INTRODUCTION

According to the European Union welfare legislation, it is necessary to render animals unconscious before slaughter (EU, 1993). Electrical waterbath stunning of broiler chickens is the standard method in commercial slaughterhouses. Various combinations of electrical waveforms as well as frequency, voltage, and amount of current are used with different effects on animal welfare and meat quality. A sinusoidal alternating current (AC) of 50 Hz with a minimum of 120 mA has been reported to induce cardiac arrest in 100% of broilers after waterbath stunning of 4 to 6 s (Wormuth et al., 1981). In recent years, different waveforms have been introduced, such as rectangular wave alternating currents or pulsed direct currents (DC), with high frequencies to improve meat quality (Wilkins et al., 1998). Application of low frequencies is considered to induce death, thus preventing recovery of the birds, and is often preferred for animal welfare reasons. However, the effect of the different electrical parameters on the induction of unconsciousness and death is not fully understood (Raj and Tserveni-Gousi, 2000). Under commercial conditions, a constant voltage is applied in multibird waterbath stunners. The actual current per bird is thus determined by the individual resistance. Although the resulting variation in effective stunning current between broilers is often discussed, the influence on the...
induction of unconsciousness has not been systematically analyzed.

Assessment of brain waves through recording of electroencephalograms (EEG) is the most reliable method to understand the states of consciousness and unconsciousness following stunning. The chicken EEG clamp (Coenen et al., 2007) is a noninvasive method that allows derivation of broiler brain waves and assessment of different electrical setups on a great number of birds.

The occurrence of a flat, isoelectric EEG with a profound reduction of electrical brainpower to less than 10% of the prestun level has been used to indicate unconsciousness in broilers (Raj et al., 2006a,b). Two brain frequency bands have been considered for assessment of isoelectricity. A profound reduction in the 2 to 30 Hz band has been associated with an overall loss of brain function (Raj and O'Callaghan, 2004a,b). The smaller band of 13 to 30 Hz has been associated with information processing ability or the ability to perceive pain. A profound reduction of the 13–30 Hz band has therefore been used as an indicator for insensibility in broilers (Raj and O’Callaghan, 2004a,b). Prinz et al. (2009) analyzed the brainpower of conscious broiler chickens in these 2 frequency bands using the chicken EEG clamp and thus established a representative baseline EEG. This baseline EEG can be used to calculate the reduction of electrical brainpower following different stunning setups. Epileptic activity before the isoelectric EEG has also been used as an indicator for adequate stunning efficiency (Schütt-Abraham et al., 1983; Raj et al., 2006a,b).

In addition to EEG analysis, physical reflexes, such as eye reflexes and the occurrence of breathing movements, poststun are often used to assess stunning effectiveness. Prinz et al. (2010a,b) and Prinz, (2009) analyzed the occurrence of different reflexes following waterbath stunning with AC and DC currents of various frequencies. In their studies, positive responses to the corneal reflex test and occurrence of spontaneous eye blinking were most reliable to indicate returning consciousness. More than 30% of eye reflexes and a marked increase of positive responses over time have been interpreted with regaining consciousness (Prinz, 2009; Prinz et al., 2010a,b). Resumption of breathing was not directly related to consciousness but indicated reversible stunning (Prinz, 2009; Prinz et al., 2010a,b). Induction of cardiac arrest in the waterbath ensures an irreversible stun (von Wenzlawowicz and von Holleben, 2001). Due to convulsions, stun-to-kill methods might be associated with meat-quality defects (Gregory, 1989).

The aim of the present study is to understand the effects of 3 different waveforms (sine wave AC, rectangular AC, and pulsed DC) on the induction of unconsciousness and death in both male and female broilers. To identify the condition of the bird following stunning, the EEG was recorded. Additionally, several spontaneous reflexes, such as breathing, eye blinking, and wing flapping, together with the corneal reflex were assessed.

