Competent Authority approving current and frequency combinations that were insufficient to stun (Raj, et al., 2006a,b,c; Prinz, et al., 2010).

EC Regulation (1099/2009) requires the use of electrical parameters that specify minimum 177 currents within a defined frequency range. These legislative requirements should be applied 178 in all processing plants that employ electrical water bath stunning, including those plants 179 supplying the Muslim community. However, it has been reported that some Halal 180 Certification Bodies are concerned about these parameters as some of the current/frequency 181 options have the potential to result in the death of birds during the stunning process. The 182 results from this study should allay the concerns of the Certification Bodies and permit the 183 continued use of electrical water bath stunning for the production of halal poultry products, 184 185 but at current and frequency combinations that encourage the effective stunning of birds prior to slaughter. 186

187

188 Conclusion

On welfare grounds this work describes how the use of specific parameters permitted by the newly required electrical combinations to stun birds produces an effective stun (EC Regulation 1099/2009) and is not contradictory to the requirements of many Halal Certification Bodies.

In summary, the four recommended combinations of current and frequency studied during this project did not induce a single cardiac arrest at stunning. Following stunning, it is also important to ensure that the requirement for both carotid arteries to be severed at slaughter (EC 1099/2009) is met because the birds stunned with the current/frequency combinations tested will have the possibility of regaining consciousness.

Subsequently to this study, an opportunity occurred at a Spanish poultry processing plant 198 (Avicola Sanchez S.A) to assess the effect of two frequency/current combinations on a 199 limited number of broilers. The study was approved beforehand by the Institutional Animal 200 Care and Use Committee (IACUC) of the Catalan Government. The trial occurred towards 201 202 the end of the normal processing shift on twelve broilers ranging between 2.0 & 2.8 kg live weight. The birds were stunned in the plant's commercial water bath stunner that was 203 204 adjusted to deliver either 600 Hz (n = 3) or 1000 Hz (n = 9), square wave AC at 200 mA per bird. Following stunning, birds were randomly removed from the processing line before neck 205 cutting, placed in a meat crate for observation and allowed to recover. The water bath 206 207 parameters were calibrated and recorded on a Vision Data Acquisition System (LDS Test & Measurement Ltd.). Current measurement was made using a PR 30 (LEM HEME ltd.) 208 209 current probe and voltage measurement using direct leads between the live water and earthed rail. 210

The application of 200 mA at 600 Hz resulted in the death of two of three birds initially tested. When the frequency was increased to 1000 Hz (200 mA per bird) all of the further nine birds tested recovered consciousness within 1 minute of the water bath stunner exit.

It is concluded that in order to meet both the legislative requirements (EC Regulation 1099/2009) for pre-slaughter stunning and the halal requirements for a live bird at slaughter, a minimum AC frequency of 1000 Hz at 200 mA per bird would be a reasonable compromise.

218 Acknowledgements

219 The help of Avicola Sanchez S.A. with the recovery trial is gratefully acknowledged. PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.255v1 | CC-BY 4.0 Open Access | received: 24 Feb 2014, published: 24 Feb 2014

221 References

- AGL Consultancy Ltd., Maple House, 50A Canada Road, Cobham, Surrey KT11 2BA
- Altman, D.G., Machin, D., Bryant, T.N. and Gardner, M.J. (2000) Statistics with Confidence
- 224 2nd edition. British Medical Journal Books, pp44-56.
- Avicola Sanchez S.A.; c/ Treball, 2-24 08940 Cornellà de Llobregat (Barcelona), Spain.
- 226 Council Regulation (EC) No. 1099/2009 on the protection of animals at the time of killing
 - 7 Fluke UK Ltd., 52 Hurricane Way, Norwich, Norfolk NR6 6JB
 - Kerbaj, R. (2009). Muslim population 'rising 10 times faster than rest of society'. The TimesJanuary 30.
 - Gregory, N.G. and Wotton, S.B. 1987. Effect of electrical stunning on the electroencaphalogram in chickens. British Veterinary Journal. 143, 175-183.
- Gregory, N.G. and Wotton, S.B. 1988. Stunning of chickens. Veterinary Record 122, 399.
- Gregory, N.G. and Wotton, S.B. 1990. Effect of stunning on spontaneous physical activity
 and evoked activity in the brain. British Poultry Science. 31, 215-220.
- 236
- Havinga, T. (2010). Regulating halal and kosher foods: different arrangements between state,
 industry and religious actors. Erasmus Law Review, 3(4), 241.
- Nakyinsige, K., Che Man, Y. B., Aghwan, Z. A., Zulkifli, I., Goh, Y. M., Abu Bakar, F., AlKahtani, H. A., & Sazili, A. Q. (2013). Stunning and animal welfare from Islamic and
 scientific perspectives. Meat Science, 95(2), 352-361.
- Prinz, S. (2009). Waterbath stunning of chickens, Effects of electrical parameters on the
 electroencephalogram and physical reflexes of broilers. PhD thesis, Radboud University
 Nijmegen.

- Prinz, S., Van Oijen, G., Ehinger F., Coenen A., Bessei, W. (2010) Electroencephalograms
 and physical reflexes of broilers after electrical waterbath stunning using an alternative
 current. Poultry Science 2010, 89: 1265-1274.
- Raj, A., O'Callaghan, M., & Knowles, T. (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(1), 7-18.
- Raj, A., O'Callaghan, M., & Hughes, S. (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(1), 19-24.

Raj, A.B.M., O'Callaghan, M and Knowles, T.G. (2006c) 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: 25-30.

Raj, A.B.M., Wilkins, L.J., O'Callaghan, M. and Phillips, A.J. 2001 Effect of electrical
stun/kill method, interval between killing and neck cutting and blood vessels cut on blood
loss and meat quality in broilers. *British Poultry Science 42:* 51-56

Rawles, D., Marcy, J. and Hulel, M. 1995 Constant current stunning of market weight
broilers. Journal of applied poultry research 4: 109-116

262 Riaz, M. N., & Chaudry, M. M. (2003). Halal food production: CRC Press.

Wilkins, L.J., Gregory, N.G., Wotton, S.B. and Parkman, I.D. 1998. Effectiveness of electrical stunning applied using a variety of waveform-frequency combinations and consequences for carcase quality in broiler chickens. British Poultry Science. 39. 511-518.

- 266 Wotton S.B. and Wilkins, L.J. 2004 Primary Processing of Poultry. In Measuring and
- Auditing Broiler Welfare. Edited by C.A. Weeks and A. Butterworth. CABI. 161-180.