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FOOD TECHNOLOGY

Duty cycle and high-frequency effects on welfare and meat quality of broilers chicken: compliance with European animal stunning regulation

Bruna Regina Pereira da Rocha[1] **Amanda Adria**[2] **Vanderlei Aparecido de Lima**[3] **Cleusa Inês Weber**[1,2*] **Alessandra Machado-Lunkes**[1]

[1]Programa de Pós-graduação em Tecnologia de Alimentos, Universidade Tecnológica Federal do Paraná (UTFPR), 85602-863, Francisco Beltrão, PR, Brasil. E-mail: cleusaines@utfpr.edu.br. *Corresponding author.
[2]Departamento de Ciências Agrárias, Universidade Tecnológica Federal do Paraná (UTFPR), Francisco Beltrão, PR, Brasil.
[3]Departmento de Química, Universidade Tecnológica Federal do Paraná (UTFPR), Pato Branco, PR, Brasil.

ABSTRACT: European market regulates that poultry electronarcosis stunning in abattoirs must be performed with a minimal required current (mA per animal) and correlative frequency (Hz), to promote animal welfare and meat quality. In this way, Brazilian abattoirs must adjust the stunning parameters so that they can meet the requirements of that market. This study evaluated the effect of stunning parameters using frequency and duty cycle variables. For this, nine treatments were performed, whose results showed that the analyzed frequencies (700, 1100, and 1500 Hz) had a higher incidence of indicators of animal welfare (AW). Thus, stunning had low efficiency in AW terms. However, the 25% and 40% duty cycles had the best stunning efficiency. There was no significant difference ($P > 0.05$) for pH 24 h, bruises, and drip loss. Color and pH 2 h were significantly affected ($P < 0.05$). The shear force was higher in birds subjected to higher frequencies and interaction between 1500 Hz and 15% and 25% duty cycles. Water-holding capacity was lower at 1100 Hz and in the 25% duty cycle interactions. The treatments affected the evaluated indicators, except for the 40% duty cycle, which had a positive influence. Therefore, the duty cycle applicability must be elucidated due to its direct influence on stunning efficiency.
Key words: electronarcosis, carcass quality, animal welfare, slaughter.

Efeito do duty cycle e altas frequências no bem estar animal e qualidade de carne de frango: atendimento do regulamento europeu de insensibilização animal

RESUMO: O mercado europeu regulamenta que a insensibilização de aves por eletronarcose nos abatedouros deva acontecer com uma corrente mínima necessária (mA por animal) e frequência correlata (Hz), com corrente e frequência alta. O intuito é promover o bem estar animal e qualidade de carne. Desta forma, os abatedouros brasileiros devem ajustar os parâmetros de insensibilização para que possam atender este mercado. O objetivo do trabalho foi avaliar o efeito dos parâmetros de insensibilização usando as variáveis frequência e *duty cycle*. Foram realizados nove ensaios. Os resultados demostraram que as frequências avaliadas (700 Hz, 1100 Hz e 1500 Hz) apresentaram maior incidência nos indicadores de Bem Estar Animal (BEA). Assim, houve baixa eficiência na insensibilização das aves para este fator. Entretanto, o *duty cycle* 25% e 40% promoveu a melhor eficiência na insensibilização das aves. Não foi observada diferença significativa ($P > 0,05$) para pH 24h, hematomas e a perda por gotejamento. A cor e o pH 2h foram afetados significativamente ($P < 0,05$) nos ensaios avaliados. A força de cisalhamento foi maior nas frequências mais altas e na interação da frequência de 1500 Hz com o *duty cycle* de 15% e 25% A capacidade de retenção de água foi menor na frequência de 1100 Hz, e na interação *duty cycle* 25%. Os ensaios estudados apresentaram comprometimento dos indicadores avaliados, exceto a variável *duty cycle* a 40%, que demonstrou influência positiva nos indicadores avaliados. Portanto, a aplicabilidade do *duty cycle* deve ser elucidada devido a sua influência direta na eficiência da insensibilização.
Palavras-chave: eletronarcose, qualidade de carne, bem estar animal, abatedouro.