MATERIALS AND METHODS

In total, 180 Ross broiler chickens, 90 males and 90 females, were raised in one flock for 7 wk. The final weight was 2.6 ± 0.2 and 2.3 ± 0.2 kg for males and females respectively. The birds were caught and kept in transport boxes for 2 to 3 h before stunning. They were then randomly allocated to 3 experimental groups. All experimental groups consisted of 10 male and 10 female broilers. The birds’ feet were fixed into a grounded metal shackle that was mounted onto a rotating stand. The shackle size was appropriate for the slaughter weight of the broilers and tapered to adjust for varying leg size. For stunning, single birds were immersed in an electrified waterbath up to the base of their wings. Three different waveforms: sine wave AC, rectangular AC, and pulsed DC, were tested with 3 constant voltage settings. Before stunning, the feet and shackle of every bird were sprayed with water to reduce the electrical resistance. The waterbath consisted of a plastic basin equipped with a metal plate as a live electrode completely covering the bottom. Salt was added to the water to keep the conductivity constant at 4 mS/cm. A constant voltage of 60, 80, or 120 V was delivered from a Meyn Quest stunning cabinet (Meyn Quest Cabinet, Meyn Food Processing Technology, Oostzaan, the Netherlands). The true root mean square current per bird was determined by the individual resistance of each broiler and was measured using a Fluke 123 industrial scope meter (20 MHz; Fluke Corporation, Everett, WA) and a Fluke 80i-110s AC/DC current probe (Fluke Corporation). All data was recorded onto a data acquisition program (Fluke View SW90W, Fluke Corporation). In all stunning groups, a low frequency was used. For technical reasons, sine wave AC was applied with 50 Hz and rectangular AC and pulsed DC with 70 Hz. The pulse width in the DC treatments was 1:1 (50% duty cycle). Average stunning time was 4.2 ± 0.8 s in all groups. Although the length of waterbaths in most commercial processing plants provides for a longer stunning time, it may be shortened to 4 s when the line speed is increased or when birds do not touch the waterbath from the beginning.

Following stunning, the chickens were fixed into the EEG device while still hanging in the shackle. Brain waves were recorded within 10 s poststun. The EEG recordings lasted for 120 s poststun and birds were subsequently killed in a box filled with carbon dioxide. During EEG recording, the occurrence of breathing, spontaneous eye blinking, and wing flapping were recorded on observation channels on the EEG, thus facilitating a direct comparison between brain activity level and physical reflexes. The occurrence of wing flapping in the first 40 s poststun was assessed as an indicator for convulsions where stunning resulted in the death of the 114 birds. Breathing was monitored through regular cloaca movements. Failure of resumption of breathing following stunning was regarded as a sign of cardiac failure. The corneal reflex was tested through the
touching of the bird’s cornea with a feather every 20 s poststun. Neck tension was tested 30 s poststun, but due to the fixation of the broilers in the EEG-clamp assessment was difficult, and this parameter has been excluded from analysis. Directly following stunning, the occurrence of tonic or clonic convulsions was assessed and recorded as described by Prinz et al. (2009).

All EEG recordings were transferred to the Brainvision analyzer (Brain Products, Gilching, Germany) using a software aid to convert Windaq-data (Dataq Instruments Inc., Akron, OH). The recordings were filtered for the broader frequency band of 2–30 Hz and for the smaller band of 13–30 Hz. They were then subdivided into 3 poststun periods: P1, 10 to 20 s; P2, 20 to 30 s; and P3, 30 to 40 s. In each period, 5 segments of 1 s were marked and a fast Fourier transformation calculated, showing the total brainpower content of every segment. The grand average fast fourier transformation of the 5 segments in each period delivered the representative brainpower in the respective period. This facilitates a comparative analysis of genuine EEG without the influence of disturbances caused by movement artifacts or manipulation of the bird during testing of physical reflexes. The brainpower thus obtained from the 3 poststun periods was expressed as a percentage of the representative baseline EEG (Prinz et al., 2009).

The relative brainpower was used to evaluate the level of unconsciousness in the broilers. A reduction in total brainpower to less than 10% of the baseline EEG in the 2–30 Hz band was regarded as a profoundly suppressed or isoelectric EEG with loss of overall brain function. The same profound reduction in the 13–30 Hz band was considered indicative of loss of sensibility (Raj and O’Callaghan, 2004a,b). Relative brainpower of more than 10% of the baseline EEG indicated inadequate stunning. In a visual assessment of the recordings, epileptiform activity was marked where the EEG showed typical spike and wave discharges with a frequency of 2 to 6 Hz (Figure 1). A characteristic chaotic EEG pattern with high amplitude and low frequency directly after stunning, followed by an isoelectric EEG could be observed in many birds. This was also regarded as an indicator for a form of unconsciousness.

