Electrical stunning in South American catfish (*Rhamdia quelen*) using direct current waveform: welfare and meat quality

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ABSTRACT: *In Brazil, humane slaughter regulation is in use since 2000; however it is not applied to fish. This paper studied parameters for electrical stunning using direct current waveform in South American catfish (Rhamdia quelen) and its subsequent effects on muscular pH and rigor mortis. Seventy fish were allocated into two groups. In group 125, fish were stunned using 125 Volts (V), 30 Hz, duty cycle of 90% and 1.3 Amp (A) applied for 30 s; in group 400, fish were stunned with 400 V, 30 Hz, duty cycle of 27%, 0.9 A, for 30 s. Unconsciousness time was determined through behavioural assessment. After slaughter, ten fish from each group were refrigerated for both measurements muscular pH and rigor mortis index (RMI) at 0, 3, 6, 24, 72 and 120 h. In 125, 14.4% (5/35) of fish were not effectively stunned, in contrast with 400 in which 100% of fish (35/35) were effectively stunned. The unconsciousness duration was higher in 400 group (87.7±16.1 s) in contrast with group 125 (66.6 ± 16.1 s). Until 6 h post mortem ninety percent of fish reached maximum rigor mortis (RMI=100%).*
Key words: *animal welfare, fish, humane slaughter, meat quality, stunning, unconsciousness.*

Insensibilização elétrica em Jundiá (*Rhamdia quelen*) usando corrente direta: bem-estar e qualidade da carne

RESUMO: *No Brasil, a normativa de abate humanitário está em vigência desde 2000, no entanto tal norma não contempla peixes. O objetivo deste trabalho foi estudar os parâmetros para insensibilização elétrica em Jundiá (Rhamdia quelen) e seus efeitos sobre o pH muscular e o rigor mortis. Setenta peixes foram alocados em dois grupos. No grupo 125, os peixes foram insensibilizados usando 125 Volts (V), 30 Hz, duty cycle of 90% e 1.3 Amp (A) durante 30 s; no grupo 400, os peixes foram insensibilizados com 400 V, 30 Hz, duty cycle of 27%, 0.9 A, durante 30 s. O tempo de inconsciência foi determinado por avaliação comportamental. Após o abate, 10 peixes de cada grupo foram refrigerados para mensurações de pH muscular e o índice rigor mortis (RMI) às 0, 3, 6, 24, 72 e 120 h. No grupo 125, 14.4% (5/35) dos peixes não foram efetivamente insensibilizados, em contraste com o grupo 400, em que 100% dos peixes (35/35) foram efetivamente insensibilizados. A duração da inconsciência foi significativamente maior no tratamento 400, igual a 87.7 ± 16.1 s em relação aos 66.6 ± 16.1 s no 125. Noventa por cento dos peixes atingiram o máximo rigor mortis (RMI=100%) dentro das 6 h pós-abate. A insensibilização elétrica em Jundiá parece ser possível usando parâmetros do grupo 400, devido à duração de inconsciência maior que 60 s.*
Palavras-chave: *abate humanitário, bem-estar animal, inconsciência, insensibilização, qualidade da carne, peixes.*

INTRODUCTION

Global production of fish, crustaceans, molluscs and other aquatic animals continued to grow and reached 170.9 million tonnes in 2016. Aquaculture production was 80 million tonnes in 2016, an increase of 5.2% from previous year (FAO, 2018a). According to website FAO statistics, in 2016, 509,285 tons of fish were produced in Brazil from aquaculture, presenting an increase of 5.4% in relation to the previous year (FAO, 2018b).

The South American catfish *Rhamdia quelen* (Osteichthyes, Teleostei, Siluriformes, Pimelodidae, Rhamdia), locally known as jundiá, is a promising species for Brazilian fish farming. The species *Rhamdia quelen* presents neotropical distribution, from Southwest Mexico to central Argentina (GOMES, et al., 2000). South American catfish production is 1,747 tons and represents 0.32% of Brazilian aquaculture (BRASIL, 2011). The estimates of South American catfish killed per year vary between 8,735 million to 582,000,

depending on the weight at slaughter (0.2 - 3 kg) (GOMES, et al., 2000). South American catfish are commonly used in polyculture with other species, such as tilapia (*Oreochromis niloticus*), common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idella*), big head carp (*Aristichthys nobilis*), silver carp (*Hypoththalmichthys molitrix*), African catfish (*Clarias gariepinus*), American catfish (*Ictalurus punctatus*) and pacu (*Piaractus mesopotamicus*) (OSTRENSKY et al., 2007). *R. quelen* lives naturally in lakes and deep wells of rivers, preferring calmer waters environments with sand and mud bottom, along the banks and vegetation. They hide among rocks and rotting logs, from which they come out at night looking for food (GOMES, et al., 2000). In experiments on larvae and fingerlings of South American catfish, marked aversion to light and search for dark places was observed (PIAIA et al., 1999).

