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Article

Electronarcosis protocols in broiler chickens for the halal market

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Abstract

This study considered the parameters of the halal market to evaluate different protocols for broiler electronarcosis regarding time to return of consciousness, bleeding efficiency, and carcass and meat quality at a broiler slaughterhouse. One hundred and eighty-four 43-day-old male broilers of the Cobb® strain were used. The experimental design used was completely randomized with 46 broilers for each protocol. The protocols used 80 V and 600 Hz, 50 V and 1000 Hz, 60 V and 1000 Hz, and 70 V and 1000 Hz. Time to return of consciousness, bleeding efficiency, bruising, presence of broken bones in pectoral muscle, and meat quality were evaluated. Birds subjected to stunning by the 50 V and 1000 Hz protocol showed the shortest time to return of consciousness after electronarcosis, and high incidence of breast hemorrhage. Birds subjected to the 60 V and 1000 Hz protocol had a higher percentage of broken bones in the pectoralis minor. The 50 V and 1000 Hz electronarcosis protocol fits better to the halal market due to the shorter time to return of consciousness and improved bleeding efficiency. This study suggests new proposals for stunning protocols to serve the halal market.

Key words: carcass quality, electric current, high frequency, meat quality, stunning

Résumé

Cette étude a considéré les paramètres du marché halal afin d'évaluer différents protocoles d'électronarcose (ou étourdissement électrique) des poulets à griller en ce qui a trait au temps de retour à la conscience, l'efficacité de la saignée, et la qualité de la carcasse et de la viande dans un abattoir de poulets à griller. Cent quatre-vingt-quatre poulets à griller mâles âgés de 43 jours de race Cobb® ont été utilisés. Le design expérimental utilisé était complètement aléatoire avec 46 poulets à griller pour chaque protocole. Les protocoles utilisés : 80 V et 600 Hz; 50 V et 1000 Hz; 60 V et 1000 Hz; 70 V et 1000 Hz. Le temps pour le retour à la conscience, l'efficacité de la saignée, les contusions, la présence de fractures aux os dans le muscle pectoral, et la qualité de viande ont été évalués. Les poulets ayant subi le protocole 50 V/1000 Hz ont montré la plus courte durée du retour à la conscience après l'électronarcose, et la plus grande incidence d'hémorragie de la poitrine. Les poulets ayant subi le protocole 60 V et 1000 Hz avaient un plus grand pourcentage de fractures d'os dans le Pectoralis minor. Le protocole d'électronarcose 50 V et 1000 Hz convient mieux au marché halal puisqu'il a la plus courte durée du retour à la conscience, et la meilleure efficacité de la saignée. Cette étude suggère de nouvelles propositions en matière de protocoles d'étourdissement pour servir le marché halal. [Traduit par la Rédaction]

Mots-clés : qualité de carcasse, courant électrique, haute fréquence, qualité de viande, étourdissement

Introduction

The Islamic religion stands out as the fastest growing in the world, thus increasing the demand for halal-certified food (Pew Research Center 2014). Among halal standards, preventing the use of electronarcosis in broiler slaughter generates many discussions, mainly regarding bird welfare (Anil 2020). Dahlan (2020) states that halal slaughter does not allow electronarcosis; instead, the bird must be still conscious and alive at the time of slaughter. According to Yardimci (2019), this improves the loss of body blood during bleeding. Notwithstanding, some Islamic countries allow birds to be subjected to superficial stunning as long as this does not cause their death (Seidler 2012), as is the case in Malaysia (Amjum et al. 2020).

In commercial broiler slaughterhouses, preslaughter electronarcosis in an immersion tank is currently the most used method. This method is convenient, economical, requires a small installation area, and can be easily adapted for commercial processing plants (Awan and Sohaib 2016). According to Raj and O'Callaghan (2004), this method allows companies to serve different markets. The methods of electrical stunning in an immersion tank may be divided into three categories: low voltage and high-frequency stunning, low voltage and low-frequency stunning, and high voltage and low-frequency stunning (Huang et al. 2014).