For statistical analysis, JMP (2007) was used. The data was submitted to Nominal Logistic Regression. All factor effects were calculated with the chi-squared likelihood ratio test with waveform, voltage, sex, and the interactions of waveform × sex and waveform × voltage as fixed factors. Body weight as a covariate was included in the statistical model. The actual current per bird was regarded as a variable factor to evaluate stunning efficiency under conditions comparable to commercial slaughterhouses, where constant-voltage waterbaths are applied. The probabilities for positive and negative EEG responses (EEG power higher or lower than 10% of prestun power) and for physical reflexes were calculated by inverse prediction and the percentage of the predicted occurrence of positive effects was plotted.

RESULTS

Analysis of the current per bird showed a significant effect for sex and waveform. The interaction of waveform × sex was not significant (Table 1). With the same constant voltage setting, female birds obtained a significantly lower current than males in all stunning groups. The same voltage setting achieved a significantly higher current with sine wave AC compared with that of rectangular AC. Application of a pulsed DC (pulse width 1:1) resulted in the lowest current per bird (Table 2).

The fast fourier transformations were calculated for a total of 164 EEG recordings, and 16 EEG traces (10 for males and 6 for females) could not be analyzed due to movement artifacts and disturbances and were treated as missing values. The occurrence of an isoelectric EEG in the 2–30 Hz band EEG in the first 40 s poststun was significantly influenced by waveform, voltage, and sex (Table 1). Increasing voltage resulted in more birds showing a profoundly suppressed EEG in this frequency band (Figure 2). When stunned with sine wave AC, the occurrence of an isoelectric EEG was more likely than with a rectangular AC, and pulsed DC showed the lowest stunning effect (Figure 2). Female birds showed significantly fewer profoundly suppressed EEG than males after stunning (Figure 2). Analysis of the 13–30 Hz band showed similar results with significant effects of waveform and voltage, whereas sex was close to the level of significance (Table 1; Figure 2).

Analysis of the EEG recordings and physical reflexes in the 3 poststun periods (P1, P2, and P3) allows for a better understanding of the stunning effect in the different groups. In P1, a significant effect of voltage was detected in the 2–30 Hz band (Table 1). In the smaller frequency band of 13–30 Hz, no factor showed a significant effect, and the interaction of waveform × sex was close to the level of significance (Table 1). With increasing voltage, more birds showed a profoundly suppressed EEG in both frequency bands (Figure 2). In P2, the effect of voltage, sex, waveform, and the interaction of waveform × sex in the 2–30 Hz band were significant (Table 1). In the 13–30 Hz band, voltage and waveform showed a significant effect, whereas sex was close to the level of significance (Table 1). In P3, again, stunning voltage, sex, and waveform showed a significant effect in the 2–30 Hz band but not the interaction of the factors (Table 1). In the 13–30 Hz band, again, voltage and waveform significantly influenced the occurrence of isoelectric EEG in the broilers (Table 1). In both frequency bands, more birds showed isoelectric EEG with higher stunning voltage. Males were more likely to obtain an isoelectric EEG than females. Sine wave AC was most effective, followed by a rectangular AC and pulsed DC (Figure 2) in obtaining an isoelectric EEG.

Analysis of the physical reflexes showed a significant effect of all factors on the occurrence of the corneal reflex at 20 s poststun (Table 1). At 40 s poststun, only
the interaction of waveform × voltage did not show a significant influence on the corneal reflex (Table 1). Birds were more likely to express a positive response when stunned with lower voltage or pulsed DC, whereas sine wave AC stunning resulted in the lowest percentage of corneal reflexes. Females showed more positive

Figure 1. Examples of electroencephalogram (EEG) traces of broiler chickens directly following waterbath stunning with different waveforms: sine wave AC in the upper panel, rectangular AC in the middle panel, and pulsed DC (with initial large-movement artifacts due to convulsions) in the lower panel. The amplitude of the y-axis is 80 μV and the area between the bold vertical lines represents 1 s.
reflexes than males. The occurrence of spontaneous eye blinking was significant for all factors in all 3 post-stun periods (Table 1). Birds stunned with higher voltage expressed less spontaneous eye blinking. Sine wave AC suppressed the occurrence of this reflex compared with that using rectangular AC (Figure 2). Birds stunned with pulsed DC showed the most spontaneous eye blinking. Females expressed more spontaneous eye blinking than males in all groups (Figure 2).

