Electrical Water Bath Stunning of Broilers: Effects on Breast Meat Quality

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The objective of this study was to investigate the influence of electrical water bath stunning treatments (pulsed direct current) on stress conditions of poultry meat. This was carried out by evaluating meat quality measured through the incidence of PSE (pale, soft, exudative) in broiler breast fillet meat. We applied the randomized factorial design 2² with three replications at the central point, with independent variables, including voltage (40, 80, 120 V) and electrical frequency (100, 400, 700 Hz). The response functions under study were pH, L*, a*, b*, WHC and incidence of PSE meat. Samples presenting with pH of ≤ 5.80 and L* of ≥ 53.0 were classified as PSE whereas those with $5.80 < \text{pH} < 6.00$ and $44.0 < \text{L}^* < 53.0$ were considered normal. The fillets taken from birds without stunning presented 54.14% of PSE meat samples, which decreased to 25.0% with stunning. The response functions L* and incidence of PSE showed regression ($P \leq 0.05$) with good adjustment of experimental data to the proposed model. The lowest incidence of PSE meat was observed when voltage and frequency were at their highest levels. In poultry slaughter, the applied electric stunning voltage of 120 V with a frequency of 700 Hz was determined to be the most effective for inhibition of PSE development.

Key words: frequency, PSE broiler chicken meat, voltage

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Introduction

In most countries, stunning of animals for slaughter is a legal requirement, where the animals for meat consumption should instantaneously be rendered insensible and remain insensitive to pain until the brain is completely unresponsive (Council Directive 93/119/ CEE, 1993). The most universally accepted means to immobilize birds before slaughter is electrical stunning (Bilgili, 1999). The state of unconsciousness induced by electricity results from the inhibition of impulses in both the reticular activating and the somatosensory systems (Heath *et al.*, 1994). The electrical current reaching the brain should be adequate enough to induce a seizure and render the bird to be insensitive to pain (Gregory and Wotton, 1989). In general, this current is less than that required for ventricular fibrillation; thus, avoiding killing by electrocution. However, while insufficient current may physically immobilize the bird, it cannot prevent its per-

ception of pain, stress, or discomfort (Fletcher, 1993). The intensity of electric current used for stunning can vary among poultry slaughterhouses. Those in the European Community recommend that the minimum current used for stunning to be 120 mA, which will induce cardiac arrest in 90% of the birds. This causes instantaneous and irreversible stunning (Poole and Fletcher, 1998), which ensures that the birds are not subjected to any conscious stress associated with recovery from the stunning. In the United States, the voltage and frequency used in the chicken stunning vary, with 77.4% of poultry slaughterhouse systems utilizing low voltage systems (10 to 25 V) with high frequency (500 Hz) (Gregory and Wotton, 1989). In these cases, the main objective of stunning is immobilization of the bird with minimal carcass damage (Heath *et al.*, 1994). In Brazil, the electrical current or the voltage applied varies among slaughterhouses, and there is currently no legal specification. This current causes generalized contractions that can have an obvious and marked effect on muscle characteristics. For example, if excessive currents or low frequencies are used, the electrical stunning has the potential to induce bleeding and broken bones as a result of strong contractions (Gregory and Wilkins, 1989; Rawles *et al.*, 1995). These stressful conditions accelerate the onset of rigor mortis, which would be

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avoided by the use of proper use of electrical stunning methodologies (Ma and Adis, 1973; Papinaho and Fletcher, 1995). Contreras and Beraquet (2001) studied the effect of various voltages (20, 40, 80 and 100 V at 60 Hz and no stunning) and frequencies (60, 200, 350, 500 and 1000 Hz at 40 V) on the efficiency of stunning, blood loss and defects in chicken carcass. At a current of 40 V and frequency of 1000 Hz, they observed that 90% of animals were stunned and blood loss was maximized. Furthermore, additional studies demonstrated that stressful conditions just before preslaughter activities promoted the development of PSE (pale, soft, exudative) meat (Mitchell and Kettlewell, 1998; Barbut *et al.*, 2008; Oba *et al.*, 2009). This was shown to be caused by the rapid decline of pH values while the carcass is still warm; thus, leading to the denaturation of myofibrillar proteins (Olivo *et al.*, 2001; Barbut, 1997) and loss of their functional properties (Kissel *et al.*, 2009).

Several studies have examined the relationship between electrical stunning and animal stress with emphasis on meat quality (Lee *et al.*, 1979; Craig and Fletcher, 1997; Northcutt *et al.*, 1998; Bilgili, 1999; Alvarado and Sams, 2000; Gregory, 2005) however thus far, this has not been established. Thus, the objective of the present study was to investigate the influence of electrical stunning on the animal's stress and the subsequent effect on meat quality.