However, considering halal slaughtering process, there are disagreements regarding the preslaughter stunning protocols to be adopted. One alternative is the use of low voltage (45 to 50 V) and high-frequency (900 to 1000 Hz) stunning, with an amperage below 120 mA, resulting in a shorter period of bird unconsciousness (Farouk et al. 2014). This association between low voltage and high frequency has physiological influences on the sodium and potassium pump of the central nervous system, minimizing the stimulation of nerve cell impulses and thus reducing ventricular fibrillation (Sabow et al. 2017). Farouk et al. (2016) states that a protocol with high frequency and low voltage meets the requirements of halal slaughter for producing a mild desensitization, that is, reversible stunning, and improves bleeding efficiency since it does not cause cardiac defibrillation and consequently cardiac arrest.

According to Nakyinsige et al. (2013), the incorrect use of stunning protocols may directly influence meat quality. For example, the acceleration of postmortem glycolysis may alter tenderness, color, water holding capacity, and cooking loss, thus affecting the life span of the product on display. According to Ali (2011), the factors that affect quality are the amount of blood remaining in the meat, often resulting from the occurrence of cardiac defibrillation; red wing tips; broken bones; hemorrhages; and lipid oxidation due to the presence of high concentrations of hemoglobin in the musculature.

In view of the scarcity of studies that determine the adequacy of stunning protocols meeting the standards of the halal market, the aim of this study was to evaluate different broiler preslaughter electrical stunning protocols for serving the halal market. For that, we analyzed the time to return of consciousness, bleeding efficiency, and carcass and meat quality.

Materials and methods

Study site and animals

The research was approved by the Ethics Committee on Animal Use (CEUA) of Federal University of Grande Dourados (protocol number 01/2020). The experiment was conducted in a broiler chicken commercial halal slaughtering plant in Dourados, Mato Grosso do Sul State, Brazil. The plant has a slaughtering capacity of 145 000 birds per day. According to the Köppen climate classification, Dourados city is located at 22°13′18.54″ south latitude, 54°48′23.09″ west longitude, and average altitude of 430 m, having a mesothermal humid climate (Cwa), with rainy summer and dry winter.

The birds used in this research were male Cobb® strain broilers, reared in Dark House system with a housing capacity of 35 000 birds and density of 14 birds/m^2. At 43 days of life, the birds were sent to a refrigeration facility after a 6 h fast. The distance between poultry houses and slaughterhouse was 23 km, with an average speed of 85 km/h and transport time of approximately 40 min. An open-sided truck was used for the transport of birds and canvas at the beginning of its body. The truck had a capacity of 540 boxes with an average of eight birds each, which is equivalent to 23 kg, totaling 4320 birds per truck.

Upon arriving at the refrigeration facility, the birds remained for 20 min in an air-conditioned waiting shed (automatic thermoregulatory system with fan activation and fogging system). The aim was to control the ambient temperature (average 17 °C) and guarantee thermal comfort of birds during unloading.

Experimental procedure

After unloading, 184 male broilers with an average weight of 2.85 ± 0.28 kg were identified with numbered security seals in the left drumstick, and 46 birds were distributed into each electronarcosis protocol.

The selected birds were hung on the air transporter of carcasses by trained employees and sent to the stunning area. Then, the electronarcosis method was used by immersing the bird's head in a tank with electrified water. A Fluxor® stunner, model UFX7, was used, with continuous quadratic current being applied in all treatments. The electronarcosis tank was made of polypropylene and was 4 m long, 60 cm wide, 90 cm high, with a capacity of 31 birds immersed in chlorinated water, without the addition of electrolytes. The immersion time of each bird in the tank was 12 s, at a rate of 140 birds/min.

The experimental design used was completely randomized with four electronarcosis protocols, in which high-frequency protocols with different voltages were tested against the European standard (low frequency and high voltage). The electronarcosis protocols used were Protocol 1: voltage of 80 V and frequency of 600 Hz (112 mA per bird) (European standard); Protocol 2: voltage of 50 V and frequency of 1000 Hz (92 mA); Protocol 3: voltage of 60 V and frequency of 1000 Hz (110 mA); and Protocol 4: voltage of 70 V and frequency of 1000 Hz (129 mA).