Failure to resume breathing indicated cardiac failure in the different stunning groups. Stunning voltage and waveform, sex, and the interaction of waveform × sex proved to be significant. Broilers were less likely to resume breathing when stunned with higher voltage or sine wave AC as compared with lower voltages or pulsed DC (Figure 2). Significantly more males failed to resume breathing (Figure 2). The results for the occurrence of wing flapping showed significantly more birds expressing wing flapping when stunned with sine wave AC compared with rectangular AC or pulsed DC (Figure 3). The effect of voltage was close to the level of significance. Higher voltage settings produced more wing flapping (Figure 3). Female broilers were significantly more likely to flap their wings than males (Figure 3). The interaction of waveform × voltage also showed a significant effect. Increasing voltage with the same waveform resulted in more birds with severe wing flapping (Figure 3).

The occurrence of epileptiform activity was significantly influenced by all factors and was generally low in all stunning groups (Table 1; Figure 4). Birds stunned with sine wave AC were less likely to express epileptiform activity than birds stunned with rectangular AC or pulsed DC. Increasing voltage suppressed the occurrence of epileptiform activity. Females generally showed more epileptiform activity than males (Figure 4). When leaving the waterbath, a high amount of birds showed tonic-clonic convulsions (81–100%, means not shown) for all stunning voltages and waveforms.

### Table 1. Results of the statistical analysis: Nominal logistic regression

<table>
<thead>
<tr>
<th>Factor effect</th>
<th>Voltage</th>
<th>Waveform</th>
<th>Sex</th>
<th>Waveform × voltage</th>
<th>Waveform × sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current</td>
<td>0.0077</td>
<td>&lt;0.0001</td>
<td>—</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>EEG, 2–30 Hz, P1 through 3, 0 to 40 s</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0138</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>EEG, 13–30 Hz, P1 through 3, 0 to 40 s</td>
<td>0.0006</td>
<td>0.0004</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>EEG, P1, 2–30 Hz</td>
<td>0.0038</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>EEG, P1, 13–30 Hz</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>EEG, P2, 2–30 Hz</td>
<td>0.004</td>
<td>0.0177</td>
<td>0.0077</td>
<td>NS</td>
<td>0.0408</td>
</tr>
<tr>
<td>EEG, P2, 13–30 Hz</td>
<td>0.0035</td>
<td>0.0052</td>
<td>0.0953</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>EEG, P3, 2–30 Hz</td>
<td>0.0002</td>
<td>&lt;0.0001</td>
<td>0.0487</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>EEG, P3, 13–30 Hz</td>
<td>0.0008</td>
<td>&lt;0.0001</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Corneal reflex 20 s</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0113</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Corneal reflex 40 s</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0041</td>
<td>NS</td>
<td>0.0004</td>
</tr>
<tr>
<td>Spont. eye P1</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0127</td>
<td>&lt;0.0001</td>
<td>0.0051</td>
</tr>
<tr>
<td>Spont. eye P2</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0038</td>
<td>0.0004</td>
<td>0.0008</td>
</tr>
<tr>
<td>Spont. eye P3</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.018</td>
<td>0.0003</td>
<td>0.0002</td>
</tr>
<tr>
<td>Breathing</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0001</td>
<td>NS</td>
<td>0.0013</td>
</tr>
<tr>
<td>Wing flapping</td>
<td>NS</td>
<td>&lt;0.0001</td>
<td>0.0249</td>
<td>&lt;0.0001</td>
<td>NS</td>
</tr>
<tr>
<td>Epileptic activity</td>
<td>0.0008</td>
<td>0.0048</td>
<td>0.0011</td>
<td>0.0013</td>
<td>0.008</td>
</tr>
<tr>
<td>Clonic-tonic convulsions</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0004</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

1EEG = electroencephalogram; Periods: P1, 10 to 20 s; P2, 20 to 30 s; P3, 30 to 40 s; Spont. eye = spontaneous eye movements.