INTRODUCTION

The high demand for proteins of high biological value, i.e., easily absorbed from food, drives the production and consumption of poultry meat (KRANJAC et al., 2018). Brazil is the world's largest exporter of chicken meat, followed by the United States and the Netherlands, within the European Community (FAO, 2021). Brazil exported 4.231 million tons of poultry meat in 2020, i.e., 31% of all production, which corresponds to US$ 6,097 million (ABPA, 2018).

Abattoirs constantly invest in structural conditions, handling, and transportation of poultry, as well as in other stages of the production chain to meet animal welfare (AW) standards (ALBRECHT et al., 2019). Companies that export chicken to the European Union must meet several AW requirements specific to the European Community Regulation No. 1099/2009, such as stunning parameters (VEISSIER et al., 2008).

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Electronarcosis is the stunning method most used in poultry slaughter due to the low cost of installation, maintenance, and effectiveness of execution (NOVOA et al., 2019). This method consists of applying a frequency (voltage) and current (amperage). Some studies have indicated that high voltages combined with high currents cause a lower incidence of carcass injuries (GIRASOLE et al., 2015). Moreover, electronarcosis has a direct impact on the quality of the final product. This is because chicken unconsciousness causes less physical movement in the bleeding stage and; consequently, fewer bruises, blood pooling, and fractures (HUANG et al., 2017; NOVOA et al., 2019).

Most electronarcosis studies assess frequency (Hz), voltage (V), and, in some cases, amperage, arguing that higher frequencies result in better meat quality results (HUANG et al., 2017; MERCOGLIANO et al., 2017). Possibly, low frequencies favor cardiac arrest in poultry, which causes bruises and muscle blood pooling (GIRASOLE et al., 2015). Conversely, the duty cycle, which consists of the relationship between the time of the highest and the lowest amplitude waves, has been little explored. Therefore, its influence on meat quality and animal welfare is not fully understood (SIQUEIRA et al., 2017, WOTTON et al., 2020).

Given the economic importance of poultry meat exports for Brazilian meat industries and the fact that European stunning parameters (EC 1099/2009) are different as commonly used in Brazil. This study assessed the frequency and duty-cycle effects of poultry stunning on animal welfare and meat quality parameters.

MATERIALS AND METHODS

Animals and stunning procedure

A batch of 52,000 Cobb birds, aged 48 days, with an average weight of 2.84 kg, was used in the experiment according to the experimental design (Table 1). The assay was performed in an industry that slaughters an average of 140,000 birds/day. The birds were divided equally in 9 treatments and the respective parameters were applied. From each treatment 100 chickens were evaluated randomly. The samples were collected in the sectors of bleeding, plucking, evisceration, and cuts of the slaughter line. The stunning was performed using a UFX 7 stunner (Fluxo Brazil), with electrodes placed into the water bath stunning, in which the birds were immersed up to the base of their wings for 3 seconds. The parameters for stunning were defined according to the limits already recommended by the European Community Regulation (EC No. 1099/2009).

After slaughter, 20 carcasses were identified for each treatment and sent to the water chiller process for about 90 min. Subsequently, breast samples (*pectoralis major*) were obtained and stored at 7 ºC for 24 h to provide adequate conditions for performing the other meat quality analyses.

All experimental procedures were approved (protocol No. 2020-07) by the Comitê de Ética no Uso de Animais (CEUA) of the Universidade Tecnológica Federal do Paraná.

Effects of the variables

The European Community Regulation (EC 1099/2009) establishes a minimum value of current

Table 1 - Experimental design for frequency (Hz) and duty cycle (%) factors analyzed at 3 levels.

