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RESEARCH ARTICLE

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Water-bath stunning process in broiler chickens: Effects of voltage and intensity

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Abstract

The effect of electrical parameters (intensity and voltage) to obtain an effective water-bath stunning in a commercial poultry slaughterhouse was studied. A total of 390 broilers were randomly divided into six experimental groups according to the intensity (150 and 200 mA) and voltage (51-60, 61-80 and 81-100 V). Statistical analysis showed a significant ($p<0.001$) effect of electrical parameters on the cloacal reflex since the response rate was 8 s for 96% of broilers. On the other hand, the stunning treatments suppressed the palpebral reflex up to 12 s in 80% of broilers. All stun-treated broilers showed breathing response after 27 s with an average time between 45 and 50 s depending on voltage and intensity of the current. Regarding voltage, the percentage of complete neck cutting increased with the increase of voltage, presenting the highest levels in broilers stunned at 81-100 V (100 and 92.8%, for 150 and 200 mA, respectively). Concerning to intensity, the best results were obtained in broilers stunned at 150 mA, showing mean percentages of 94.83 and 87.30%, for 150 and 200 mA, respectively. The bruises on wings were significantly ($p<0.001$) affected by voltage, observing the highest values in broilers slaughtered at lower voltages. An opposite trend was observed on bruises under the wings since the lowest voltages showed ($p<0.001$) lower levels (1.34 and 1.42%, for 150 and 200 mA, respectively). Finally, bruises on dorsal winds and on back were significantly ($p<0.001$) influenced by intensity level, showing the lowest percentages in broilers stunned at 150 mA.

Additional keywords: animal welfare; reflex response; bruise; neck cutting; ocular reflex.

Abbreviations used: B (breathing); BB (bruises on back); BDW (bruises on wings dorsal); BUW (bruises under wings); BW (bruises on wings); C (contingency coefficient); CR (cloacal reflex); NT (neck tension); PR (palpebral reflex); R (recovery of consciousness).

Authors' contributions: Conceived, designed and performed the experiments: LFPG, MN, LV and IGT. Animals rearing: MN and AL. Analyzed the data: MN, NC, IGT, and LV. Contributed reagents/materials/analysis tools: MN, AL, and LFPG. Wrote the paper: MN, NC, LV, IGT and JML. All authors read and approved the final manuscript.

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Introduction

During the last years, animal welfare concerns have gained importance for producers, consumers, and retailers of animal-based food products, mainly in the European Union (EU) (Faucitano *et al.*, 2017). In this regard, the EU approved the Council Regulation (EC) No 1099/2009 on the protection of animals at the time of the killing. Nowadays, EU regulation has approved the use of captive bolt, head electrical stunning, head-to-body electrical stunning, electrical water bath and carbon dioxide methods as stunning methods for poultry industry (Berg & Raj, 2015).

According to Bourassa *et al.* (2017), electrical water bath stunning is commonly utilized in commercial poultry slaughter. Several parameters such as the frequency, the intensity, the voltage, and the current type (alternating current or direct current) are the most important electrical factors that can be modified to enhance the stunning effectiveness and obtain high-quality meat. In this regard, the European Community established a minimum current of 120 mA for stunning, which should induce cardiac arrest in 90% of broilers.

Concerning frequency, high frequencies (>800 Hz) are less effective at stunning and require higher current intensities (Prinz *et al.*, 2010; Girasole *et al.*, 2016), medium frequencies (between 600 and 400 Hz) improve carcass quality and decrease breast muscle hemorrhaging in broilers (Wilkins *et al.*, 2000), and low frequencies (<200 Hz) cause high muscle contractions and consequent rupture of small blood vessels in the skin and/or flesh (Gregory, 1989). According to Girasole *et al.* (2016), frequencies above 1,200 Hz are not recommended to broiler electronarcosis since the return to consciousness is very fast, which do not guarantee the maintenance of the state of unconsciousness and the insensibility to pain until death, causing animal suffering. So, frequencies higher than >300 Hz are commonly used in poultry slaughterhouses to preserve carcass quality (Gazdziak, 2007).