In each electronarcosis protocol, 10 birds were analyzed for period of unconsciousness, 10 birds were used to assess the bleeding efficiency, and the other birds (26 birds) were used to assess the presence of bruises, petechiae, breast bone fracture, and breast meat quality.

The birds subjected to electronarcosis were bled individually and manually when leaving the stunning tank (1.5 s after stunning) by trained staff following the religious precepts, since the processing plant is qualified for halal slaughter. During the experiment, the average line speed was 140 birds/min, totaling 8160 birds slaughtered per hour. After bleeding, the birds were directed to the bleeding tunnel where they remained for a period of 3 min.

Then, the birds were scalded in water, plucked, automatically eviscerated, and cooled by immersion in water (precooling at 12 °C and cooling at 2 °C), remaining immersed for 1 h 20 min. After cooling, carcasses were evaluated for the presence or absence of hemorrhages in the wings, breast, and thighs. The carcasses were cut and the breasts were deboned for the analysis of the presence of hemorrhages in the breast muscle (pectoralis major and pectoralis minor), being stored at a temperature of 5 °C. After 24 h postmortem, the presence of fractured breast bones and deboned breast weight were analyzed. The breasts were separated into right and left fillets and analyzed for pH, physical measurements, color, drip loss, and water holding capacity (right fillet of the pectoralis major muscle). The left fillet was frozen in a freezer at −18 °C for further analysis of thawing and cooking loss and shear force.

Variables evaluated

The period of unconsciousness was assessed immediately after electronarcosis. For this, 10 birds were randomly selected and identified, analyzing the characteristic signs of adequate stunning. In the unconscious period, the tonic phase occurs when the bird shows an arched neck, wings closed to the body, constant involuntary tremors in the body and wings, eyes open with dilated pupils, legs extended and absence of rhythmic breathing. After the tonic phase, the clonic phase begins quickly with leg movement, neck relaxation, disordered wing movements, and absent rhythmic breathing and corneal reflex. The total phase comprises the time of the tonic and clonic phases.

For this analysis, 10 birds were placed on the floor, standing on their limbs in a stationary position, with the neck extended forward, facilitating breathing. The duration of the tonic and clonic phases and the time to return of consciousness were recorded. The birds were analyzed individually. After the return of unconsciousness, the birds were destined for humane slaughter, not being used for any further analysis.

For bleeding efficiency evaluation, another 10 birds were selected and weighed before being hung on the slaughter line, then subjected to the electronarcosis protocol, removed from the slaughter line, and hung on fixed hooks. Immediately after that, the two jugular and carotid veins were manually cut and the animals were left to bleed for 3 min. Afterward, the birds were again weighed to obtain postbleeding weight, and the percentage of blood loss was calculated. To improve the accuracy, water absorption during the passage through the stunning tank was estimated. For that, 10 birds were weighed before and after stunning to measure water absorption during this process. The mean value was used as an estimate of water absorption during the test, and this value was subtracted from the bled bird weights to calculate blood loss with calculations according to the methodology proposed by Contreras and Beraquet (2001). Twenty-six birds were randomly selected for the assessment of bruising on thighs, wings, and breasts (pectoralis major), following the presence or absence classification of Ludtke et al. (2010). The assessment of bruising on the deep pectoral muscle (pectoralis minor) was performed after the extraction of the carcass musculature. The classification considered the extent of the injury caused: absent (= 0 cm), mild (0–1 cm), moderate (1–2 cm), and severe ($\geq$2 cm).

The presence of broken bone in the pectoralis major muscle was analyzed by palpating the muscle so as to feel the presence of fragments of the coracoid bone. The classification, which considered the absence or presence of broken bone, followed the methodology recommended by USDA (2002).

Breast meat quality

The breasts (pectoralis major) were weighed on a semianalytical scale after deboning and removal of the pectoralis minor. Breast fillet was sized with the aid of a digital caliper, following the methodology of Mudalal et al. (2014), with measurements of fillet width (mm), length (mm), and thickness (cm). The final pH of the breast fillet was measured in triplicate using a digital pH meter (Texto, model 205) directly on the pectoralis major muscle. The color of breast fillets was determined using a portable Minolta spectrophotometer CR 400, in the CIELab system, in which the parameters L^* (brightness), a^* (red intensity), and b^* (yellow intensity) were measured in three different points on the ventral surface of the pectoralis major muscle according to the methodology proposed by Van Laack et al. (2000).