	---Encoded---		---Real---		
Treatment	X_1	X_2	Frequency (Hz)	Duty Cycle (%)	Current (mA)
1	-1	-1	700	15	200
2	-1	1	700	40	200
3	-1	0	700	25	200
4	1	1	1500	40	200
5	1	-1	1500	15	200
6	1	0	1500	25	200
7	0	0	1100	25	200
8	0	1	1100	40	200
9	0	-1	1100	15	200

SUBTITLE: X_1 – Frequency; X_2 – Duty Cycle.

(200 mA), applied to poultry subjected to stunning when the frequency is applied between 400 and 1500 Hz. Also, considering the data on this subject (HUANG et al., 2017; NOVOA et al., 2019; XU et al., 2019), a factorial design with 2 factors (X_1: Frequency, X_2: Duty cycle) and 3 levels were used, totaling 9 treatments (Table 1).

Poultry stunning efficiency

The electrical stunning efficiency was evaluated immediately after the stunning step was performed in the abattoir. The chickens were rated while slaughter was in operation. The slaughter operators were previously trained for this evaluation. We verified the unconsciousness indicators: coordinated wing flapping, vocalization, voluntary blinking, occurrence of arched necks (in 'S' shape), and rhythmic breathing (BORZUTA et al., 2019). Thus, the presence (1) or absence (0) of these animal welfare indicators was used to establish the efficiency of each treatment.

Meat quality

After the defeathering step a group of slaughter operators, previously trained, rated chicken carcasses with red color hematomas on the chest and in the joint between the wings and the chest, as well as wing fractures. The red color was visually quantified because in this tone, according to Gregory (1992), the elapsed time of the bruise is approximately 2 min. Other colors were disregarded for having a minimum formation time of 12 h (GREGORY, 1992), which ensures that the stunning process did not cause this type of hematoma. The results of these analyses were classified as present (1) or absent (0) to establish the incidence of these anomalies in each treatment.

The color was determined using a calibrated colorimeter (model CR400/410, Konica Minolta, Japan). All readings were performed in the internal region of the pectoralis major muscle, which was subjected to three analyses to obtain values of L^* (luminosity), a^* (green/red), and b^* (blue/yellow).

The pH was measured using a portable digital pH meter (Testo, Germany) by inserting the electrode into the posterior surface of the *pectoralis major* muscle at three different points in the same sample. The readings were performed 2 h and 24 h *post-mortem*.

The shear force was determined using a texture analyzer TA.XT-Plus (Stable Microsystems Ltd., England). The samples in the form of cubes measuring 1.0 x 1.0 x 2.0 cm^3 (height, width, length) were subjected to a Warner-Bratzler blade at a speed of 5.0 mm s^{-1}. The results were expressed in Newton (N).

The water-holding capacity was obtained from 0.5 g of sample cut into cubes. The cubes were placed between two filter papers (Whatman™ model, Ge Healthcare, USA) and then between two acrylic plates. A standard weight of 10 kg was placed on the acrylic plates and the pressure was maintained for 5 min. Afterward, the sample was weighed to assess the amount of water retained compared to the initial weight of the sample (BARBUT, 1996).

The drip loss was determined using samples in the form of cubes with approximately 100 g. The samples were placed in inflated and closed plastic bags, which were hung inside a refrigerator at 4 °C for 48 h. Then, the samples were removed and weighed again to calculate the percentage of loss (HONIKEL, 1998).

Statistical analysis

The Shapiro-Wilk normality test was performed on the data set. This test showed that the data are not normal ($P > 0.05$). For this reason, the data were fitted to the gamma probability distribution using generalized *linear* models. The data were analyzed using the S*P*SS software (version 20.0), considering a significance level of 95%. To assess meat quality, the results were analyzed using the gamma probability distribution model (Generalized Linear Model - GLM). Regarding stunning efficiency, the results were assessed by the binomial model (GLM). Subsequently, for pairwise comparison, the results were subjected to the Bonferroni test (ROSSI, 2018).