Recently, with the intensification in animal production, concern about animal welfare has grown, most markedly in western culture. Animal welfare debate has tended to focus on terrestrial species. However, in a study on Norwegian perception about animal welfare ELLINGSEN et al. (2015) noted that fish welfare was considered important by the respondents. Additionally, highly educated Latin Americans consider fish as sentient animals and believe that fish should be included in the regulations of humane slaughter (RUCINQUE; et al., 2017). The slaughter methods used in fish are a critical point that can cause high degree of suffering in the large number of individual fish slaughtered annually for human consumption. Thus, in Norwegian and Swedish humane slaughter regulations, stunning of farmed fish is mandatory (NORWAY, 2009; RÖCKLINSBERG, 2015). However, humane slaughter regulation is applied in Brazil since 2000, although not applied to fish species (BRASIL, 2000). Public consultations to update such regulation were carried out and as a research group we contributed with information to include fish; however, in the last update in 2018 fish were not included.

Killing methods such as hypothermia in ice followed by evisceration and exsanguination cause high degree of suffering in conscious animals (ROBB & KESTIN, 2002). In Brazil, most fish slaughterhouses apply hypothermia in ice during 20 min or more prior to bleeding, by cutting the gills (OLIVEIRA FILHO et al., 2014). Other methods considered as inhumane such as asphyxia, exsanguination, evisceration or filleting in conscious fish are also used (RUCINQUE et al., 2017). Conversely, humane slaughter is characterized by two steps of interventions: stunning, which is applied to induce unconsciousness and insensibility to pain stimuli for sufficient duration to secure that an animal does not recover during bleeding, and induction of death by bleeding (TERLOUW et al., 2016). It is evident that fish slaughter in Brazil is not humane.

Electricity is widely used for stunning poultry and red meat animals that are killed for food. The principle of electrical stunning is to disrupt the electrical activity in the brain. The technique involves placing electrodes on either side of the head to pass a current of sufficient intensity through the brain (TERLOUW et al., 2016). Effective electrical stunning is associated with the induction of seizure or a generalized epileptiform insult (COOK et al., 1995). In such state, animals are unconscious; and therefore, unable to sense pain (ROBB & ROTH, 2003). The determination of unconsciousness can be achieved through electroencephalography (EEG) (ROBB et al., 2000) or behavioural assessment (KESTIN, et al., 2002).

In terms of meat quality aspects, *rigor mortis* and muscular pH are practical on-field indicators. Increased muscular activity, stress at slaughter and the consequent endocrine responses influence fish *postmortem* biochemical processes, mostly the anaerobic glycolysis and ATP degradation rate. This in turn influences the onset and release of rigor mortis, which is related to the involution rate of fish freshness. In this way, pre-slaughter stress leads to undesirable quality changes mostly in physical and freshness parameters (HUSS, 1995; POLI et al., 2005). The close interrelationship between endocrine acute stress responses and fish post-mortem biochemical processes suggested the relevance of not only haematic stress indicators like plasma cortisol, lactate and glucose, but also tissue stress indicators such as muscular pH, lactate and ATP and its catabolites. The natural muscle pH of live fish is just above 7.0, typically about 7.3; however, this falls markedly after death as the fish goes through *rigor mortis* and glycogen is converted to lactic acid (HUSS, 1995; POLI et al., 2005). When fish is on complete *rigor mortis*, the filleting process tends to be less efficient and slower. The delay on the installation of rigor mortis facilitates the manual work of filleting, optimizing the time and procedure for the operator (POLI et al., 2005).

Thus, considering the relevant negative consequences of fish suffering both for animal welfare and product quality, this paper studied parameters for electrical stunning using direct current waveform in South American catfish (*Rhamdia quelen*) and its subsequent effects on muscular pH and rigor mortis.

MATERIALS AND METHODS

Animals and location

Fish evaluated in this study (*Rhamdia quelen*) belonged to the "Estação de Pesquisa em Piscicultura e Ecologia de Espécies Nativas" (Research Station in Fish Farming and Ecology of Native Species) of ITAIPU Binacional hydropower plant, in the city of Foz do Iguaçu, Southwest of Parana, Brazil, where they were raised in cages located in the water reservoir. The experiments were carried out in September 2015. The methodology reported by RUCINQUE et al. (2018) as detailed below was followed.

The Itaipu reservoir has a flooded area of 1,350 km² and three licensed aquaculture parks accounting for 24.9 ha for native fish farming (SILVA et al., 2012). Eighty male and female fish were raised during four months in cages of 7 m³ at a final density of 40 kg/m³. Twenty-four hours before starting the experiment, healthy fish were randomly allocated and transported to an aquaculture laboratory (S 25° 25′ 46.678″ O 54° 34′ 51.873″). Transport lasted 30 min, then fish were placed into 2.4 m³ (1.88×1.48×0.89 m) concrete tanks at a density of 17 fish/m³; fish were not fed during 48 hours prior to experiments, following the common pre-slaughter fasting practice in the industry. Fasting is used to reduce both the gut contend and the charge of gut microbiota. The water in the tank came from a reservoir and it was constantly renewed by a continuous flux (10 L s⁻¹). Water quality parameters during the experiment were pH of 7.57 ± 0.37; dissolved oxygen 5.44 ±

0.61 mg L⁻¹, and temperature 20.84 ± 1.15 °C. The photoperiod was natural, 12 h of light and 12 h of darkness (RUCINQUE et al., 2018).