Material and Methods

Animals

The experiment was conducted during the summer season in a commercial slaughterhouse in the southwest region of the state of Mato Grosso, Brazil. The Cobb mixed-sex broilers, aged 44 days were routinely prepared for slaughter after a 6-h fast prior to harvesting. The birds were manually harvested with 8–10 animals per cage, and received an overall shower mist before truck transportation (Simões *et al.*, 2009; Langer *et al.*, 2010). During transportation, the average speed was 60 km/h and the distance from the farm to the commercial slaughterhouse was 40 km. During truck reception at the slaughterhouse, the birds received an ambient temperature ($30.5^{\circ}\text{C} \pm 2$) water mist and ventilation for 40 min before slaughter.

Animal Slaughter and Sample Collection

Before slaughter, birds were divided into 2 groups: The first group was under the treatment of water shower mist and electrical stunning (WtS) ($n=30$) and the other without stunning (WoS) ($n=30$). The electrical stunning was performed with a stunner machine (FX 3.0 CC, Industrial Electronic Flux) under pulsed direct current, with the chicken's head submerged in a vat with clean water. The electrical parameters were as follows: voltage=80 V and frequency=400 Hz for 14 s per bird. The automated bleed was performed within 2 s of stunning. The WoS birds were hung and immediately bled manually, which took approximately 5 min. Subsequently, the activities followed the standard industrial practices consisting essentially of scalding at 58°C for 2 min, defeathering, mechanical evisceration, and cooling, first in a pre-chiller (8 to 16°C for 12 min) followed by a

chiller (0 to 4°C for 50 min), after which the carcasses were moved forward using a cold-water counter flow design. The breast fillet samples (*Pectoralis major*) were collected approximately 1 h and 30 min after death and stored at 4°C for 24 h prior to the analysis of pH, color (L^* , a^* and b^*), and water holding capacity (WHC).

Experimental Design

We used a 2^2 full factorial experimental design with three replicates at the center point, where the independent variables were: voltage and electrical frequency, levels -1 , 0 and $+1$, for a total of 7 assays (Table 1). The assays were conducted randomly and each assay used 30 chickens. The response surface methodology (RSM) was used to evaluate the effects of voltage (x_1) and frequency (x_2) and their interaction on the dependent variables or responses. The response functions evaluated were: pH, L^* , PSE percentage and water holding capacity. The analysis of variance (ANOVA) and the coefficients of determination (R^2) were used to compare the fit of the models against the experimental data. The repetitions at the central point provided the degrees of freedom to calculate the pure error and, subsequently, the lack of fit test. All analyses were carried out and all graphs were created using the STATISTICA program for Windows, version 7.0 (Statsoft, 2004). The model for each response was expressed in the form of Equation 1, where $\hat{Y}$ =response, x_1 and x_2 =codified variables voltage and frequency, respectively, and β =estimated coefficient on the response surface, and e =pure error.

$$\hat{Y} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{x_1 x_2} + e \quad (\text{Equation 1})$$

To compare the results between the WtS and WoS treatments we used the Student's *t*-test at 5% probability (STATISTICA 7.0 software, Statsoft, 2004). For the incidence of PSE measurement, we used the binary variation (1 and 0) where 1 means normal and 0 means PSE meat.

pH and Color Measurement

The pH values were determined by inserting electrodes into the breast muscle using a contact pH meter system (Testo, Model 205). The analysis was performed in triplicate

Table 1. Experimental assay for the 2^2 full factorial design with three replications at the center point (C)

Assay	Independent Variables	
	Voltage $x_1(X_1)$	Frequency $x_2(X_2)$
1	-1 (40 V)	-1 (100 Hz)
2	$+1$ (120 V)	-1 (100 Hz)
3	-1 (40 V)	$+1$ (700 Hz)
4	$+1$ (120 V)	$+1$ (700 Hz)
5 (C)	0 (80 V)	0 (400 Hz)
6 (C)	0 (80 V)	0 (400 Hz)
7 (C)	0 (80 V)	0 (400 Hz)

x_i —Encoded value; X_i —real value.

24 h post-mortem as previously described by Olivo *et al.* (2001).

A colorimeter (Minolta CR 400) was used to evaluate the color parameters on the posterior surface of the fillets, including L* (lightness), a* (red component), and b* (yellow component), with three different reading points at the sample surface (Soares *et al.*, 2002).