In order to verify that broilers do not present any signs of consciousness or sensibility in the period between the end of the stunning process and death, some authors (Coenen *et al.*, 2007; Prinz *et al.*, 2012) recommend assessing brain waves through electroencephalogram recording. However, this method is difficult to apply in commercial slaughterhouses. So, modifications in the behaviour of broilers (such as spontaneous blinking and swallowing, head shaking, and wing flapping), physical reflexes (cessation of breathing, onset of seizures, and fixed eye, for instance) and physiological signs (response to external stimulus such as corneal reflex and response to pain stimulus such as comb or toe pinching) are the common indicators used by the operators to verify that broilers do not present any signs of consciousness (Erasmus *et al.*, 2010; Prinz *et al.*, 2012). With regard to post-stunning injuries, wings are the main part of the carcass affected and bruises are the most common type of trauma (71.42% of the total traumas, averaging 1.0 to 3.0 cm in diameter) (Siqueira *et al.*, 2017).

In recent years, the several combinations of the electrical parameters at slaughter, such as intensity, frequency, voltage or different waveforms; have been used under commercial processing conditions to enhance broiler welfare and avoid meat downgrading conditions. However, more studies are needed to optimize the water-bath stunning electrical parameters to improve animal welfare in poultry slaughterhouses. Therefore, the aim of the present work was to assess different electrical parameters (intensity and voltage) to obtain an effective water-bath stunning in a commercial poultry slaughterhouse. In order to evaluate the state of consciousness of broilers cloacal reflex (CR) and palpebral reflex (PR), neck tension (NT), rhythmic breathing (B) and total recovery (R) were observed. Moreover, the influence of the tested electrical parameters on broilers carcass damage (bruisers on wing) was also evaluated.

Material and methods

Animals

The experiment was conducted in a commercial slaughterhouse (Coren) located in Ourense (Spain) equipped with electrical water-bath for bird stunning. A total of 390 Ross broiler males with an average age of 45 days (range 36 to 51 days) and a final live weight of 3.2±0.3 kg was used. The birds were caught and kept in transport boxes for 2-3 h before stunning. They were then randomly divided into six experimental groups according to intensity and voltage. All experimental groups consisted of 65 broilers.

Slaughter process and sampling

A commercial stunner (LINCO, Trige, Denmark) with an alternating current and frequency of 395 Hz was used to alter the stunning intensity and voltage levels of the electric current provided to the water bath. For stunning, single broilers were immersed up to the base of their wings in the electrified water bath. The slaughter line speed was set up to ensure that stunning time was superior to 7 s for each bird. Two different intensities (150 and 200 mA) combined with three different voltages (51-60, 61-80 and 81-100 V) were tested. The six treatments tested were:

Trial 1: intensity = 150 mA and voltage = 51-60 V
Trial 2: intensity = 150 mA and voltage = 61-80 V
Trial 3: intensity = 150 mA and voltage = 81-100 V
Trial 4: intensity = 200 mA and voltage = 51-60 V
Trial 5: intensity = 200 mA and voltage = 61-80 V
Trial 6: intensity = 200 mA and voltage = 81-100 V

These selected parameters are in agreement with those required in Annex I of the Regulation (EC) N.1099/2009 (EC, 2009). The state of consciousness, as a result of an ineffective or poor stun of the broilers, was evaluated after stunning. The frequency of CR and PR -as well as NT, B, and R of the broilers- was assessed by a visual grading system. For each of the listed reflexes or behaviors, the necessary times to initiate them (latencies) were recorded. After the stunning process, the broilers were removed from the shackles and put on its side on a table. The CR is an involuntary behavior, which consists of a sporadic sucking movement of the cloacal lips, and it can be an indicator of consciousness grade. The PR was assessed as described by Erasmus *et al.* (2010) through touching the medial canthus of the eye. The appearance of neck tension was considered when the manipulator raised the broiler head and the bird could hold it itself (Mouchoniere *et al.*, 1999). The B was determined by observing movement in the vent area, whereas, the R was accounted for broilers that recovered the absolute conscious state.