A 100 g sample of the pectoralis major muscle was used for drip loss analysis, according to an adapted methodology described by Rasmussen and Andersson (1996). The analysis of water holding capacity was according to the methodology proposed by Hamm (1960).

The fillets were frozen for 2 weeks at −18 °C. After this period, the fillets were placed in a cooling chamber at a temperature of 5 °C for 24 h for thawing, and thawing loss percentage was calculated. For weight loss by cooking, the breast fillet samples were placed in plastic containers and cooked in a water bath at 85 °C for 30 min, until reaching a final internal temperature of 75–80 °C according to the methodology of Honikel (1987).

For shear force, the samples used to determine weight loss by cooking were used. These samples were cut into cobblestone shapes with dimensions of 1 cm × 1 cm × 2 cm in quintuplicate. Then, they were placed with the fibers oriented perpendicular to the Warner–Bratzler blades coupled to the texture analyzer TA.XT plus (Stable Micro Systems), with a blade descending speed of 200 mm/min, and values expressed in kg/force/cm^2 (AMSA 2015).

Statistical analysis

The results of the analyses carried out after slaughter and those regarding meat quality were evaluated for residual normality using the Shapiro–Wilk test, and for homogeneity of variances using the Levene's test. Subsequently, the results were subjected to analysis of variance (ANOVA) through the MIXED procedure of SAS (version 9.3). When a significant effect was observed, means were compared using the Tukey's test. To analyze the results regarding the return of consciousness, Pearson's correlation coefficients between these variables were estimated. For this purpose, the following intervals were used as classification values for the correlation: $r = 0.10$–0.30 (weak); $r = 0.40$–0.60 (moderate); $r = 0.70$–1.00 (strong) (Rumsey 2016). Since the frequency results for hemorrhages did not comply with the assumptions of homogeneity of variances and residual normality, they were evaluated nonparametrically using the χ^2 test. The significance level used for all analyses was 5%.

Table 1. Tonic phase duration, clonic phase duration, total period of unconsciousness, and percentage of blood loss in broilers subjected to different electronarcosis protocols.

Protocol	Tonic phase (s)	Clonic phase (s)	Total unconsciousness (s)	Blood loss (%)
80 V/600 Hz	30.60 b	56.36 a	93.40 a	12.64 b
50 V/1000 Hz	16.60 c	30.50 b	47.10 b	16.08 a
60 V/1000 Hz	41.50 a	48.40 a	89.90 a	15.18 a
70 V/1000 Hz	38.11 a	49.11 a	87.22 a	14.95 a
SEM*	1.773	2.638	3.975	0.289
P value	<0.0001	0.0016	<0.0001	<0.0001

Note: Frequencies followed by different lowercase letters differ by the Tukey's test at 5% significance.
*Standard error of the mean.

Results

The live weights of preslaughter broilers subjected to different electronarcosis protocols did not differ from each other ($P = 0.2594$). The 60 V/1000 Hz and 70 V/1000 Hz protocols showed the longest tonic phase duration, followed by the 80 V/600 Hz protocol, with the 50 V/1000 Hz protocol showing the shortest duration (41.50, 38.11, 30.60, and 16.60 s, respectively, SEM 1.773; $P < 0.0001$) (Table 1).

The protocols that resulted in the longest clonic phase duration in broilers were 80 V/1000 Hz, 70 V/1000 Hz, and 60 V/1000 Hz (56.36, 49.11, and 48.40 s, respectively, SEM 2.638; $P = 0.0016$), while the 50 V/1000 Hz protocol showed the shortest clonic phase duration (30.50 s). When analyzing the total period of unconsciousness of birds after electronarcosis, the 50 V/1000 Hz protocol resulted in the shortest period in relation to the other three protocols that did not differ from each other (47.10 s, and 93.40, 89.90, and 87.22 s, 80 V/600 Hz; 60 V/1000 Hz, and 70 V/1000 Hz, respectively, SEM 3.975; $P < 0.0001$). For bleeding efficiency, electronarcosis protocols using 1000 Hz (16.08%, 15.18%, and 14.95%, SEM 0.2890; $P < 0.0001$), regardless of the voltage used, showed higher blood loss compared with the standard protocol (80 V/600 Hz, 12.64%).