Classification of Chicken Fillets

The samples were classified as either PSE or normal meats based on pH and L* values as described by Soares *et al.* (2002). Thus, the fillets with $L^*_{24h} \geq 53.0$ and $pH_{24h} \leq 5.80$ values were classified as PSE, while samples under $44.0 < L^*_{24h} < 53.0$ and $5.80 < pH_{24h} < 6.00$ values were considered normal.

Water Holding Capacity (WHC) Measurement

WHC was determined based on the technique described by Hamm (1960). Samples weighing 2.00 ± 0.10 g were carefully placed between two filter papers and left under a 10 kg weight for 5 min. The samples were weighed again and the WHC was calculated and expressed as a percentage of water exuded using the formula: $WHC (\%) = 100 - [(initial\ weight - final\ weight) / (initial\ weight)]$ (Wilhelm *et al.*, 2010).

Results and Discussion

Table 2 shows the average values of pH, color (L*, a* and b*), WHC, and PSE incidence of chicken fillets from birds subjected to WtS [conditions = 80 V, 400 Hz (ES)] and those from WoS conditions. Compared to WoS samples, WtS samples showed post-mortem pH values that were significantly higher ($P \leq 0.05$), 5.87 compared to 5.82, respectively. These results are consistent with a previous study (Craig and Fletcher, 1997), which observed that fillets from WtS broilers under high current (125 mA, 50 Hz, 5 s) or low voltage (11 V, 500 Hz, 10 s) showed higher pH values than those from non-stunned birds. The lower pH value observed for fillets from WoS birds was directly related to stress experienced at the time of bleeding, which promotes an increased rate of glycolysis in muscles. The application of electrical stunning has been associated with a decreased rate of post-mortem glycolysis. This is due to lower stress re-

sulting from birds showing less fighting movements at the time of sacrifice (Lee *et al.*, 1979; Thomson *et al.*, 1986; Murphy *et al.*, 1988). The pH response obtained experimentally (Y) and the estimated model ($\hat{Y}$) are shown in Table 3, while Table 4 presents the analysis of variance for models generated for the response functions.

The chicken fillets from slaughtered WoS birds showed L* values significantly higher (54.91) than those from WtS birds (52.53) (Table 2). Alvarado and Sams (2000) observed higher L* values for turkey filets that were slaughtered under WoS conditions comparing to turkey meat samples from WtS. Furthermore, Craig *et al.* (1999) also found that fillets samples from WtS (11 V, 500 Hz for 10 s and 125 mA, 60 Hz for 5 s) had lower L* values when compared to the control group of WoS. The results of our pH and L* determination studies on fillet samples from WoS birds indicate that these animals suffered profound stress at the time of sacrifice as measured by the incidence of PSE meat.

Fillets of slaughtered chickens from the WoS group had a* values (Table 2) significantly higher samples from the filets of broilers slaughtered under WtS conditions, showing more reddish coloration. These results are consistent with the study of Takahashi *et al.* (2009), which found that fillets from chickens slaughtered without stunning were redder (higher a* value) than fillets from electrically-stunned chickens (40 and 90 V/800 Hz).

It is possible that the reddish coloration of fillets from WoS chicken was the result of the incomplete removal of blood from the muscle at slaughter. In fact, less blood loss was observed in carcasses from WoS chickens in relation to WtS under at 20, 40, 80, and 100 V with a frequency of 60 Hz and also varying the frequency of 60, 200, 350, 500 and 1000 Hz at a constant voltage of 40 V (Contreras and Beraquet, 2001). In contrast, stunning the animal by any method produces a rise in blood pressure in the arteries, capillaries, and veins and causing a transient increase in heart rate, which are all factors that favor successful bleeding (Thornton, 1969).

In contrast to the L* and a* values, b* values did not differ ($P > 0.05$) between treatment groups (Table 2). Our results were similar to those reported by others who have evaluated different stunning methods, including: electrical (13 Hz and 500 mA for 7 s), gas (70% Argon and 30% CO₂), and no electrical stunning (Alvarado *et al.*, 2007); in addition to electrical stunning with low voltage (11 V, 500 Hz for 10 s) and high current (125 mA, 60 Hz for 5 s) and no electrical stunning (Craig *et al.*, 1999).

In relation to the WHC values, they were not significantly different between treatment groups (Table 2), although the chicken fillets from WtS birds presented lower pH and higher L* values. Takahashi *et al.* (2009) found that fillets from WoS chickens presented lower WHC values in comparison to fillet samples from WtS chickens under high and low voltage (40 V and 90 V/high frequency for both 800 Hz).