For the neck cutting stage, the number of injured blood vessels was verified by *post-mortem* examination. A ventral cut across the throat, close to the head, is necessary to obtain a successful severance of both common carotid arteries and both external jugular veins. After visual inspection, the animal batch was automatically slaughtered by exsanguination through a neck cutting up to 150 s. The carcasses were then scalded, plucked and eviscerated in the same processing line. The appearance of four different bruises (Fig. 1) were assessed and recorded by a trained panel, differentiating between bruises on wings (BW), bruises on under wings (BUW), bruises dorsal wings (BDW) and bruises on back (BB).

Statistical analysis

All results were analyzed by SPSS 23.0 programme for Windows (IBM Corporation, NY). Correlations between variables and final live weight ($p<0.05$) were determined by Pearson´s linear correlation coefficient. Data of qualitative traits including reflex response (CR, PR, NT, B, and R) were calculated as latency intervals and were plotted as frequency percentage. The effect of current parameters (intensity and voltage) and the interaction voltage × intensity in R were evaluated with a cross-tabulation by chi square test. The contingency coefficient (C) was used to compare the magnitude of the association between different currents and chicken reflex response. An analysis of variance (ANOVA) of one way was performed to determine differences between stunning currents and each bruise frequency. Duncan's t-test was used to analyses the least squares mean (LSM) for a significant level $p<0.05$.

Results

The effect of electrical treatments in stunning indicators (CR, PR, NT, B, and R) of broilers is shown in Fig. 2. Statistical analysis showed a significant ($p<0.001$) effect of current parameters on CR, since the response time was 8 s for 96% of broilers. Although the results of the contingency coefficient estimation showed a slight association (C=0.13), a total of 14.5% of chickens stunned with voltages over 80 V showed CR after 8 s, while this percentage was reduced to 2.5% and 4.2% for broilers stunned at 51-60 and 61-80 V, respectively. Regarding intensity, statistical analysis did not indicate significant differences on CR among samples. A similar pattern was observed for PR, with an average latency time of 11.6±1.3 s (Fig. 2). The stunned treatments suppressed this reflex for up to 12 s in 80% of broilers. However, the application of current with high voltages increased ($p<0.001$) the delay of PR, since the response rate was over 12 s for 53.8% and 43.2% of stunned chickens of trials 3 and 6, respectively (Fig. 2).