Six fish were used in a pilot study for testing the efficacy of currents to induce unconsciousness (Table 1). Hence, seventy 70 fish of 0.12 ± 0.04 kg and a total length of 22.73 ± 2.00 cm were allocated into two groups, 125 and 400. Rosner´s formula for comparing two means was applied to define the minimum sampling size of 35 fish in each group (ROSNER, 2010), considering a 95.0% confidence interval and a power of 90.0%. The values for mean and standard deviation of unconsciousness time for two different types of current (51 ± 26) and (37 ± 10) for stunning in tilapia were obtained from LAMBOOIJ et al. (2008).

In group 125, fish were stunned with the input parameters: pulsed direct current waveform, 125 Volts (V), 30 Hz and duty cycle equal to 90% applied for 30 s. In group 400, fish were stunned with pulsed direct current waveform, 400 V, 30 Hz and duty cycle equal to 27% applied for 30 s. The output parameters were calculated and displayed by the equipment. Electrical parameters for each group were established according to the pilot study as well as the equipment capacity (KOLZ, 2008), with the aim of comparing two different values of voltage regarding to duration of unconsciousness (Table 1).

Electrical stunning

The backpack electrofisher (Smith Root LR-24, USA https://www.smith-root.com/electrofishers/lr-24) was used for electrical stunning

Table 1 - Electrical parameters used in the pilot study to test stunning in six South American catfish (*Rhamdia quelen*).

Waveform	Input parameters			Output parameters – Average (peak)			Application time (s)	Results	
	Tension (V)	Frequency (Hz)	Duty cycle (%)	Power Watts (W)	Tension (V)	Current (A)		Effective stunning	Duration of unconsciousness (s)
Smoot direct current	205	-	-	295 (296)	193 (193)	1.3 (1.3)	30	Not	0
Pulse direct current	125	30	30	127 (160)	123 (123)	1.0 (1.3)	30	Not	0
Pulse direct current	205	50	70	266 (440)	204 (204)	1.3 (2.2)	15	Not	0
Pulse direct current	400	30	25	308 (440)	204 (204)	1.5 (2.2)	30	Yes	46
Pulse direct current	125	30	90	142 (157)	123 (127)	1.2 (1.3)	30	Yes	60
Pulse direct current	400	30	27	288 (1075)	319 (320)	0.9 (3.4)	30	Yes	50

within a 75 H × 56 W × 40 H cm plastic box serving as stunning tank, containing 80 L of fresh water coming from the concrete tanks. The distance between the ring-shaped and the wire-shaped electrodes was 40 cm. Water parameters were controlled to remain at 20 °C, dissolved oxygen >6 mg L^{-1}, pH between 6.5 and 7.5 and conductivity of 0.700 mS (RUCINQUE et al., 2018). Small quantities of salt were added to set the conductivity to the required level (LINES & KESTIN, 2004). Water measurements were using the Horiba U-50 Series Multiparameter Water Quality Meters.

Fish were removed from the concrete tanks using a hand net and placed in the stunning tank. Fish swam freely during 30 s before stunning; they were stunned in pairs. A protocol to determine the unconsciousness duration through behavioural evaluations was followed (KESTIN et al., 2002) (Table 2). The same observer evaluated all fish to avoid confusing effects due to inter-observer variations. The process of electrical stunning for all animals was recorded with a digital camera (Sony SteadyShot DSC-W320). Subsequently, the recordings were then analyzed to confirm the unconsciousness duration time for each fish, and the first observed consciousness indicator was registered. Immediately

after the observation of the return to consciousness, each fish was placed in a plastic aquarium with 35 L of fresh water and 300 mg L^{-1} of benzocaine. When fish stopped opercular movements, which corresponds to stage 6 of anaesthesia (LAMBOOIJ et al., 2008), they were bled through gill-cutting in ice, for 3 min. The carcasses were subsequently measured and weighed. The recordings were analysed to confirm the unconsciousness duration time for each fish, which was considered as ended when the first indicator of consciousness appeared, considering swimming, equilibrium, response to handling, response to painful stimuli, vestibulo-ocular reflex VOR and breathing (RUCINQUE et al., 2018).

Muscular pH and rigor mortis

First 10 fish from each group were identified and refrigerated in plastic boxes with ice. Both muscular pH and *rigor mortis* index (RMI) were measured at 0, 3, 6, 24, 72, 120 and 144 hours after slaughter. The proportion of ice per fish was 2:1. For pH determination, a 1 cm-cut was performed in the skin to access the epaxial muscle, in order to insert the electrode linked to the pH meter (MS TECNOPON, mPA 2010). A new cut

Table 2 - Protocol to determine the unconsciousness duration through behavioural evaluations after electrical stunning in South American catfish (*Rhamdia quelen*), modified from Kestin et al., 2000.