RESULTS AND DISCUSSION

The coordinated wing flapping, rhythmic breathing, and vocalization showed no statistical difference between treatments ($P > 0.05$). However, the interaction between frequency and duty cycle for "arched neck" was significant ($P < 0.05$) (Table 2). The 1100 Hz frequency resulted in a higher number of birds with slightly arched necks (in 'S' shape), indicating poor poultry stunning (Table 2).

The frequency and duty cycle significantly influenced ($P < 0.05$) the occurrence of voluntary blinking (Table 2). The frequencies of 1500 and 700 Hz and the 15% duty cycle provided the highest occurrence of voluntary blinking (Table 3). However, the interaction of factors was not significant in the treatments evaluated (Table 2). There was low stunning efficiency, similar to a study that applied a frequency of 650 Hz, 100 mA, and 50% duty cycle, obtaining a high incidence of "voluntary blinking" (SIQUEIRA et al., 2017).

Table 2 - Effect test for stunning model using duty cycle (%) and frequency (Hz) factors for arched neck, voluntary blinking, wing fractures, coordinated wing flapping, rhythmic breathing, vocalization and hematomas incidence in broilers chicken.

Dependent variable	Source of variation	Wald Q-square hypothesis test	DF	P
Arched neck	Intercept	240640.00	1	< 0.0001
	F	2049.00	2	0.3590
	DC	0.35	2	0.8390
	F*DC	8970.00	3	0.0300
Voluntary blinking	Intercept	281708.00	1	< 0.0001
	F	15903.00	2	< 0.0001
	DC	18973.00	2	< 0.0001
Wing fractures	Intercept	249057.00	1	< 0.0001
	F	19487.00	2	< 0.0001
	DC	0.69	2	0.7070
	F*DC	19705.00	4	0.0010
Coordinated wing flapping	Intercept	0.00	1	1.0000
	F	0.00	2	1.0000
	DC	0.00	4	1.0000
	F*DC	0.00	2	1.0000
Rhythmic breathing	Intercept	75319.00	1	< 0.0001
	F	*	-	-
	DC	2599.00	1	0.107
	F*DC	*	-	-
Vocalization	Intercept	93336.00	1	< 0.0001
	F	3198.00	1	0.074
	DC	3198.00	1	0.074
	F*DC	*	-	-
Hematomas	Intercept	149636,00	1	< 0.0001
	F	3069.00	2	0.216
	DC	1626.00	2	0.444
	F*DC	985.00	2	0.611

SOURCE: The author (2021).

SUBTITLE: DF – Degree of freedom; P value in bold – Significant to the confidence interval of 95% (P < 0.05). F – Frequency; DC – Duty Cycle; F*DC - Frequency*Duty Cycle; * – unable to compute due to numerical problems.

The 700 Hz frequency and the interaction between 1500 Hz and 40% duty cycle significantly (P < 0.05) influenced the occurrence of wing fractures (Table 3). There was a higher number of wing fractures when the frequency of 700 Hz was applied, which compromised the carcass quality and yield. Conversely, a lower occurrence of wing fractures was observed when frequencies of 1100 and 1500 Hz were used. Contrary to what was found in our research, a frequency of 750 Hz and a current of 200 mA resulted in a lower number of carcass injuries (GIRASOLE et al., 2015) and a high frequency of 1200 Hz (GIRASOLE et al., 2016) caused poor stunning.

There was no statistical difference (P > 0.05) for chest hematomas (Table 2), pH 24 h *post-mortem*, drip loss and L^* color parameter (Table 4). All frequency levels evaluated (700 Hz, 1100 Hz and 1500 Hz) resulted in bruises and blood pooling presence, including the internal pectoralis major muscle part. According to SABOW et al. (2017), the use of high frequencies can reduce the incidence of fractures and hemorrhages because the contraction force, caused by muscle stimulation, is lower in these cases.

The color analysis showed that the frequency of 1500 Hz significantly influenced (P < 0.05) the variable a^*, whereas b^* was influenced

Table 3 - Parameters estimation for stunning model evaluating frequency and duty cycle factors for arched neck, voluntary blinking and wing fractures in broilers chicken.