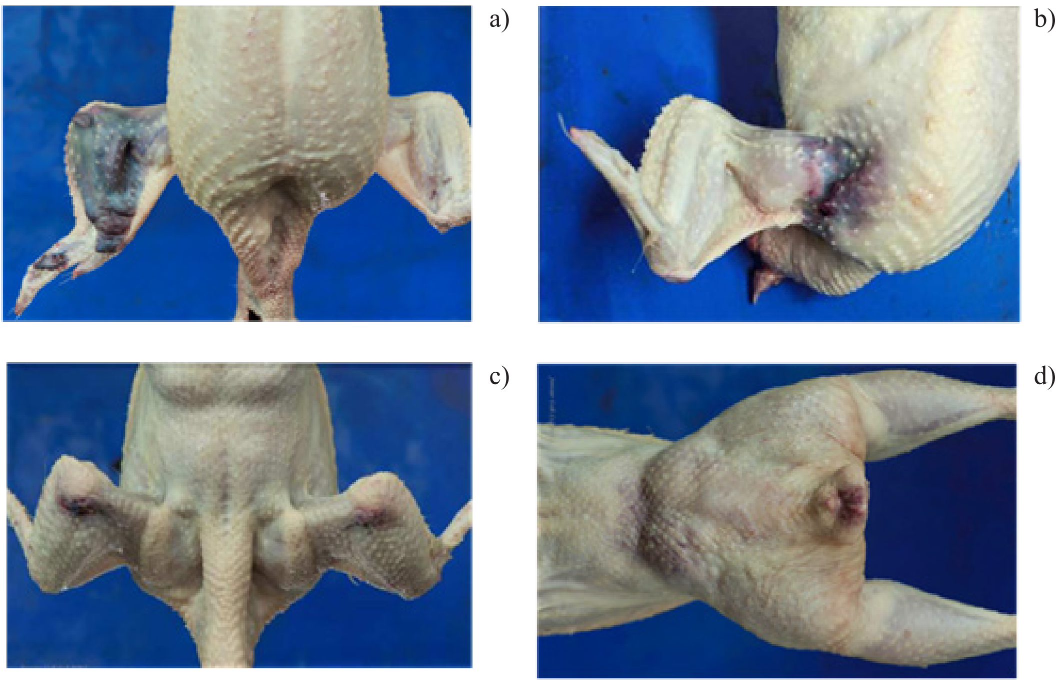

Figure 1. Evaluation of carcass damage through the bruises appearance: bruise on wing, BW (a); bruise under wing (b); bruise on dorsal wing (c); bruise on back (d).