	--------Self-initiated behaviour--------		-----------Response to stimuli----------		-----------Clinical reflexes-----------	
	Swimming	Equilibrium	Handling	Prick	Eye roll	Breathing
Behaviour / reflex	Swimming behaviour	Righting ability	Response to handling	Response to prick on lip	Vestibule-ocular reflex VOR	Rhythmic opercular activity
Procedure	Observe spontaneous swimming behaviour	Invert fish, observe righting response	Attempt to catch by tail and administer tail pinch, observe response	Prick lightly on lip with enough pressure to cause pricking sensation to human, observe response	Observe eye movement when fish is rolled from side to side through the perpendicular	Observe opercula for rhythmic movement
Recovery of consciousness after shift off the current	Slow or abnormal swimming, eg, upside down or normal swimming	Slow to right or Quickly rights	Only slow or feeble response after tail pinch(s) or immediate vigorous escape attempt on first touch/pinch	Slow and reduced response or head shake or escape attempt	Partial VOR or one eye shows VOR or eyes roll relative to the head while attempting to remain upright when fish is rolled	Slow or irregular movement or regular opercula movement

was made in each measurement, alternating the left and right muscles. The pH electrode was calibrated every 10 measurements.

Rigor mortis index was calculated according to BITO et al. (1983), using the following formula:

$$RMI\ (\%) = [(L_0 - L_T)/L_0] \times 100$$

Where L_0 (cm) is the vertical distance between the base of the caudal fin and the table surface, measured immediately after slaughter, and L_T (cm) is the vertical distance between the base of the caudal fin and the table surface at selected time intervals (CONCOLLATO et al., 2014).

For the other 25 fish of each group, carcasses were filleted following the normal processing procedure. This procedure was done by trained personnel, so that they could identify haemorrhages and spinal fractures in the fillets or carcasses (RUCINQUE et al., 2018).

Statistical analyses

The Shapiro-Wilk test was used to verify the normality of data in each variable. The variables unconsciousness duration time and latency until the first spasm presented normal distribution, and the variables number of spasms, muscular pH and *rigor mortis* index did not show a normality pattern. For comparisons between treatments, the t-test and the Mann-Whitney test were applied to parametric and non-parametric data, respectively. The Kruskal-Wallis test was used to compare different measurement times within the same group. The Fisher's exact test was used to compare proportion of animals performing specific behaviours between treatments. Analyses were performed using Minitab software, version 17.

RESULTS AND DISCUSSION

In group 125 a total of 14.4% (5/35) fish were not effectively stunned, in contrast with group 400, in which 100% of fish (35/35) were effectively stunned ($P = 0.054$). The five fish from group 125 that were not effectively stunned were excluded from subsequent behavioural assessments. The lower voltage used in group 125 may have influenced the final current in a critical manner. In African catfish (*Clarias gariepinus*), voltages of 223, 225, 232 and 264 V at 50 Hz applied for 1 s in a tank with fresh water did not cause unconsciousness (LAMBOOIJ et al., 2006). Particularly, four of these fish (4/5) were not effectively stunned (group 125); they were located in the lower left corner as shown in figure 1. Probably, in this position, the current did not effectively pass through the brain.

Immediately after the current was turned on fish instantly lost behavioural responses and reflexes which were indicators of consciousness: fish stopped swimming, lost equilibrium, did not respond to painful stimuli, stopped opercular movements and did not show any vestibulo-ocular reflex. Such results are in agreement with studies in tilapia (LAMBOOIJ et al., 2008), salmon (ROBB & ROTH, 2003) and trout (ROBB et al., 2002). When the electrical current was turned off, most of fish (73.3% in 125; 80.0% in 400) presented open mouth and operculum, with no difference between groups ($P = 0.763$). Subsequently, fish showed spasms along the length of the body. The proportion of fish that had spams was similar between groups ($P = 0.204$), with a total of 30.0% in 125 and 48.6% in 400. The median (min-max) number of spasms was similar between groups ($P = 0.978$), with 6.0 (2.0–17) spasms for 125, and 6.0 (1.0–28) for 400. The latency until the first spasm was different among groups ($P < 0.01$) with mean equal to 24.0 ± 7.6 s for 125 and 47.9 ± 16.5 s for group 400. Spasms ceased when fish performed breathing movements. Data of the assessed post-stunning behaviour are presented on figure 2.