### Table 2. Means and SD of the current obtained by male and female broilers in response to different waveforms

<table>
<thead>
<tr>
<th>Waveform</th>
<th>Voltage</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sine wave AC</td>
<td>60</td>
<td>72 ± 8</td>
<td>52 ± 10</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>92 ± 14</td>
<td>73 ± 6</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>174 ± 22</td>
<td>138 ± 15</td>
</tr>
<tr>
<td>Rectangular AC</td>
<td>60</td>
<td>67 ± 6</td>
<td>48 ± 7</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>91 ± 12</td>
<td>71 ± 9</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>153 ± 21</td>
<td>117 ± 15</td>
</tr>
<tr>
<td>Pulsed DC (duty cycle 1:1)</td>
<td>60</td>
<td>61 ± 7</td>
<td>36 ± 10</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>78 ± 17</td>
<td>57 ± 10</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>128 ± 24</td>
<td>103 ± 15</td>
</tr>
</tbody>
</table>

1AC = alternating current; and DC = direct current.
DISCUSSION

The aim of the study was to determine the different effects of 3 waveforms on the induction of unconsciousness and death considering 3 different voltage settings. First, it is very obvious that the same constant voltage resulted in a significantly different electrical current being delivered to the birds, depending on waveform and sex. Birds stunned with voltage of a sine wave AC resulted in higher amperage per bird than that from the application of a rectangular AC voltage. Birds stunned with pulsed DC of the same voltage obtained the lowest current (Table 2). Bilgili (1992) reported that higher voltage was necessary with pulsed DC to...
obtain the same amperage when compared with AC in a pilot processing plant.

Female broilers obtained a significantly lower current in all stunning groups than male birds treated with the same constant voltage (Table 2). The lower BW of female birds did not cause the distinction, given that this factor was corrected in the statistical analysis. Wornuth et al. (1981) did not find a different resistance between male and female broilers in their study, while laying hens showed a significantly higher resistance. This was explained by the considerably thinner legs and the tough skin of laying hens compared with broilers. It should be considered that laying hens are considerably older than broiler chickens. Rawles et al. (1995) reported a significantly higher voltage use for females to obtain the same current as compared with that used for males using 36- to 50-d-old broilers. Corresponding to the results presented here, BW could be excluded as the cause for the distinction in their study. However, a physiological difference with more highly resistant abdominal fat in female broilers has been discussed (Rawles et al., 1995). In the present study, it is thought that the higher resistance of female broilers might have been caused by their thinner legs. Although the feet and shackle were wet before stunning, male broilers with thicker legs may have a tighter contact, thus improving conductivity. However, the variation is a cause for concern, as under practical conditions, numerous broilers of both genders are stunned together. Multibird waterbath stunners might therefore cause even higher differences in current per bird, with potential effects on stunning efficiency and meat quality. Constant-current stunners should therefore be considered to overcome the physical variation between broilers. First attempts to develop such systems have been reported from the United Kingdom (Sparrey et al., 1993). The effect of different amounts of current on stunning efficiency should now be discussed.

Acceptable stunning setups should induce unconsciousness in at least 90% of the animals. The occurrence of a profoundly suppressed isoelectric EEG with less than 10% of the baseline EEG has been used to determine unconsciousness and insensibility in broilers (Raj and O’Callaghan 2004a,b; Raj et al., 2006a). In addition, a sound reduction of positive responses to the corneal reflex test and spontaneous eye blinking have been identified as tools to assess stunning efficiency (Prinz, 2009; Prinz et al., 2010a,b). Figure 2 shows the effect of the different electrical waveforms on these parameters. Sine wave AC achieved the best stunning results, whereas application of a rectangular AC was less effective (Figure 2). Pulsed DC treatments showed the lowest stunning efficiency (Figure 2). Assessment of the development of brain waves and reflexes over time following different waveform treatments will reveal a better understanding of their effect on the induction of unconsciousness.

In the groups stunned with sine wave AC, a low voltage of 60 only achieved an acceptable stunning result in male broilers. Although the percentage of birds in this

![Figure 3. Percentage of wing flapping in the different stunning setups, obtained from the predicted values of the nominal logistic regression. AC = alternating current; DC = direct current.](https://academic.oup.com/ps/article-abstract/91/4/998/1578255)