Dependent variable *	---------------Fatcor---------------	-----------------DF-----------------	------------------P------------------
Arched neck	Intercept	1	< 0.001
	F1100	1	< 0.001
	F1500	1	0.711
	F700	-	-
	DC15	1	0.074
	DC25	1	0.345
	DC40	-	-
	F1100*DC15	1	< 0.001
	F1100*DC25	-	-
	F1500* DC15	1	0.222
	F1500* DC25	1	0.107
Voluntary blinking	Intercept	1	0.000
	F1500	1	< 0.001
	F700	1	0.002
	F1100	-	-
	DC15	1	< 0.001
	DC40	1	0.056
	DC25	-	-
Wing fractures	Intercept	1	< 0.001
	F1500	1	0.496
	F700	1	0.014
	F1100	-	-
	DC15	1	0.699
	DC40	1	0.163
	DC25	-	-
	F1500*DC15	1	0.075
	F1500*DC40	1	0.002
	F700*DC15	1	0.051
	F700*DC40	1	0.896

SOURCE: The author (2021).

SUBTITLE: DF – Degree of freedom; P value in bold – Significant to the confidence interval of 95% (P < 0.05). F – Frequency (Hz); DC – Duty Cycle (%); F*DC - Frequency*Duty Cycle. *Parameters estimation for coordinated wing flapping, rhythmic breathing, vocalization and hematomas are not showed because these dependent variables had no significance on Wald Q – square hypothesis test for stunning model.

(P < 0.05) by the frequency of 1100 Hz and the 25% duty cycle (Tables 4 and 5). The highest mean value of a^* was observed for the interaction between the frequency of 1500 Hz and the 15% duty cycle (Table 4). Regarding b^*, the highest values were recorded when the frequency of 1100 Hz was applied, or in the interaction between 1100 Hz and 25% duty cycle.

For L^* values no significant difference was observed among the treatments. However, the values were between 54.53 ± 3.99 and 57.90 ± 2.77. The chicken fillets are considered normal between $44.00 < L^* < 53.00$ (BARBUT et al., 2005). According to CARVALHO et al. (2018), $L^* > 53.00$ characterize the PFN (Pale, firm, non-exudative) incidence (Table 5). Following regulation EC No. 1099/2009 and the former EU regulation on stunning, SIRRI et al. (2017) evaluated the effect of a 400 Hz frequency, with current intensities of 150 mA/poultry and 90 mA/poultry, on

Table 4 - Meat quality evaluation for stunning using duty cycle (%) and frequency (Hz) factors for water-holding capacity, texture, drip loss, color parameters L^*, a^* and b^*, pH 2h and pH 24h in broilers chicken.