The behaviours observed in this study were also reported by VEIT et al. (2017) using sinusoidal waveform, different frequencies (50, 100, 300, 500 and 1000 Hz) and two electrical fields (128 or 256 V) in a tank (66 × 43 × 19 cm, L × W × H) with fresh water during 5 s, also in *Rhamdia quelem*. Fish remained electro-immobilized and showed a tonic phase of epileptiform insult, with open mouth and opercula and fins away from the body. Effective stunning leads to tonic/clonic seizures, which are considered a sign for unconsciousness (EFSA, 2004). Electrical stunning can provoke immediate loss of
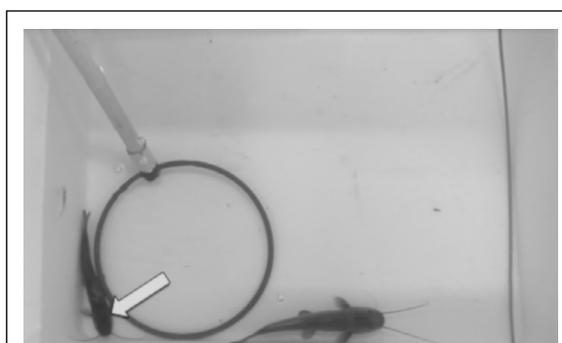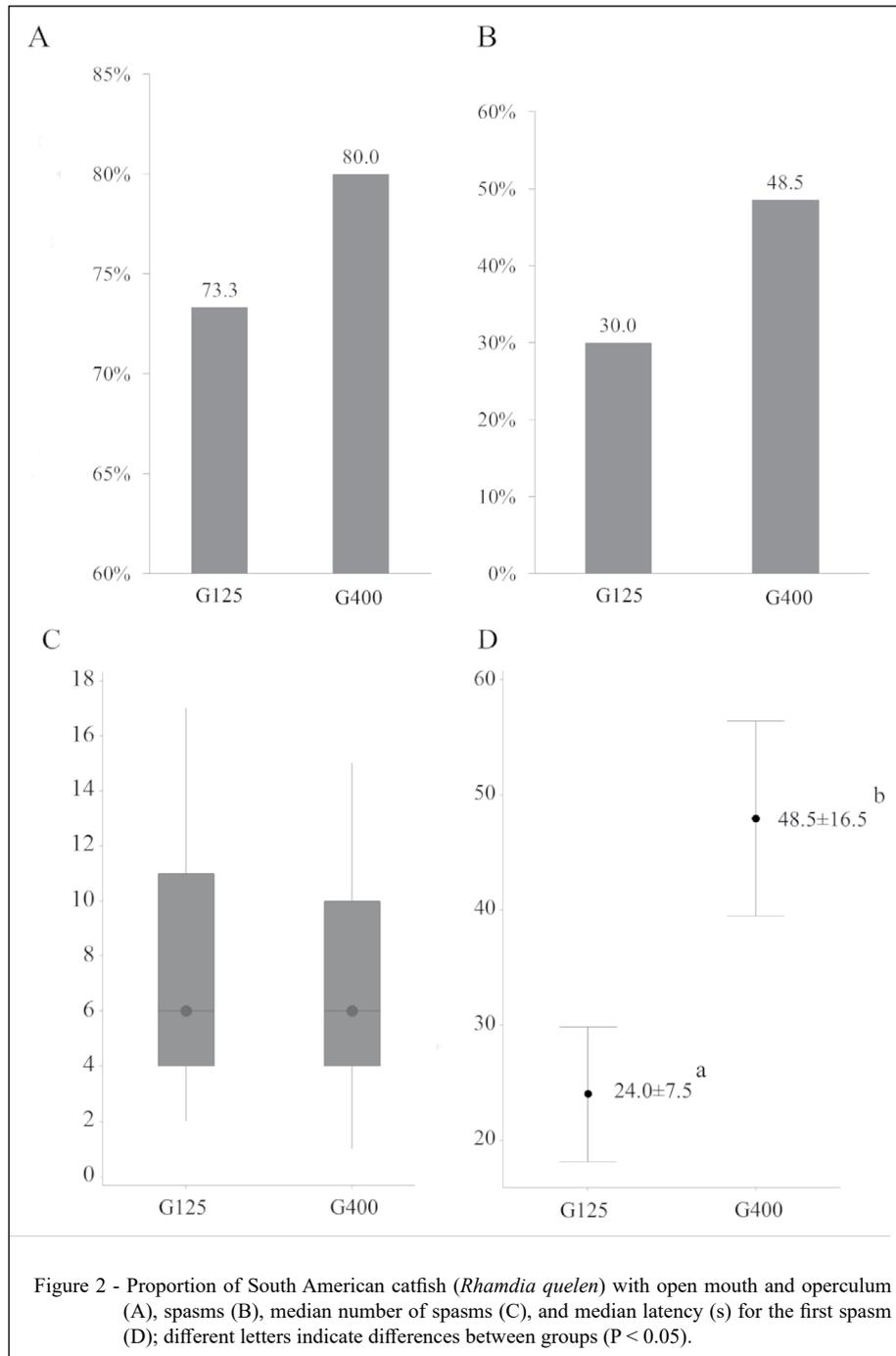

Figure 1 - The arrow indicates the position of 4/5 South American catfish (*Rhamdia quelen*) that were not effectively stunned.

consciousness in fish; it is based on the induction of a general epileptiform insult, called grand mal or seizure-like state, caused by an electrical current going through the brain (LAMBOOIJ et al., 2010). This epileptic process is characterized by rapid and extreme depolarization of the neuronal membrane potential (LAMBOOIJ et al., 2014). The

brain is unable to respond to stimuli, and there is a consensus that a vertebrate animal is unconscious and thus cannot experience pain during a generalized epileptiform insult (LAMBOOIJ et al., 2014).