There was a significant and positive correlation between the total period of unconsciousness and the clonic phase in all electronarcosis protocols used. In other words, the longer the clonic phase, the longer the total period of unconsciousness (Table 2).

Analyzing the protocols individually, the birds that were stunned at 80 V/600 Hz obtained a strong and positive correlation ($r = 0.96$) between total period of unconsciousness and clonic phase duration. This also occurred for the birds that were stunned with the 60 V/1000 Hz and 70 V/1000 Hz protocols, with a strong and positive correlation in both treatments ($r = 0.93$ and 0.96, respectively). There was also a significant positive correlation, although moderate ($r = 0.80$), for the 50 V/1000 Hz electronarcosis protocol, indicating that the clonic phase duration had a moderate influence on the total period of unconsciousness.

There was also a significant positive and moderate correlation ($r = 0.68$) between tonic phase duration and total period of unconsciousness in the 70 V/1000 Hz protocol, indicating that the longer the tonic phase, the longer the total period of unconsciousness. A significant positive and moderate correlation ($r = 0.79$) was observed between blood loss and the clonic phase of birds subjected to the 50 V/1000 Hz electronarcosis protocol, which shows us that the longer the clonic phase, the higher the blood loss of that bird. Likewise, a significant positive and strong correlation ($r = 0.88$) was observed between total period of unconsciousness and blood loss, showing that the longer the total period of unconsciousness, the higher the blood loss. For the other protocols, no significant correlation was found between the parameters.

For the percentage of hemorrhage in the wings, thighs, and pectoral muscle, there was no significant difference between the electronarcosis protocols tested (Table 3). However, the 50 V/1000 Hz protocol showed the highest incidence of hemorrhagic breasts compared with the 60 V/1000 Hz and 70 V/1000 Hz protocols (34.62%, 11.54%, 3.85%, respectively, SEM 1.773; $P = 0.0341$) that obtained the lowest frequencies of breast hemorrhage.

When assessing the presence of broken bone in the pectoral muscle, the 60 V/1000 Hz protocol showed a higher occurrence of this defect (61.54%; $P = 0.0176$), and the other protocols did not differ significantly from each other.

There were no significant differences in the live weight ($P = 0.2938$) of birds and in fresh breast weight ($P = 0.1609$) between the electronarcosis protocols (Table 4). No differences were found for meat quality variables, except for drip loss, in which the 70 V/1000 Hz electronarcosis protocol presented the lowest value compared with the 60 V/1000 Hz protocol (4.05 and 5.61, respectively; SEM 0.16; $P = 0.0053$). The other protocols (80 V/600 Hz and 50 V/1000 Hz) did not differ between themselves and the others.

Discussion

Some Muslim countries (Malaysia, Oman, Kuwait, Bahrain) already allow electronarcosis as long as it promotes superficial loss of consciousness so that birds do not die before bleeding. However, this type of electronarcosis (high frequency and low voltage), which leads to a period of unconsciousness of at most 30 s, generates discussion on animal welfare (Rehman and Shahbaz Shabbir 2010).

The 50 V/1000 Hz (94 mA) electronarcosis protocol of the present study stood out for presenting the shortest period of unconsciousness in relation to the other protocols. This result agrees with Berg and Raj (2015), who compared a high frequency (950 Hz) and medium voltage (70 V) protocol with a low frequency (400 Hz) and low voltage (40 V) protocol.

Table 2. Pearson's correlation coefficient between unconsciousness phases (tonic/clonic), total period of unconsciousness, and blood loss in broilers subjected to different electronarcosis protocols.