The incidence of PSE fillets was evaluated according to the variation binary (1 and 0), where 1 means normal and 0 means PSE. These values differed significantly among the

Table 2. Values of pH and color (L*, a*, b*), water holding capacity (WHC) and the PSE incidence in broiler fillets with (WtS, 80 V and 400 Hz) and without stunning (WoS)

	WtS	WoS
pH _{24h}	5.87 ^a ± 0.08	5.82 ^b ± 0.09
L* _{24h}	52.53 ^b ± 1.80	54.91 ^a ± 2.42
a* _{24h}	3.15 ^b ± 0.75	4.50 ^a ± 0.57
b* _{24h}	5.04 ^a ± 1.20	5.01 ^a ± 1.07
WHC _{24h}	65.45 ^a ± 2.28	63.99 ^a ± 4.84
PSE Incidence ¹	0.75 ^a	0.43 ^b

^{a, b} Means followed by different letters in the same row differ by the Student's t-test at 5% probability ($p \leq 0.05$).

¹ Mean of binary variation, where: 1 - Normal and 0 - PSE.

Table 3. 2^2 full factorial with the independent variables (voltage and frequency) and the experimental response functions (Y) and estimated ($\hat{Y}$) model for the evaluation of electrical stunning on broiler chicken meat quality

Independent variables														
Assay	Voltage	Frequency	pH		L*		Color a*		b*		WHC ¹ (%)		PSE Incidence (%)	
			Y	$\hat{Y}$	Y	$\hat{Y}$	Y	$\hat{Y}$	Y	$\hat{Y}$	Y	$\hat{Y}$	Y	$\hat{Y}$
1	−1 (40 V)	−1 (100 Hz)	5.80 ±0.13	5.80	53.28 ±2.11	53.38	3.80 ±0.86	3.57	5.47 ±1.64	5.33	65.19 ±2.68	65.46	56.67	59.17
2	+1 (120 V)	−1 (100 Hz)	5.85 ±0.11	5.84	53.44 ±2.09	53.54	3.91 ±0.71	3.67	4.35 ±1.16	4.22	66.99 ±6.45	67.27	43.33	45.83
3	−1 (40 V)	+1 (700 Hz)	5.75 ±0.13	5.74	55.79 ±2.80	55.79	3.83 ±1.11	3.59	5.73 ±1.08	5.59	61.11 ±3.12	61.38	70	72.50
4	+1 (120 V)	+1 (700 Hz)	5.88 ±0.11	5.88	52.75 ±2.08	52.86	3.95 ±0.94	3.71	5.82 ±1.24	5.69	64.01 ±3.61	64.28	20	22.50
5	0 (80 V)	0 (400 Hz)	5.78 ±0.11	5.81	54.34 ±2.02	53.92	3.56 ±1.05	3.64	4.20 ±0.98	5.21	65.82 ±2.54	64.60	56.67	50
6	0 (80 V)	0 (400 Hz)	5.78 ±0.12	5.81	54.12 ±2.33	53.92	3.66 ±0.92	3.64	5.49 ±1.11	5.21	65.10 ±2.40	64.60	53.33	50
7	0 (80 V)	0 (400 Hz)	5.85 ±0.09	5.81	53.72 ±2.19	53.92	2.77 ±0.74	3.64	5.39 ±1.02	5.21	63.98 ±2.82	64.60	50	50

x_i : Coded Value; X_i : Real Value; ¹ WHC: Water Holding Capacity; ² SF: Shear Force.

Table 4. Analysis of variance for response functions pH, L*, a*, b*, WHC and PSE incidence of broiler fillets

Response	DF	SS	MS	F	p	R ²	P of Lack of fit
pH	6	0.01	0.004	2.16	0.44	0.74	0.0005
L*	6	5.47	1.82	18.69	0.06	0.95	0.1
a*	6	0.013	0.004	0.018	0.93	0.01	0.50
b*	6	0.46	0.15	0.885	0.46	0.53	0.17
WHC	6	18.33	6.11	7.12	0.27	0.88	0.71
PSE Incidence	6	1,363.98	454.66	40.88	0.10	0.94	58.33

DF=degree of freedom, SS=sum of square, MS=mean square

slaughtered chicken fillets from the WtS and WoS treatment groups (Table 2). The lowest mean was observed for the WoS treatment, indicating a higher incidence of PSE. The occurrence of PSE in chicken fillets from WtS and WoS birds is shown in Fig. 1. For slaughtered WtS chickens (80 V and 400 Hz), we observed that the incidence of PSE was 25%, which was much lower than those from WoS birds (57.14%).