Fatcor	----WHC----		---Texture---		--Drip loss--		----pH 2h----		---pH 24h---		------L^*------		-------a^*------		-------b^*------	
	Mean ± SD	CV (%)	Mean ± SD	CV (%)	Mean ± SD	CV (%)	Mean ± SD	CV (%)	Mean ± SD	CV (%)	Mean ± SD	CV (%)	Mean ± SD	CV (%)	Mean ± SD	CV (%)
F700	59.38 ± 2.09	3.51	19.63 ± 7.49	38.15	3.75 ± 1.09	29.06	6.58 ± 0.22	3.30	6.07 ± 0.18	2.96	55.41 ± 3.19	5.75	2.17 ± 1.32	60.83	9.78 ± 1.80	18.40
F1100	63.25 ± 4.74	7.49	23.12 ± 6.89	29.80	3.71 ± 0.58	15.63	6.69 ± 0.14	2.11	6.07 ± 0.15	2.47	56.50 ± 2.31	4.09	2.55 ± 0.96	37.64	11.17 ± 1.43	12.80
F1500	62.89 ± 3.69	5.86	22.82 ± 7.97	34.92	3.59 ± 1.33	37.04	6.46 ± 0.18	2.83	6.08 ± 0.13	2.13	56.17 ± 2.89	5.14	2.87 ± 1.76	61.32	10.20 ± 1.49	14.60
DC15	61.27 ± 2.80	4.57	22.13 ± 6.93	31.31	3.57 ± 0.67	18.77	6.65 ± 0.23	3.54	6.09 ± 0.18	2.95	54.94 ± 2.87	5.22	2.46 ± 1.44	58.53	9.87 ± 1.56	15.81
DC25	63.97 ± 4.18	6.53	22.53 ± 8.42	37.37	3.58 ± 0.78	21.79	6.56 ± 0.18	2.83	6.06 ± 0.14	2.31	57.22 ± 2.60	4.54	2.48 ± 1.16	46.77	10.98 ± 1.68	15.30
DC40	60.27 ± 4.12	6.84	20.92 ± 7.36	35.18	3.90 ± 1.47	37.69	6.51 ± 0.19	2.96	6.07 ± 0.14	2.31	55.91 ± 2.61	4.67	2.64 ± 1.60	60.61	10.31 ± 1.62	15.71
F700*DC15	59.57 ± 2.06	3.46	19.36 ± 6.74	34.81	3.58 ± 0.69	19.27	6.82 ± 0.12	1.75	6.07 ± 0.22	3.62	54.53 ± 3.99	7.32	1.91 ± 0.75	39.26	8.89 ± 1.60	17.99
F700*DC40	58.67 ± 1.93	3.29	20.79 ± 8.21	39.49	3.86 ± 1.64	42.48	6.54 ± 0.13	2.03	6.04 ± 0.17	2.81	56.45 ± 2.57	4.55	2.28 ±1.82	79.82	9.75 ± 1.73	17.74
F700*DC25	59.90 ± 2.28	3.81	18.74 ± 7.42	39.59	3.81 ± 1.79	46.98	6.39 ± 0.12	1.93	6.10 ± 0.13	2.13	55.24 ± 2.67	4.83	2.34 ± 1.18	50.42	10.71 ± 1.65	15.41
F1500*DC40	62.68 ± 3.25	5.19	20.34 ± 7.73	38.00	4.10 ± 1.99	48.54	6.67 ± 0.09	1.32	6.08 ± 0.11	2.14	55.77 ± 3.18	5.7	2.85 ± 1.89	66.32	10.12 ± 1.52	15.02
F1500*DC15	60.61 ± 2.78	4.59	23.15 ± 5.55	23.97	3.29 ± 0.61	18.54	6.39 ± 0.09	1.42	6.12 ± 0.14	2.63	54.83 ± 1.74	3.17	3.14 ± 2.08	66.24	10.13 ± 1.05	10.36
F1500*DC25	65.38 ± 3.57	5.46	24.96 ± 9.53	38.18	3.39 ± 0.95	28.02	6.31 ± 0.11	1.77	6.03 ± 0.13	2.48	57.90 ± 2.77	4.78	2.61 ± 1.26	48.28	10.35 ± 1.86	17.97
F1100*DC25	66.66 ± 3.12	4.68	23.88 ± 6.83	28.60	3.55 ± 0.60	16.90	6.63 ± 0.14	2.15	6.04 ± 0.15	2.15	57.31 ± 2.27	2.27	2.50 ± 1.07	42.8	11.87 ± 1.14	9.60
F1100*DC40	59.46 ± 5.50	9.24	21.62 ± 6.02	27.84	3.74 ± 0.50	13.37	6.70 ± 0.10	1.54	6.08 ± 0.13	1.81	56.72 ± 1.80	3.17	2.82 ± 0.93	32.98	11.06 ± 1.36	12.30
F1100*DC15	63.63 ± 1.84	2.89	23.87 ± 7.59	31.80	3.84 ± 0.66	17.19	6.74 ± 0.16	2.43	6.08 ± 0.16	2.29	55.46 ± 2.51	4.52	2.34 ± 0.86	36.75	10.59 ± 1.51	14.25
P																
Frequency (Hz)	< 0.001		< 0.001		0.837		< 0.001		0.920		0.061		0.024		< 0.001	
Duty Cycle (%)	< 0.001		0.098		0.400		< 0.001		0.501		0.666		< 0.001*		< 0.001	
F * DC	0.004		0.010		0.654		< 0.001		0.436		0.325		0.4970		0.219	

SOURCE: The author (2021).