Protocol	80 V/600 Hz				50 V/1000 Hz				60 V/1000 Hz				70 V/1000 Hz			
	Tonic	Clonic	TPU[a]	BL[b]	Tonic	Clonic	TPU[a]	BL[b]	Tonic	Clonic	TPU[a]	BL[b]	Tonic	Clonic	TPU[a]	BL[b]
Tonic	1	−0.3984*	−0.1457	−0.3593	1	−0.1907	0.2938	0.3078	1	−0.4857	−0.1320	−0.5330	1	0.4806	0.6824	−0.1345
		(0.2540)**	(0.6880)	(0.3079)		(0.5979)	(0.4100)	(0.3870)		(0.1547)	(0.7163)	(0.1127)		(0.1904)	(0.0428)	(0.7299)
Clonic		1	0.9654	0.3981		1	0.8823	0.7964		1	0.9306	−0.0893		1	0.9690	0.4827
			(<0.0001)	(0.2546)			(<0.0007)	(0.0058)			(<0.0001)	(0.8063)			(<0.0001)	(0.1882)
Total			1	0.3273			1	0.9230			1	−0.3244			1	0.3643
				(0.3560)				(0.0001)				(0.3604)				(0.3350)

[a]Total period of unconsciousness.
[b]Blood loss.
*Correlation coefficient.
**Significance level.

Similarly, Prinz et al. (2010) mentioned that the use of high frequency (1000 Hz) in association with low voltage (40 V) may induce a short period of unconsciousness. Furthermore, there was a significant positive correlation between total period of unconsciousness and blood loss in the high-frequency electronarcosis protocol (1000 Hz).

Such low voltage and high frequency (50 V/1000 Hz) protocol promoted superficial desensitization. According to Terlouw et al. (2016), when passing through the central nervous system, the electric current is of sufficient intensity (1 mA for birds) to depolarize neurons throughout the bird's brain from the release of excitatory amino acids (glutamate) in the extracellular space, causing the depolarization of nerve cells and allowing calcium to enter. This causes episodes of voluntary epilepsy and consequently loss of consciousness, also promoting muscle contraction, which proves that the bird is in the subsequent phase known as the tonic phase. According to Xu et al. (2011), the higher the frequency and the lower the voltage, the shorter the duration of this depolarization (0.2 and 0.4 ms), allowing the bird to regain consciousness quickly.

This depolarization of the nervous system reduces nerve impulses in the bird's body. The measurement of these impulses is possible through indicators of the tonic and clonic phases, with the use of electroencephalograms or by measuring time (Llonch et al. 2015), observing the characteristic signs of each phase. In the present study, the methodology used to assess depolarization consisted of analyzing the characteristic signs of the tonic and clonic phases. The time of each phase was measured, with the 50 V/1000 Hz protocol having the shortest duration of the two phases compared with the other tested protocols.

Sabow et al. (2017) showed similar results for total period of unconsciousness. The authors reported physiological aspects of the tonic phase, in which the bird has an epileptiform activity (voluntary epilepsy). In other words, all brain cells fire nerve impulses together in a harmonized pattern (hypersynchronous activity), thus reducing the basic reflexes of movement and causing muscle contraction rigidity, leading to minimal movement. Then, in a fraction of seconds, the second phase (clonic phase) begins. This phase comprises a suppression of brain activity, which reduces the activities of the central nervous system, with greater release of chemical substances (neurotransmitters) that stimulate nerve cells. This reduces the seizure state of the bird, with the recovery of respiratory movements, eye reflexes, and, in the third and last phase, the return of consciousness.

Ludtke et al. (2010) observed a shorter period of unconsciousness of birds when using low voltage (40 V) in association with high frequency (850 Hz). Prinz et al. (2010) showed that birds subjected to electronarcosis with low frequency (400 Hz) and moderate voltage (65 V) protocols had a longer clonic phase compared with birds subjected to high frequency (1500 Hz) and medium voltage (75 V) protocols.

In the present study, birds subjected to the 50 V/1000 Hz electronarcosis protocol had the shortest period of unconsciousness, which reduced the risk of bird death before the bleeding process was completed. In addition, birds subjected to electronarcosis protocols with high frequency (1000 Hz)

Table 3. Frequency of hemorrhage in the breast, thigh, and wings, and presence of broken bones in the pectoral muscle in broiler carcasses subjected to different electronarcosis protocols.