When measured on the EEG, this insult consisted of relatively small waves increasing in amplitude in the tonic phase and decreasing in

Figure 2 - Proportion of South American catfish (*Rhamdia quelen*) with open mouth and operculum (A), spasms (B), median number of spasms (C), and median latency (s) for the first spasm (D); different letters indicate differences between groups (P < 0.05).

frequency in the clonic phase to result ultimately in a period of strong depression of electrical activity as described in pigs, sheep, calves and fish (LAMBOOIJ et al., 2006). The general epileptiform insult on the EEG of broiler chickens, ostriches and eels is characterized by a tonic/clonic phase and an exhaustion phase (LAMBOOIJ et al., 2004). Probably, the behaviours observed in our study for South American catfish (*Rhamdia quelen)* indicate a tonic/clonic phase and, thus, unconsciousness. Such finding can improve fish welfare at slaughter, due to the possibility of replacing the use of hypothermia in Brazilian abattoirs (MENDES, 2015; OLIVEIRA FILHO et al., 2014; PEDRAZZANI et al., 2009). PEDRAZZANI et al, (2009) observed a median time of 750 s for the loss of all behavioural indicators of consciousness in Nile tilapia subjected to hypothermia, indicating a high degree of suffering. Therefore, methods that meet human slaughter principles must be validated to replace the hypothermia at slaughter of fish in Brazil.

The open operculum and mouth in the absence of spasms are suggestive of tonic phase and the spasms reflect the onset of clonic phase. It was not possible to identify an exhaustion phase because when fish ceased spasms, they initiated breathing movements. In African catfish (*Clarias gariepinus*) stunned with 291 ± 5 V and 1.60 ± 0.11 A during 1 s in fresh water, the characteristics of a general epileptiform insult on the EEG were observed. The tonic, the clonic and the exhaustion phase were 7 ± 3, 12 ± 7 and 9 ± 6 s on the EEG, respectively (LAMBOOIJ et al., 2006). The latency to first spasms in South American catfish was longer in group 400, possibly related to the higher voltage used as compared to group 125. In trout exposed to electrical field using a sinusoidal waveform and 50 Hz, (ROBB et al., 2002), the proportional relationship between current magnitude (V) on survival and the time to recovery of opercular movements was also demonstrated. The post-stunning behaviours observed, open mouth and operculum, spams and latency until the first spasm, suggest effective unconsciousness in South American catfish (*Rhamdia quelen*) under the described experimental conditions. However, before the application of electrical stunning at pre-slaughter, the electrical parameters for induction of unconsciousness should be validated measuring brain activity through EEG, considering an exposition time of at most 1 s.

A total of 89% (58/65) of fish showed breathing as first response after electrical stunning. Breathing seems to be a promising indicator,

considering the correlation between the incidence of recovery of Visual Evoked Responses through EEG and initiation of opercular movements in Atlantic salmon (ROBB & ROTH, 2003). Other first behavioural responses in the present study were swimming (4/65), eye roll (2/65) and equilibrium (1/65). Hence, opercular movements seem to constitute an important behavioural indicator of consciousness recovery after electrical stunning in South American catfish, which can be easily applied in the field.

According to figure 3, the mean duration of unconsciousness was less in 125 (62.6 ± 15.4 s) in contrast with 400 (87.7 ± 21.6 s) (*P* < 0.01). The unconsciousness duration time was higher in 400, possibly due to higher voltage (400 V) in contrast with 125 (125 V). In a study on South American catfish (*Rhamdia quelen*) submitted to electrical stunning VEIT et al. (2017) observed that all fish recovered responses to painful stimulation and VOR after 8.5 to 57.3 s, LAMBOOIJ et al. (2006) reported 28 ± 8 s of unconsciousness in African catfish stunned through a voltage of 291 ± 5 V, 1.60 ± 0.11 A during 1 s. LAMBOOIJ (2014) reported that 40 s may be a suitable period of unconsciousness to facilitate exsanguination and onset of death in unconscious animals. In broiler chickens, the minimum duration of 60 s of unconsciousness is required for effective
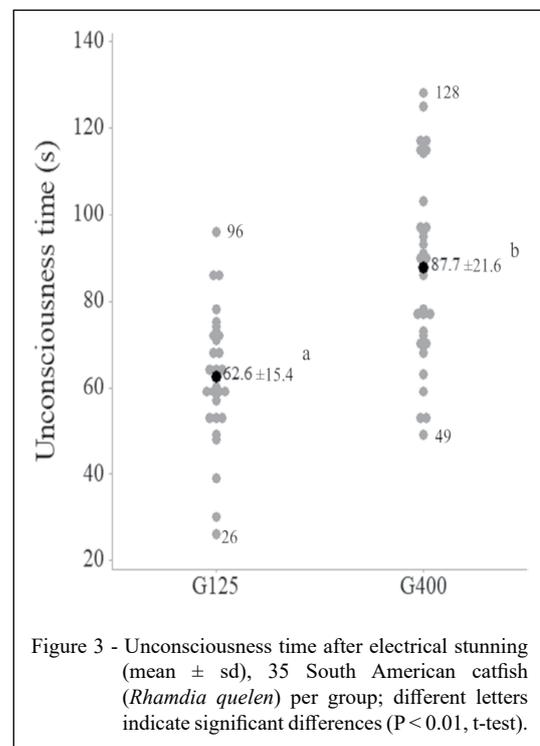