![Figure 4. Occurrence of an epileptiform or chaotic EEG (low-frequency epilepsy and slow-wave disturbance) in the different stunning groups, obtained from the predicted values of the nominal logistic regression. AC = alternating current; DC = direct current; EEG = electroencephalogram.](https://academic.oup.com/ps/article-abstract/91/4/998/1578255)
group with considerable brain activity in the 2–30 Hz band is slightly elevated in P1, it rapidly decreases in P2 and P3. This trend has been observed before (Prinz, 2009; Prinz et al., 2010a,b) and has been interpreted with the occurrence of epileptiform activity, followed by a profound EEG reduction (Raj and O’Callaghan, 2004a,b). The results of the 13–30 Hz band, however, show a profound EEG suppression in 90% of the broilers in P1, which can be interpreted with insensibility to pain (Raj et al., 2006a,b). The reflexes support this conclusion, as corneal reflex and occurrence of spontaneous eye blinking are reduced to less than 30% of the birds. Female birds treated with the same constant voltage did not show a comparable reduction of brain activity and physical reflexes (Figure 2). Their stunning result was inadequate, with less than 90% showing a profound suppression of the EEG in the 2–30 Hz band in all periods, and in P1, it was also shown in the 13–30 Hz band. The difference might have been caused by the significantly lower stunning current of 52 mA obtained by the females compared with 72 mA for the males (Table 2). Increasing the voltage setting to 80 V delivered a similar current of 73 mA to female broilers (Table 2). This group resulted in adequate stunning efficiency with a profoundly suppressed EEG in the 2–30 and 13–30 Hz bands in more than 90% of the birds (Figure 2). Moreover, corneal reflex and spontaneous eye blinking could only be observed in very few animals (Figure 2). It can therefore be concluded that stunning current, not voltage has the main influence to render broiler chickens unconscious. In the present study, sine wave AC of 70 mA proved sufficient to achieve unconsciousness in more than 90% of broilers. A minimum of 80 V of sine wave AC must therefore be applied in commercial slaughterhouses with an application time of at least 4 s to ensure adequate stunning results in female broilers. Failure of resumption of breathing in the majority of birds treated with this voltage indicates the induction of cardiac failure (Figure 2). This effect of low frequency sine wave AC has been observed before (Wilkins et al., 1998).

Broilers stunned with a rectangular AC of 70 Hz showed an overall lower stunning efficiency compared with that shown when using sine wave. A constant voltage of 60 V did not achieve a profound suppression of the EEG in male and female birds (Figure 2). Although almost all birds resumed breathing following stunning, the majority of birds also expressed spontaneous eye blinking at 40 s poststun (Figure 2). This is in contrast to the results of male broilers treated with sine wave AC of 60 V and might be caused by the lower current obtained by the broilers that received rectangular AC (Table 2; Figure 2). When voltage is increased to 80, male broilers show a profound suppression of the EEG in both the 2–30 Hz and 13–30 Hz bands in more than 90% of the birds in P2 and P3 (Figure 2). However, in both frequency bands, P1 indicates considerable brain activity in 20% of broilers. As previously discussed, this might be explained with epileptiform activity or a generally deranged state of the brain caused by the current flow. Corneal reflexes and spontaneous eye blinking could only be observed in a few birds (Figure 2), supporting the conclusion that this group was effectively stunned. Moreover, only 30% of the broilers were able to recover from the stunning process. Female broilers treated with the same setup were not adequately stunned according to the criteria set out in this study (Figure 2). A rectangular AC of 80 V is therefore only sufficient to render male broilers unconscious. This distinction might be caused by the lower stunning current of female broilers with 71 mA, compared with 91 mA per male bird when treated with 80 V. Adequate stunning efficiency of female broilers could be achieved with 120 V of rectangular AC, resulting in a current of 117 mA per bird (Figure 2). It can therefore be concluded that a minimum of 90 mA must be applied to achieve unconsciousness in 90% of the broilers treated with rectangular AC of 70 Hz. This value is considerably higher than the 70 mA minimum current established for sine wave AC. The waveform, therefore, has a major influence on stunning effectiveness. The more gradual rate of voltage change of a sine wave AC with excursion in positive and negative directions compared with the sharp vertical unilateral rise of a DC has been discussed as a possible cause (Raj, 2006).