SUBTITLE: SD – standard deviation; CV - coefficient of variation; WHC – Water-holding capacity; F*DC - Frequency*Duty Cycle. P value in bold – Significant to the confidence interval of 95% ($P < 0.05$) on Wald Q – square hypothesis test for stunning model.

chicken meat quality and found no significant difference for color parameters (L^*, a^*, b^*), similar to the results recorded by HUANG et al. (2014) and HUANG et al. (2017), under different stunning conditions.

The pH 2 h values were influenced by the frequency and duty cycle, as well as by the interactions between them (Table 4). The pH 2 h *post-mortem* was significantly influenced ($P < 0.05$) by the frequencies

Table 5 - Parameters estimation for stunning model evaluating frequency and duty cycle factors for a* and b* color parameter, pH 2h, texture and water-holding capacity in broilers chicken.

Dependent variable *	----------------Factor----------------	------------------DF-----------------	-------------------P-------------------
b*	Intercept	1	< 0.001
	F1100	1	< 0.001
	F1500	1	0.105
	F700	-	-
	DC25	1	< 0.001
	DC40	1	0.111
	DC15	-	-
a*	Intercept	1	< 0.001
	F1100	1	0,538
	F1500	1	0,604
	DC25	1	0,220
	DC40	1	0,058
	F1100* DC25	1	0,058
	F1100* DC40	1	0,014
	F1500* DC25	1	0,016
	F1500* DC40	1	< 0.001
pH 2h	Intercept	1	< 0.001
	F1100	1	< 0.001
	F700	1	< 0.001
	F1500	-	-
	DC15	1	0.032
	DC25	1	< 0.001
	DC40	-	-
	F1100* DC15	1	0.487
	F1100* DC25	1	< 0.001
	F700* DC15	1	< 0.001
	F700* DC25	1	< 0.001
Texture	Intercept	1	0.711
	F1100	1	0.017
	F1500	1	0.789
	F700	-	-
	DC15	1	0.845
	DC25	1	0.468
	DC40	-	-
	F1100* DC15	1	0.656
	F1100* DC25	1	0.958
	F1500* DC15	1	0.014
	F1500* DC25	1	0.024
Water-holding capacity	Intercept	1	< 0.001
	F1100	1	0.534
	F1500	1	0.002
	F700	-	-
	DC15	1	0.476
	DC25	1	0.334
	DC40	-	-
	F1100* DC15	1	0.084
	F1100*DC25	1	0.002
	F1500*DC15	1	0.107
	F1500*DC25	1	0.482

SOURCE: The author (2021).

SUBTITLE: DF – Degree of freedom; P value in bold – Significant to the confidence interval of 95% (P < 0.05). F – Frequency (Hz); DC – Duty Cycle (%); F*DC - Frequency*Duty Cycle. b* - color parameter; a* - color parameter; pH 2h – pH obtained 2 hours postmortem. * Parameters estimation for dripp loss, pH 24h and L* are not showed because these dependent variables had no significance on Wald Q – square hypothesis test for stunning model.

of 1100 and 700 Hz and the 15% and 25% duty cycles, as well as by the interactions between 1100 Hz and 25% duty cycle, 700 Hz and 15% duty cycle, and 700 Hz and 25% duty cycle (Table 4). Frequencies of 1100 and 700 Hz and duty cycles of 15% and 25% caused a greater pH 2 h decline. Thus, the interactions between 700 Hz and 15% duty cycle and between 1500 Hz and 25% duty cycle resulted in the highest (6.82 ± 0.1) and lowest (6.31 ± 0.11) means of pH 2 h, respectively (Table 4). This may be because these frequencies were the lowest ones, as well as the duty cycle, which reduces the stunning efficiency due to higher poultry stress (HUANG et al., 2014).