Figure 3 - Unconsciousness time after electrical stunning (mean ± sd), 35 South American catfish (*Rhamdia quelen*) per group; different letters indicate significant differences (P < 0.01, t-test).

stunning, assuming that a neck cut is performed within 30 s after stunning (HINDLE et al., 2010). Nevertheless, fish should be bled immediately following stunning to guarantee that the fish does not recover. Thus, stunning of South American catfish for humane slaughter may be possible considering such parameters: pulsed direct current waveform, 400 V, 30 Hz, duty cycle of 27% and 0.8 A applied for 30 s, since they induced unconsciousness that lasted 87.7 ± 21.6 s. The use of electroencephalography (EEG) for validation of electrical parameters for stunning together with the precautionary principle approach is suggested for further studies before on-field application of our results. The observation of behaviour does not allow differentiation between electro-immobilization or paralysis and real stunning with the loss of consciousness induced by the current. Knowledge regarding the duration of unconsciousness is also needed, as fish must be unconscious during the bleeding process until death.

Probably, the current peak has an important role because it was equal to 3.3 A in 400, coinciding with longer unconsciousness (87.7 ± 21.6 s), different from 1.3 A in 125 (62.6 ± 15.4 s) (Figure 4). In South American catfish stunned through different currents and frequencies, VEIT et al. (2017) observed a significant effect of frequency on the apparent stun duration. DASKALOVA & PAVLOV (2015) studied the effectiveness of tail-first dry electrical stunning for turbot (*Scophthalmus maximus*) and common sole (*Solea solea*). The authors used a current peak of 2.39 ± 0.91 A for turbot and 1.22 ± 0.68 A for sole applied by 1 s to induce unconsciousness during 15.1 ± 9.4 s and 13.6 ± 12.2 s for turbot and sole, respectively (DASKALOVA & PAVLOV, 2015). Thus, the results on unconsciousness duration time in 400 in this study may be related to the current peak.
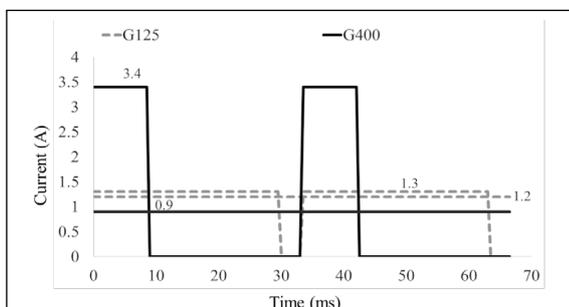
Immediately after slaughter the pH values (median, min-max) were 6.65 (6.20-6.90) for 125 and 6.70 (6.40-7.10) for 400 (Figure 5) (*P* = 0.236). Similar values (6.50 to 6.75) were observed in (*Rhamdia quelem*) submitted to electrical stunning (VEIT et al., 2017). Higher values were observed in African catfish slaughtered by cranial spiking, the initial pH of the muscle immediately after death was 7.10. It decreased to 6.00 within 4 days, and then gradually increased to 7.25 at the lapse of 18 days storage period, in which the fish were organoleptically unacceptable (ADOGA et al., 2010). MANTHEY et al. (1988) also reported values of pH equal to 6.2 ± 0.2 and 6.1 ± 0.5 at 24 hours and 10 days after slaughter, respectively, in European catfish (*Silurus glanis*) stunned with a blow to the head, eviscerated and bled. Other methods of humane slaughter, as percussion, improve the quality of meat (ROBB & KESTIN, 2002). In Atlantic cod (*Gadus morhua* L.) stunned by CO_2, lower pH values after slaughter were observed, in comparison with fish stunned by percussion (KRISTOFFERSEN et al., 2006). Therefore, electrical stunning in South American catfish does not seem to significantly alter the values of muscle pH.

The values of pH for South American catfish were similar in 125 and 400 in all the measurement times (p > 0.05). The initial reduction in pH could be attributed to *post mortem* glycolysis which led to accumulation of lactic acid, which in turn lowered the pH of the muscle (HUSS, 1995). Brazilian regulation requires a pH of less than 7.0 in the muscle of fish (BRASIL, 2017), therefore the values of muscular pH in South American catfish are in concordance with such regulation. Our results showed that electrical

Figure 4 - Current diagram (average and peak) of parameters used in 35 South American catfish (*Rhamdia quelen*) per group.
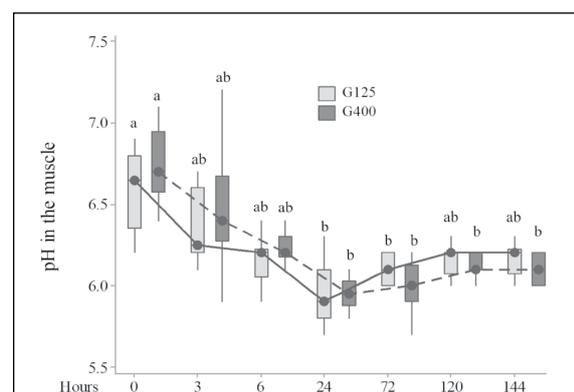
Figure 5 - Muscle pH values, in 10 South American catfish (*Rhamdia quelen*) per group; different letters indicate difference between intervals of measurement (P < 0.01, Kruskal-wallis test).

stunning does not significantly affect the pH values in South American catfish, as they are in the range allowed by the Brazilian legislation.