Pulsed DC stunning seemed less effective than AC for 4 s, with neither males nor females obtaining adequate stunning results with 60 or 80 V. An increase to 120 V resulted in a profound suppression of the EEG in more than 90% of male broilers for both frequency bands (2–30 and 13–30 Hz; Figure 2). Although the percentage of birds with considerable brainpower in P1 was slightly higher, this corresponds with the findings for male broilers stunned with sine wave AC of 60 V and rectangular AC of 80 V, respectively, and could be explained by the occurrence of epileptiform activity or a deranged state of the brain caused by the current flow. The occurrence of spontaneous eye blinking could only be observed in very few birds for up to 40 s poststun, supporting the conclusion of good stunning effectiveness (Figure 2). Moreover, at 20 s poststun, the corneal reflex could not be elicited in more than 70% of the birds, indicating deep unconsciousness. In the later corneal reflex test at 40 s poststun, a rapid return of positive responses could be observed in birds that resumed breathing, indicating progressive recovery. Although spontaneous eye blinking was still markedly reduced, fast and efficient bleeding would be necessary for this stunning setup to prevent recovery of the birds before death from bleeding. Raj (2006) suggests a minimum of 40 s of unconsciousness following waterbath stunning to prevent recovery of the birds during bleeding.

Female broilers treated with the same constant voltage of 120 V of pulsed DC did not show adequate stunning efficiency (Figure 2). They only received a stunning current of 103 mA, compared with 128 mA for
males (Table 2). It can therefore be concluded that a minimum stunning current of 130 mA would be necessary to achieve unconsciousness in more than 90% of broilers treated with a pulsed DC current. This value is considerably higher than the necessary current established for sine wave or rectangular AC.

The effectiveness of pulsed DC for waterbath stunning has been questioned before (Raj et al., 2006b,c). Raj (2006) explains the lower stunning efficiency of pulsed DC with the current flow in one direction only, whereas AC flows in positive and negative directions. Results of previous studies (Prinz, 2009; Prinz et al., 2010b), on the other hand, show no difference in stunning efficiency of pulsed DC and rectangular AC waterbath stunning. In their study, a pulsed DC of 70 Hz rendered more than 90% of broilers unconscious with minimum currents of 80 mA. The distinction might be caused by the longer stunning time of 10 s (Prinz, 2009; Prinz et al., 2010b). This might have caused a longer duration of insensibility in the broilers than 4 s of stunning time in the present study. Raj et al. (2006b,c) used a shorter stunning time of 1 s with currents of 100, 150, and 200 mA. If the application time of a pulsed DC influences the duration of insensibility, birds might have recovered shortly after leaving the waterbath in the present study, thus leading to inadequate stunning results in the EEG analysis. The longer stunning time of 10 s, on the other hand, resulted in a longer period of insensibility, with a profoundly suppressed EEG for up to 40 s poststun. The influence of stunning time on the duration of unconsciousness after pulsed DC stunning needs further investigation. From the results of the present study, a 4-s stunning time with a pulsed DC can only be recommended with more than 120 V for commercial slaughterhouses.

Application of AC does not show this effect of stunning time. The results presented here correspond with findings of Prinz et al. (2009), who analyzed the effectiveness of frequency and the amount of a rectangular AC on the EEG of broilers. A current of 100 mA per animal showed an adequate stunning effect when applied for 10 s, as compared with more than 90 mA in the present study. Raj et al. (2006a) also confirmed the effectiveness of 100 mA with up to a 200-Hz sine wave AC after 1 s of stunning time. The results presented here show an even higher effectiveness of sine wave AC of 50 Hz, where currents between 70 and 80 mA per bird achieved a profound suppression of the EEG in more than 90% of the broilers.

The occurrence of epilepsy before the profound suppression of the EEG has been interpreted to indicate good stunning efficiency in broiler chickens (Schütt-Abraham et al., 1983). Raj et al. (2006a,b) found a high percentage of epileptiform activity in broilers stunned with AC, whereas pulsed DC resulted in fewer birds with an epileptic EEG. Prinz et al. (2010a,b) only detected epileptic activity in less than 10% of the birds with both, rectangular AC or pulsed DC. They concluded that the lower rate of epilepsy could be explained with the difference in stunning time (Prinz et al., 2010a,b). Whereas Raj et al. (2006a,b) stunned for 1 s, the current was applied for 10 s in the studies of Prinz et al. (2010a,b). Duration of epileptic activity has been reported from 9 to 17 s after the onset of current flow (Gregory and Wotton, 1987; Raj et al., 2006a) With 10 s of stunning time, a considerable amount of epileptic activity might be terminated in the waterbath or during transfer of the birds to the chicken EEG clamp before EEG recording (Prinz et al., 2010a,b). This can be supported by findings of the present study, where epileptic activity could be detected in a considerably higher number of birds for both AC and DC stunning as compared with that of the previous studies (Prinz et al., 2010a,b; Figure 4). With a shorter stunning time of 4 s, epileptic activity might have lasted for a sufficient time to be recorded. Moreover, a high percentage (81–100%) of birds showed clonic-tonic convulsions when leaving the waterbath in the present study. This has been associated with the occurrence of epilepsy in the brain (Schütt-Abraham et al., 1983). The level of epileptic activity during or shortly after waterbath stunning might therefore be considerably higher than the percentage identified on the EEG recordings.