The ANOVA for the models generated is presented in Table 4. The response functions pH, a*, b*, and WHC presented a nonsignificant regression, indicating that the model inadequately fits the experimental data (Table 4). It is likely that the pH response function was not significant due to the small variation observed for this response of 5.75 (Assay 3) to 5.88 (Assay 4) (Table 3). Other reports also have demonstrated that different electrical stunning systems did not affect meat pH 24 h post-mortem (Thomson *et al.*, 1986; Papinaho and Fletcher, 1995; Papinaho *et al.*, 1995). The response function a* varied from 2.77 (Assay 7) to 3.95

(Assay 4) (Table 3), while the response function b* ranged from 4.20 (Assay 5) to 5.82 (Assay 4). The WHC showed a variation of 61.11% (Assay 3) to 66.99% (Assay 2), which is supported by previous data in a study by Craig *et al.* (1999). This study found no significant differences in the loss of water for cooking stunned chicken breast fillets under high current (125 mA, 60 Hz, 5 s) or low voltage (11 V, 500 Hz, 10 s).

For response function L*, it was observed that assay 4 (120 V, 700 Hz) resulted in the lowest value (52.75), while assay 3 (40 V, 700 Hz) presented the highest value (55.79), indicating a higher influence of stress in birds treated with lower voltage. An analysis of variance of this response function (Table 4) showed that the regression was significant and the lack of fit was not significant. The overall coefficient of determination (R^2) was 0.9481, indicating that 94.81% of the variation was explained by the model, meaning the experimental data fit the model well. Equation 2 shows the proposed model for the effect of variables codified voltage

(x_1) and frequency (x_2) on the lightness of the fillets.

$$L^* = 53.92 - 0.72x_1 + 0.46x_2 - 0.8x_1x_2$$

Equation (2)

where: L^* =lightness, x_1 =Voltage (V); x_2 =Frequency (Hz), *Significant ($P \leq 0.05$)

It was observed that the effect of voltage (x_1) was significant and negative, indicating that the increased tension caused a decrease in the L^* value. The response surface (Fig. 2) showed that lower L^* values were observed when the voltage and frequency were used at higher levels (i.e., 120 V and 700 Hz). In contrast, higher L^* values were ob-

Fig. 1. Occurrence of PSE vs. normal meat in breast fillets from WtS and WoS broiler chickens.

served with the lower voltage (40 V) and higher frequency (700 Hz), indicating that these conditions lead to a lower quality of chicken meat.

In relation the response function PSE incidence, it was observed that assay 4 (120 V, 700 Hz) demonstrated the lowest incidence (20%), while the assay 3 (40 V, 700 Hz) presented the highest (70%) (Table 3). The tests with the lowest level of the variable voltage (40 V) had the highest incidence of PSE, regardless of the level of applied frequency (assays 1 and 3, with 56.67% and 70%, respectively). The analysis of variance of this response function was significant and lack of fit was not significant (Table 4). The overall coefficient of determination (R^2) was 0.9442, indicating that the model explained 94.42% of the variation (which means a good fit of the model to experimental data). Equation 3 shows the proposed model for the effect of variables codified voltage (x_1) and frequency (x_2) on the PSE incidence.

$$\text{PSE Incidence} = 50 - 15.84x_1 - 2.5x_2 - 9.17x_1x_2$$

Equation (3)

where: x_1 =Voltage (V); x_2 =Frequency (Hz); * Significant ($P \leq 0.05$).

We observed that the effect of voltage (x_1) was significant and negative, indicating that the increased voltage caused a reduction in the incidence of chicken PSE fillets. Furthermore, the interaction between voltage and frequency was

Fig. 2. Response surface of voltage (x_1) and frequency (x_2) effect applied in electrical stunning on the lightness (L^*) of broiler chicken fillet meat.

Fig. 3. Response surface of the effect of voltage (x_1) and frequency (x_2) applied in electrical stunning on the incidence of broiler chicken PSE meat.

negative and significant. The response surface (Fig. 2) showed that the lowest incidence of PSE was in the range of both higher electrical voltage and frequency, suggesting that when broiler chickens were stunned at 120 V and 700 Hz, they suffered less stress, thus resulting in better meat quality.

In conclusion, the application conditions of electric stunning under voltage of 120 V and frequency of 700 Hz before bleeding resulted in best quality of breast fillet meat.

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