Until 6 h *post mortem* ninety percent of fish reached maximum rigor mortis (RMI=100%) (Figure 6). The delay in the onset and resolution of rigor mortis extend the shelf life of the fish (TEJADA, 2009). During pre-rigor phase, fish flesh remains in a state of freshness. Deterioration sets in when relaxation starts, in post-rigor period. In general, the slower the rate of progress of *rigor mortis*, the longer is the shelf-life of the fish (POLI et al., 2005). A prolongation of pre-rigor period can be achieved by delaying the onset of *rigor mortis*, decreasing the rate of progress of *rigor mortis* or by both, which are obviously of great commercial importance.

Increased muscular activity, stress at slaughter and endocrine responses may highly influence fish *post mortem* biochemical processes, mostly the anaerobic glycolysis and ATP degradation rate (HUSS, 1995). Delay the onset of *rigor* is particularly important as fish meat in pre-rigor state is considered good for the market (ISLAMI et al., 2014). In a total of 90.0% of fish in 125 and 60.0% in 400 the onset resolution of *rigor mortis* occurred at 72 h, without significant differences (*P* = 0.381). In African catfish slaughtered by cranial spiking, ADOGA et al. (2010) reported that complete relaxation (35%) occurred within 12 h after death, with no sign of spoilage, since fish deterioration begins only after the resolution of *rigor mortis* (POLI et al., 2005). Nevertheless, ADOGA et al. (2010) observed African catfish in acceptable sensorial condition up to 12 days in ice storage, despite onset the resolution of *rigor* 12 hours after death. Humane
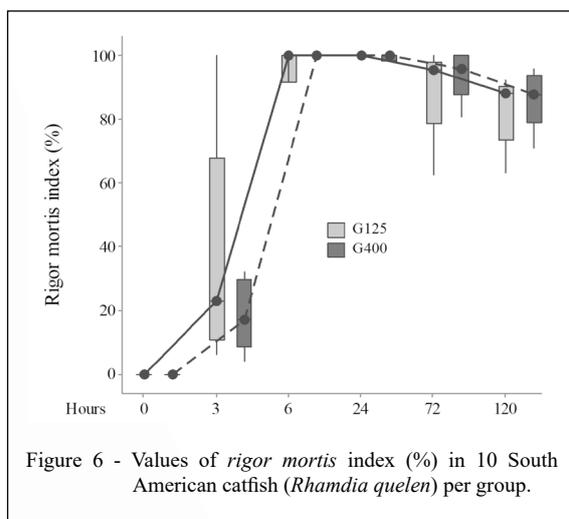
slaughter methods can delay the onset and resolution of *rigor mortis* in comparison to stunning with CO_2 (POLI et al., 2005; ROTH et al., 2002). In addition, the pre-slaughter process which involves fasting, crowding and transport from net cages to stunning tanks might influence muscle energy reserves and thus rigor mortis resolution. Electrical stunning in South American catfish accelerates the resolution of *rigor mortis*. It is important that future studies determine the quality of the meat for human consumption.

Carcass damage as muscle haemorrhages or broken vertebral column might occur when electrical stunning is used, especially when fish are immersed in water during stunning (VAN DE VIS et al., 2014). In a study on South American catfish stunned using a sinusoidal waveform with 5 s of duration, different frequencies (50, 100, 300, 500, or 1000 Hz) and electrical fields (128 or 256 V m⁻¹), VEIT et al, (2017) reported spinal fractures in 62% of fish. No problems were observed in the carcass quality in our study. Fillets and carcass did not show any haemorrhages, blood spotting in the meat, external damage or vertebral fracture. Perhaps, the electric parameters used are safe in terms of avoiding bleeding or fractures in the carcass.

New technologies are required to implement methods that meet the criteria for humane slaughter in fish, allowing for complete substitution of immersion in water and ice used in Brazil. New studies assessing unconsciousness by EEG as well as searching for methods with longer unconsciousness duration are recommended.

CONCLUSION

Electrical stunning in South American catfish (*Rhamdia quelen)* is possible through pulse direct current, 400 Volts, 30 Hz, duty cycle of 27% and 0.9 A applied for 30 s, and the unconsciousness thus produced seems to last an adequate period of time to allow for death, avoiding the risk of the animals return to a conscious state. Additionally, this stunning method did not cause significant alterations on muscle pH values. The electrical parameters employed seem safe in terms of avoiding muscle bleeding or fractures in the carcass. Validation of our results with electroencephalographic studies is warranted.

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Figure 6 - Values of *rigor mortis* index (%) in 10 South American catfish (*Rhamdia quelen*) per group.

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BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

This experiment was approved by the Ethics Committee for Animal Use of the Agricultural Sciences Campus of the Universidade Federal do Paraná (Universidade Federal do Estado do Paraná (UFPR), Brazil), during session on August 27, 2015, and it is registered under protocol number 058/2014.

DECLARTION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, 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 of the final version.

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