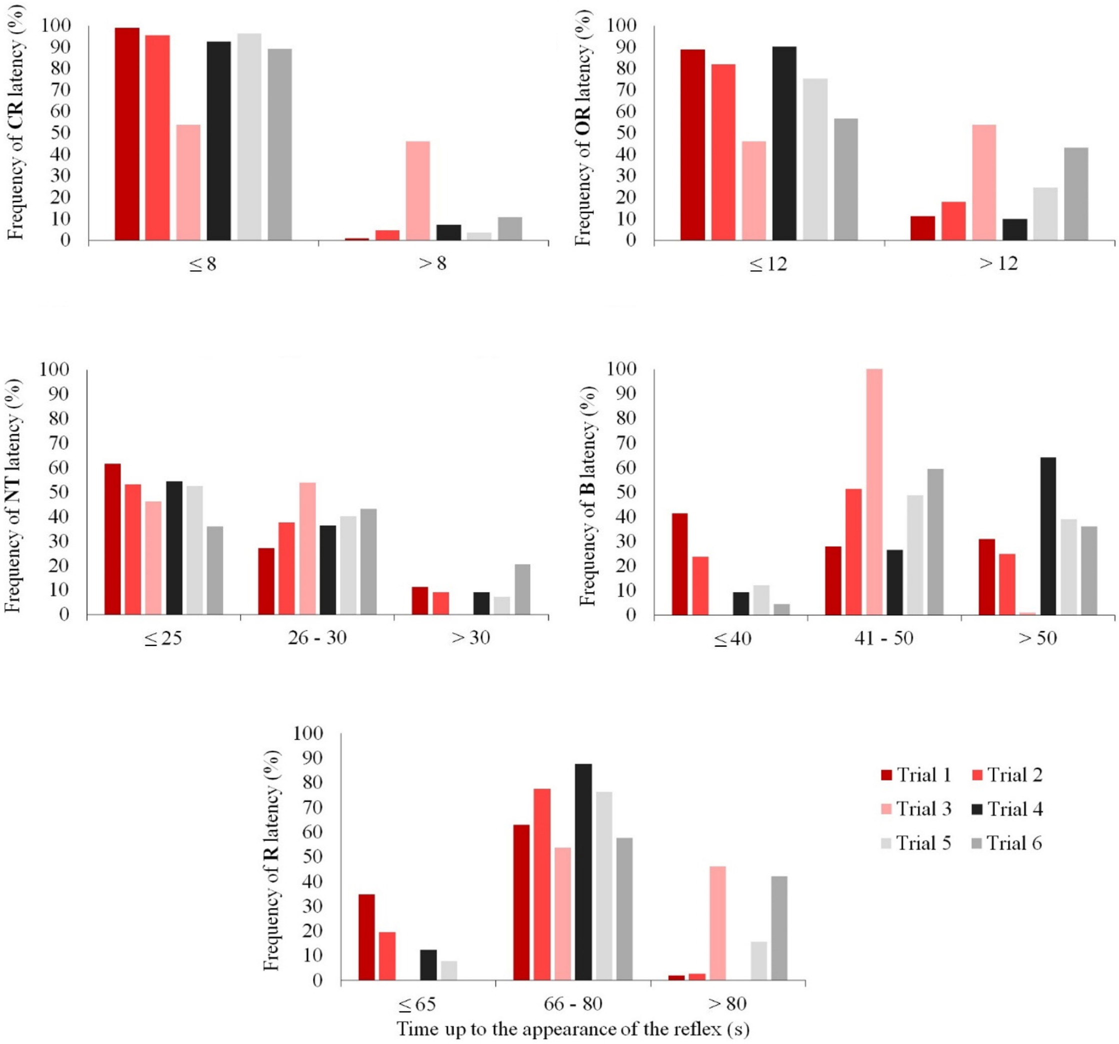

Figure 2. Effect of electrical parameters on cloacal reflex (CR), palpebral reflex (PR), neck tension (NT), rhythmic breathing (B) and total recovery (R) (mean values of 65 samples in each trial). Trials: 1: intensity = 150 mA and voltage = 51-60 V; 2: intensity = 150 mA and voltage = 61-80 V; 3: intensity = 150 mA and voltage = 81-100 V; 4: intensity = 200 mA and voltage = 51-60 V; 5: intensity = 200 mA and voltage = 61-80 V and 6: intensity = 200 mA and voltage = 81-100 V.

Immediately after stunning, chicken carcass showed relaxed appearance and absence self-controlled movement of the head. So, the absence of tension in the neck muscle is commonly used to indicate unconsciousness. After 25 s of stunning, 38% of the analyzed broilers showed neck tension. Regarding trial 6 (80-100 V and 200 mA), latencies over 30 s were observed for 20.7% of broilers, whereas lower incidences (9.3%) were observed by those of trial 4 (51-60 V and 200 mA).

Although the first breathing response was observed after 27 s of stunning, the average time for this consciousness indicator was the range of 45 and 50 s depending on intensity and voltage (Fig. 2). An interaction was observed between currents (p<0.001, C=0.27), since 32.4% of stunned chickens presented breathing recovery times over 50 s with significant differences associated with intensity and voltages (C=0.20 and C=0.24, respectively). In this regard, chickens that were subjected to high intensity stunning (200 mA) needed more time to recover breathing (40.5% needed more than 50 s). On the other hand, the R time after stunning process ranged from 60 to 99 s. Moreover, 46.2% of broilers that were stunned by trial 6 (81-100 V and 200 mA) did not show recovery signals before 80 s (Fig. 2). From the data in Fig. 2, it is apparent that birds treated with trials 3 and 6 had higher latencies (both with voltage over 80 V). So, the voltage used in the stunning process was associated with the unconsciousness duration (p<0.001; C=0.24).

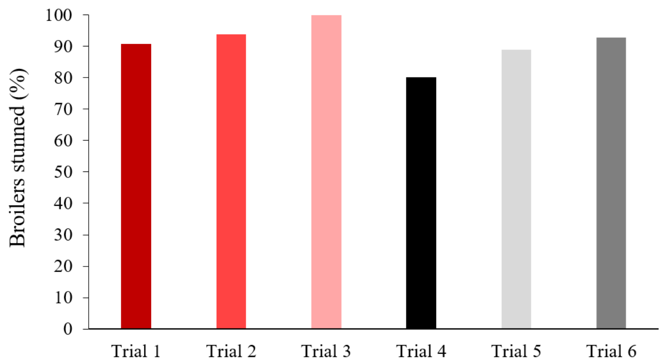

Figure 3. Effect of electrical parameters on complete neck cutting step, expressed as percentage of broilers stunned (%) (mean values of 65 samples in each trial). See trials in Figure 2.

Figure 3 shows the effect of electrical parameters in the complete net cutting step of stunned broilers. Regarding voltage, the percentage of broilers subjected to complete neck cutting augmented by increasing voltage, presenting the highest levels in broilers stunned at 81-100 V (100 and 92.8%, for 150 and 200 mA, respectively). Concerning to intensity, the best results were obtained in broilers stunned at 150 mA, showing mean percentages of 94.83 and 87.30%, for 150 and 200 mA, respectively. Finally, the results obtained from the cross-tabulation analysis showed a complete neck cutting rate of 91% of all studied broilers.