Stun-to-kill methods have welfare advantages as recovery of birds during bleeding is prevented (von Wenzlawowicz and von Holleben, 2001). Electrical stunning that induces cardiac fibrillation might, on the other hand, cause meat-quality defects (Gregory, 1989). Induction of cardiac arrest in conscious birds has been questioned due to possible welfare concerns (Raj and Tserveni-Gousi, 2000). Resumption of breathing does not necessarily coincide with sensibility but indicates the ability of the bird to recover from stunning (von Wenzlawowicz and von Holleben, 2001). In the present study, failure of resumption of breathing following stunning was assessed as an indicator of cardiac failure caused by the stunning method. From Figure 3, it is clear that pulsed DC showed a considerably lower effect on the induction of death than either sine wave or rectangular AC. This confirms findings of Kuenzel and Ingling (1977). However, insensibility could be achieved in broilers stunned with a pulsed DC without inducing cardiac failure (Figure 3). This corresponds with findings of Prinz et al. (2010a,b), who observed a profound suppression of the EEG following pulsed DC stunning with 70 Hz in more than 90% of the birds, although most animals resumed breathing. With increasing current levels, however, birds encountered cardiac failure. In another study on the effect of a rectangular AC, most broilers encountered cardiac failure when stunned with 70 Hz and a minimum of 100 mA. Lower currents resulted in the resumption of breathing without a profound suppression of the EEG (Prinz et al., 2010a). The present study confirms these findings, as applica-
tion of a low frequency AC was either too low to cause a profound suppression of the EEG in 90% of the broilers or induced death in the majority of birds (Figure 2).

The occurrence of wing flapping in the first 40 s post-stun was assessed as an indicator for convulsions where stunning resulted in death of the birds (Figure 4). Sine wave AC stunning caused a very high proportion of wing flapping for all 3 voltage settings, followed by rectangular AC (Figure 3). Pulsed DC, on the other hand, only resulted in a maximum of 40% wing flapping for the highest stunning voltage of 120 V (Figure 3). This level is slightly lower compared with a previous study on pulsed DC stunning of 10 s (Prinz et al., 2010b). It can moreover be observed that the level of wing flapping was higher in females in the present study (Figure 3). On the other hand, fewer females encountered death, probably due to the lower stunning current. The effect of high stunning currents alone on the occurrence of death, probably due to the lower stunning current. The effect of high stunning currents alone on the occurrence of wing flapping must therefore be questioned. Further studies should investigate the effect of different stunning currents and waveforms on meat quality.

**Conclusion**

The same constant true root mean square voltage delivers a significantly different amount of true root mean square current per broiler, depending on sex and current waveform. Female birds have a higher resistance in the electrical waterbath, independent of the lifetime weight. This raises welfare concerns regarding multi-bird waterbaths operated at constant voltage and variable current. As stunning effectiveness is determined by stunning current, constant-current stunners should be considered instead of constant-voltage stunners.

The necessary stunning current to achieve unconsciousness varies significantly between waveforms. Although sine wave AC showed good stunning results with 70 to 80 mA, 90 to 100 mA per bird were necessary for rectangular AC. A pulsed DC (50% duty cycle) of 130 mA achieved the same effect. For pulsed DC, stunning time seems to influence the duration of unconsciousness, whereas this seems less important for AC. A stunning time of longer than 4 s is highly recommended for commercial slaughterhouses using pulsed DC.

Low frequency-alternating currents induce death in most birds that show adequate stunning efficiency. These setups can therefore only be recommended for stun-to-kill methods. Moreover, the occurrence of wing flapping as an indicator for convulsions is significantly higher for AC. Pulsed DC stunning can reduce the induction of death by the stunning operation. Wing flapping as an indicator for convulsion is significantly lower with DC stunning. It must however be assured that birds are adequately stunned for a sufficient period to prevent recovery. Meat quality effects of the different stunning setups must be analyzed separately.

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