Variable		80 V/600 Hz	50 V/1000 Hz	60 V/1000 Hz	70 V/1000 Hz	*P* value
Wing hemorrhage		69.23%	76.92%	69.23%	50.00%	0.2029
Breast hemorrhage		15.38% ab	34.62% a	11.54% b	3.85% b	0.0341
Thigh hemorrhage		30.77%	26.92%	30.77%	15.38%	0.5417
Broken bone in the pectoral muscle		34.62% b	26.92% b	61.54% a	23.08% b	0.0176
	Score					
Deep pectoral muscle hemorrhage*	0	23.08%	0.00%	11.54%	19.23%	0.1771
	1	23.08%	26.92%	11.54%	23.08%	
	2	30.77%	46.15%	30.77%	26.92%	
	3	23.08%	26.92%	46.15%	30.77%	

Note: Frequencies followed by different lowercase letters differ by the χ^2 test at 5% significance.
*0, absent; 1, mild; 2, moderate; 3, severe.

Table 4. Quality of breast meat in broilers subjected to different electronarcosis protocols.

Variable	80 V/600 Hz	50 V/1000 Hz	60 V/1000 Hz	70 V/1000 Hz	SEM*	*P* value
Height (mm)	35.40	33.48	35.23	34.54	0.34	0.1808
Width (mm)	7.94	7.91	8.00	7.89	0.05	0.9257
Thickness (cm)	1.73	1.74	1.76	1.71	0.00	0.3806
pH	6.00	5.96	5.94	5.99	0.01	0.4053
L*	44.51	44.86	44.98	45.13	0.21	0.7497
a*	1.97	2.1	1.77	2.08	0.07	0.3909
b*	6.52	6.65	6.64	7.22	0.12	0.1814
Drip loss (%)	5.10 ab	5.09 ab	5.61 a	4.05 b	0.16	0.0053
WHC[a] (%)	34.42	34.79	35.08	34.84	0.31	0.9034
TL[b] (%)	7.01	5.29	6.76	6.14	0.24	0.0504
WLC[c] (%)	26.71	27.22	26.84	26.32	0.32	0.8132
SF[d] (Kg/cm^2)	1.86	1.75	1.88	1.76	0.04	0.6429

Note: Means followed by different lowercase letters differ by the Tukey's test at 5% significance.
[a]Water holding capacity.
[b]Thawing loss.
[c]Weight loss by cooking.
[d]Shear force.
*Standard error of the mean.

showed greater bleeding efficiency. In this regard, Sabow et al. (2016) found increases pressure in the cardiorespiratory system, which may favor bleeding, reducing the amount of blood in the muscles when using high frequency and low voltage due to the low risk of cardiac arrest with the use of high frequency.

It is well known that the preslaughter management of birds can directly influence the quality of broiler carcasses. Among these managements are the electronarcosis protocols to be used by companies, which at the end of the procedure can result in carcass hemorrhages, bruising in cuts such as wings, breast, and thighs, and the presence of broken bones. The incidence of breast hemorrhage was higher when using the 50 V/1000 Hz protocol compared with the 60 V/1000 Hz and 70 V/1000 Hz protocols. According to Farouk et al. (2014), this occurs because bird exposure to high-frequency electronarcosis (1000 Hz) in association with low voltage, with the animals remaining inside the tank for more than 13 s, may exacerbate muscle contraction, resulting in high-intensity hemorrhagic lesions. However, Sabow et al. (2017) reported that the use of low frequency (400 Hz) exacerbates muscle contractions leading to hemorrhages caused by high pressure in the arteries. According to the authors, this breaks blood vessels and leads to blood leakage between the connective tissue and the subcutaneous fat of the tissue that covers the entire musculature.

Notwithstanding, Farouk et al. (2014) reported that the higher blood loss in the halal method, as found in this study, increases the meat quality of poultry slaughtered through this method, in spite of muscle bruising. These researchers demonstrate this fact by the lower hemoglobin concentration in meat compared with the concentration found when using other methods or protocols for electronarcosis. This may be explained by the higher blood losses, decreasing hemoglobin concentration in meat, providing less lipid oxidation.