The appearance of bruises in different parts of wings were studied separately, in order to determine the relation of some carcass damage with the stunning current applied. Table 1 shows the effect of electrical parameters used in the incidence of BW, BUW, BDW, and BB of stunned broilers. The BW incidences were significantly (p<0.001) affected by voltage used. The highest BW values were observed in broilers slaughtered at a higher voltage in the range of 81-100 V (11.4 and 13.1%, for currents of 150 and 200 mA, respectively). An opposite trend was observed on BUW incidences wherein the lowest voltages (at 51-60 V) showed significantly (p<0.001) lower BUW levels (1.34 and 1.42%, for currents of 150 and 200 mA, respectively). These percentages suggest a negative correlation between voltage and BW incidences and a positive correlation between voltage and BUW incidences.

On the other hand, BDW and BB incidences were significantly (p<0.001) influenced by intensity, showing mean BDW percentages of 9.09 and 11.16% for 150 and 200 mA, respectively and average BB values of 9.15 and 10.70% for 150 and 200 mA, respectively. These percentages suggest a positive correlation between intensity and BDW and BB incidences.

Table 1. Effect of electrical parameters on bruises (expressed as %) in broilers stunned at six different current parameters (mean values of 65 samples in each trial).

Trial		BW	BUW	BDW	BB
1		15.4^{a}	1.34^{a}	9.86^{abc}	9.75^{ab}
2		14.0^{bd}	1.38^{a}	9.38^{ab}	9.12^{ab}
3		11.4^{c}	3.90^{b}	8.05^{a}	8.59^{a}
4		14.6^{ab}	1.42^{a}	10.8^{bc}	10.5^{ab}
5		14.5^{ab}	2.13^{c}	11.1^{bc}	10.7^{ab}
6		13.1^{d}	3.00^{d}	11.6^{c}	10.9^{b}
SEM		0.046	0.013	0.073	0.077
Sig.	V	***	***	ns	ns
	I	ns	ns	***	**
	V × I	***	***	ns	ns

BW: Bruises on wings. BUW: Bruises under wings. BDW: Bruises on dorsal wings. BB: Bruises on back. V: Voltage of stun. I: Intensity of current. See trials in Figure 2. [a-d] Means within the same column that have no common letters differ significantly by Duncan´s test (p<0.05). SEM: Standard error of mean. Sig.: Statistical significance difference of multivariant ANOVA; ns: not significant; ** p<0.01; * p<0.001.

Discussion

The number of animals into the bath and their body characteristics influence the effectiveness of stunning. This means that in the same slaughter line, broilers with differences in weight or size (Berg & Raj, 2015), sex (Prinz *et al.*, 2012), fat content or plumage condition (Shields & Raj, 2010) can receive unequal currents. It is worth mentioning that statistical analysis did not show significant (p>0.05) differences in live weights among stunned broilers.

Cors *et al.* (2015) reported that the detection of brainstem reflexes does not allow any conclusions about unconsciousness, but certainly the absence does. So, reflex evaluation can help to determine stun effectiveness, although they are considered unreliable. The results of this study suggest that there is an association between the stunning parameters and the appearance of CR, but it is not a reliable indicator of a successful water-bath stun. This is in accordance with the Humane Slaughter Association and Council of Justice to Animals (HAS, 2015), since in their publication about electrical water-bath stunning of poultry, they indicated that CR is not a sign of recovery. On the contrary, Barbosa *et al.* (2016) reported that the cloacal movement is considered an indirect indicator of rhythmic breathing.

On the other hand, the absence of corneal reflex is considered a reliable indicator of deep unconsciousness in poultry (Gregory, 1989; Prinz *et al.*, 2010; Erasmus *et al.*, 2010), but a positive response does not necessarily mean that the animal is able to perceive pain. The presence of ocular reflexes such as corneal reflex or nictitating membrane reflex indicates the bird is alive but do not necessarily indicate it is conscious (Siqueira *et al.*, 2017). In addition, Prinz *et al.* (2010) suggested that under practical field conditions a maximum amount of about 30% of birds with a positive corneal reflex can be accepted as effective stunning treatment. Taking into account this level, in our study, all stunned broilers presented a suppression of the corneal reflex above 70%.