The pH 24 h *post-mortem* did not differ significantly between the treatments (P > 0.05). Similar results were found by HUANG et al. (2017), applying frequencies of 500, 600, 700, 800, and 900 Hz. Studies using lower parameters of current (100 mA) and frequency (300 and 650 Hz) obtained mean pH values of 5.77 (SIQUEIRA et al., 2017). However, the pH 24 h post-mortem was between 6.03 ± 0.13 and 6.12 ± 0.14 (Table 4), outside the ideal range (5.7–5.9) for poultry meat after rigor mortis (ALI et al., 2005). These pH values indicate the PFN anomaly incidence (5.8 < pH < 6.1) (CARVALHO et al., 2018).

Other studies have reported that in recent years several abattoirs have observed that chicken fillets have shown a coloration with high L^* values, but do not have the characteristic pH of PSE (Pale, Soft, Exudative), the PFN (KAMINISHIKAWAHARA et al., 2016; CARVALHO et al., 2018). Study evaluating electrical and gas stunning, a high incidence of PFN was also observed (SILVA-BUZANELLO et al., 2018). It is worth mentioning that the tests of the present study were carried out the same batch of birds.

The texture was influenced by the frequency and the interaction between frequency and duty cycle (Table 4), with significant values for the frequency of 1100 Hz and the interactions between 1500 Hz and 15% duty cycle and 1500 Hz and 25% duty cycle (P < 0.05) (Table 5). The shear force ranged from 18.74 to 24.96 N. These values are higher than that (9.23 N) reported by SILVA-BUZANELLO et al. (2018), who applied a frequency of 400 Hz and 150 mA, and may be related to the use of higher frequencies and current intensities. Similar results were found when higher frequencies associated with higher currents reduced the shear force (XU et al., 2011). Analyzing the effects of electronarcosis stunning on turkeys, PARTECA et al. (2020) observed that the treatments with the highest mean pH values also resulted in the highest shear force values, corroborating the data found in the present study for both indicators.

The frequency of 1500 Hz and the interaction between 1100 Hz and 25% duty cycle were statistically significant (P < 0.05) for water-holding capacity (WHC) (Table 5). The values of WHC were between 58.87 and 66.99%, lower than those (79.58–82.57%) found by SMALDONE et al. (2021) who applied a frequency of 1400 Hz and a current of 200 mA. The WHC values obtained by applying a frequency of 1100 Hz, both for this parameter individually and combined with the duty cycle, demonstrated that all averages were higher than the frequency of 700 Hz, showing that the lower the frequency, the higher the WHC.

CONCLUSION

The AW parameters, voluntary blinking, presence of bowed neck and wing fracture evaluated, the frequencies applied in this study (700 Hz, 1100 Hz, and 1500 Hz) and duty cycle of 15%, 25%, and 40% did not ensure a good stunning efficiency. The 40% duty cycle, individually evaluated, had the best influence on stunning efficiency.

As for meat quality, color and pH 2 h parameters were affected by the applied frequencies and duty cycles. The shear force was higher at higher frequencies. The water-holding capacity was lower at 1100 Hz and in the 25% duty cycle interactions. However, the presence of the PFN anomaly was observed in the entire bird batch studied.

Thus, it can be understood that the electrical parameter configurations assessed here – frequency (700, 1100, and 1500 Hz) and duty cycle (15%, 25%, and 40%) – were not satisfactory for poultry stunning and consequently not for the quality of the final product either. However, the 40% duty cycle, individually evaluated, positively influenced the indicators of meat quality and animal welfare, suggesting that other configurations of this parameter may contribute to a more efficient stunning process and better conditions for the final product quality.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved the final version.

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