In view of the results obtained in this study, the 60 V/1000 Hz protocol stood out significantly in relation to the others for the presence of broken bones in the breast. On the contrary, Siqueira et al. (2017) reported that the incidence of broken bones in protocols with the use of low frequencies (500 Hz), regardless of the voltage used, resulted in a higher number of broken bones due to the force of muscle contraction when receiving the electrical discharge. However, Girasole et al. (2016) stated that the use of high frequency

(1200 Hz) causes superficial desensitization and thus the bird will likely have its conscience recovered before the bleeding process is complete. This leads the animal to struggle, and may result in carcass fractures.

In the present study, the only meat quality parameter that was altered by the tested stunning protocols was drip loss, with a higher percentage in the breast meat of birds subjected to the 60 V/1000 HZ electronarcosis protocol compared with the 70 V/1000 Hz protocol. Such findings are compatible with those of Huang et al. (2014), in which poultry carcasses subjected to high-frequency and low voltage electronarcosis protocols had lower drip loss. Similarly, Xu et al. (2011) reported that birds subjected to protocols with medium frequency (400 Hz) and medium voltage (65 V) had higher drip loss compared with birds subjected to high-frequency protocols (1000 Hz) with voltage of 60 V.

Neither the pH of the broiler breast meat nor meat color was influenced by the different electronarcosis protocols. Such results are similar to those of Huang et al. (2014). The other parameters, such as cooking loss, shear force, and water holding capacity, behaved similarly among the protocols used by Huang et al. (2017), who tested low frequency (750 Hz) and high frequency (1150 Hz) with different voltage patterns (15, 25, and 35 V), with no differences in the aforementioned measurements.

The export of chicken meat to the European Union comprises a strong appeal to the issues of animal welfare and humane slaughter of farm animals (Ludtke et al. 2010). For this market, standards include low frequency and voltage, similar to the 80 V/600 Hz protocol used in this study, in which the aim was to reach an amperage of at least 100 mA. According to studies by Prinz et al. (2012), such a protocol increases the period of unconsciousness (above 60 s) of birds, with a high risk of cardiac defibrillation due to the lack of transmission of nerve impulses to the neuromuscular cells of the heart. This increases the risk of cardiac arrest, thereby decreasing bleeding efficiency, which may directly affect meat quality. Novoa et al. (2019) also reported these findings. The authors state that ventricular defibrillation prevails with the use of low frequency (500 Hz), which may be associated with increased carcass damage such as bruising on breast muscles and wing tip hemorrhage. Nevertheless, Shields and Raj (2010) reported that this longer lasting loss of consciousness means that the bird does not recover its reflexes before the final bleeding process, so the bird does not struggle at the time of bleeding. This leads to a final product without risk of bleeding in cuts and without the presence of broken bone fragments in chicken meat.

These results are similar to those of the present study for the 80 V/600 Hz electronarcosis protocol. The period of unconsciousness, as well as clonic phase and tonic phase duration, was longer in this protocol compared with the 50 V/1000 Hz protocol. For breast meat quality, the 80 V/600 Hz protocol did not differ statistically from high-frequency protocols. However, when assessing the presence of breast bruising, this protocol did not differ from the other protocols.

An important point to be considered when recommending an electronarcosis protocol for the halal market is that, according to the Koran, birds must not undergo any kind of stunning, that is, animals must be bleeding in a state of consciousness. Some countries on the other hand tolerate superficial stunning (Nakyinsige et al. 2013). In Brazil, stunning aims to meet the requirements of animal welfare (Gomide et al. 2006). Attempting to meet halal exigencies and respecting requirements of animal welfare, the low voltage (50 V) and high-frequency (1000 Hz) electronarcosis protocol demonstrated the capacity of stunning birds according with both the welfare and the halal market requirements.

Conclusion

The low voltage (50 V) and high frequency (1000 Hz) electronarcosis protocol is indicated for the halal market due to the shorter time to return of unconsciousness and improved bleeding efficiency, in addition to not interfering with physicochemical parameters.

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The data that support this study will be shared upon reasonable request to the corresponding author.

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