Absence of NT has been used as an insensibility sign (Erasmus *et al.*, 2010) and loss of consciousness (Gerritzen *et al.*, 2004) after stun process, whereas the recovery of this reflex has been related to the control of muscle tone and, therefore, to the central nervous system (Gibson *et al.*, 2016). Our mean NT values were lower than those found by Wilkins *et al.* (1999) who observed that the average time to the NT appearance was 52 s. In addition, higher NT values were found by Mouchoniere *et al.* (1999) when studying the effect of a 300 Hz current in turkey males (around 80 s). These differences could be due to the experience of the observer and the evaluation system used in order to assess this indicator (Raj & Tserveni-Gousi, 2000). In this regard, Wilkins *et al.* (1999) stopped the evaluation when chickens were able to balance on their feet, whereas Mouchoniere *et al.* (1999) waited up to the appearance of corneal reflex and -with turkey put on side on the floor- could sustain its own head.

During the current's transfer in the chicken body, muscle contraction and rhythmic breathing stop (Shields & Raj, 2010). The recovery of this capacity is one of the first indications of consciousness regain, so, it has been assessed in several studies. Bilgili (1992) concluded that the rhythmic breath did not achieve normal levels up to 60-75 seconds post-stun. However, in our study, B values were more similar than those reported by Prinz *et al.* (2010), who found that 66% of stunned chickens recovered their breath after 40 s (stunned at 400 Hz and 150 mA). On the contrary, Wilkins *et al.* (1999) obtained average times of 15 s for the appearance of rhythmic breath in birds stunned with a constant current and 500 Hz.

Hindle *et al.* (2010) established a 60 s period of unconsciousness for a successful stun process. So, applying different combinations of current intensity and voltage resulted in correctly stunning of our broilers. Moreover, more than 40% of broilers stunned with the current of high voltages (trials 3 and 6) presented the longest R periods (above 80 s). These values can be considered adequate to keep the insensibility during the slaughter step. In particular, the suppression of reflex responses was improved by high-intensity currents and voltages trials.

On the other hand, electrical stunning methods can also cause the death of chickens due to ventricular fibrillation, which may be associated with increased incidence of carcass damage, such as red wingtips and hemorrhaging (Gregory & Wilkins, 1989). Similarly, bruises caused by poor stunning can generate defects in meat and reduce quality (Sante *et al.*, 2000). According to Gregory (1989), it is difficult to discriminate, under commercial conditions, bruises as effects of electrical stunning from other intervening factors, such as hanging, catching, and picking. In addition, the loss of commercial value during poultry slaughter has also been associated with convulsions. In this case, these bruises are generated by the severe wing flapping and uncontrolled movement of the muscles (Gerritzen *et al.*, 2004). Consequently, the meat obtained from these animals must be sold as low-quality cuts.

Hamdy *et al.* (1961) suggested that 90% of all bruises on broilers occur within the last 12 h that the birds are alive and most of them are due to initial steps in slaughter processing, such as stunning. In this context, our results obtained for BUW incidences are in agreement with data reported by other authors who observed that using high voltage currents (over 100 V) resulted in significantly more hemorrhages in the breast (Veerkamp & Vries, 1983) and bruised wing joints (Heath, 1984; Bilgili, 1999). However, Ali *et al.* (2007) noticed that the application of an alternate current with moderate voltage (53 to 63 V) presented better results on carcass damage. This finding is in disagreement with data obtained in our study since the application of high voltage (90 V) showed the lowest BW incidents. Regarding intensity, our outcomes are in agreement with data reported by Gregory & Wilkins (1989) who found that that electrical stunning of broilers at the higher frequency (1,500 Hz) caused fewer convulsions and hemorrhages than at lower frequency (50 Hz).

The absence of response in palpebral reflex, neck tension, breathing and signs of recovery are key factors to ensure a free pain slaughter. The results obtained, using two different intensities and three different voltages, showed that high intensities (200 mA) and low voltages (51-60 V) offered the best combination of electrical parameters to effectively stun the majority of broilers. Finally, the results observed for BW and BUW indicated a significant influence of voltage, whereas BDW and BB depended on the intensity of the current.

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