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Impact of electrical stunning on fish behavior and meat quality of pacu (*Piaractus mesopotamicus*)

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ABSTRACT. Electrical stunning is considered one of less stressful fish stunning method. However, for its efficiency, it is important to determine the best way of application of the electrical variables. The aim of this study was to evaluate the effect of different voltages (100, 150, and 200 V) of alternating current (AC) or direct current (DC), frequency of 50 Hz, for 180 s exposure, concerning the aspects of behavior and meat quality of pacu (*Piaractus mesopotamicus*). Fish subjected to AC remained unconscious longer, evaluated by behavior analysis, when compared to DC. The pacu subjected to 100 AC, 200 AC and 100 DC showed less bleeding in the fillets. The lightness (L★) of the fillets were higher in 150 AC, 150 DC and 100 DC, followed by 200 and 100 AC. The redness (a★) in pacu fillets were moderate in treatments 200 and 150 AC and less intense in treatments 100 DC and 100 AC. The yellowness (b★) was higher in fish fillets treatment 150 DC and lower in 100 AC. Therefore, the 200 V alternating current (AC) used in water stunning caused apparent unconsciousness of up to 4 min. after exposure and less bleeding in fillets, thus it is a suitable stunning method to be recommended for the pacu processing industry.

Keywords: fillets color, slaughter, native species, fish.

Impacto do atordoamento elétrico no comportamento e na qualidade da carne do pacu (*Piaractus mesopotamicus*)

RESUMO. O atordoamento elétrico é considerado um dos métodos de abate de peixes menos estressantes. No entanto, para eficiência deste método, é importante determinar a melhor combinação de variáveis elétricas. O objetivo deste estudo foi avaliar o efeito de diferentes voltagens (100, 150 e 200 V) de corrente elétrica alternada (CA) ou contínua (CC), frequência de 50 Hz, por 180 s de exposição, nos aspectos de comportamento e de qualidade da carne de pacus (*Piaractus mesopotamicus).* Os peixes submetidos à CA permaneceram mais tempo inconscientes, o que foi avaliado pela análise comportamental, do que aqueles submetidos à CC. Os pacus submetidos a 100 CA, 100 CC e 200 CA apresentaram menor hemorragia nos filés. A luminosidade dos filés (L★) foi maior nos tratamentos 150 CA, 150 CC e 100 CC, seguidos dos de 200 CA e 100 CA. A intensidade de vermelho (a★) nos filés dos pacus foi moderada nos tratamentos 200 CA e 150 CA e menos intenso nos tratamentos 100 CC e 100 CA. A intensidade de amarelo (b★) foi maior nos filés dos peixes submetidos ao tratamento 150 DC e menor em 100 CA. Portanto, a corrente alternada com 200 V usada no atordoamento de pacus causa aparente inconsciência por até 4 min. após exposição e pouca hemorragia nos filés, sendo, assim, um método recomendado para a indústria de processamento do pacu.

Palavras-chave: cor dos filés, abate, espécie nativa, peixe.

Introduction

In most cases, stunning and slaughter methods used by fishing industry are considered stressful. These methods include asphyxia in air or ice, gills cut, decapitation, gutting (with live animals), salt bath, and thermal shock in ice and water, the latter being the most used. However, stunning and slaughter methods by cranial percussion, cutting the spinal cord followed by gills cut, overdose of anesthetics and electrical shock can be called humane methods because they cause rapid stunning and less animal suffering (Van de Vis et al., 2003).

Slaughtering can be subdivided into two stages; the first, when the fish is stunned, and the second with the death itself. Stunning can occur by several forms, such as thermal shock, electric shock, overdose of anesthetic, cranial percussion, or asphyxia. The slaughter of fish can be performed by gills cut, bleeding, evisceration, decapitation, and cranial percussion. Fish can be subjected to both stages or directly to the second stage (Lines, Robb, Kestin, Crook, & Benson, 2003).

Electric shock is one of stunning / slaughtering methods most studied in several fish species (Nordgreen, Slinde, Moller, & Roth, 2008). It consists in passing an electrical current in water or directly on fish until complete loss of consciousness (Van de Vis et al., 2003). It is considered humane because it is fast and apparently causes less suffering than other slaughtering techniques. However, several factors may influence the effectiveness of this technique, such as type of electrical current (alternating current - AC or direct current - DC) (Robb & Roth, 2003), voltage (Lines et al., 2003; Robb & Roth, 2003), frequency (Hertz) (Lines et al., 2003; Lines & Kestin, 2005), duration of electrical current (seconds) (Lines et al., 2003; Nordgreen et al., 2008), and water conductivity (Lines & Kestin, 2004). In sea bass (*Dicentrarchus labrax)* subjected to electrical stunning (40 to 120 V and 50 to 400 Hz) for 10 s, no recovery of consciousness was observed after 20 min. (Zampacavallo et al., 2015). In tench (*Tinca tinca)* subjected to electrical stunning (51 V, 30 s) the onset of rigor mortis was more rapid when compared with the application of cranial percussion, live chilling, or CO_2 narcosis, but without compromising other quality parameters such as meat pH, color, and texture (Gasco, Gai, Rotolo, & Parisi, 2014). Therefore, studies on various factors of humane methods in the slaughter, such as electric shock are of great relevance.

Pacu (*Piaractus mesopotamicus)* belongs to the family Characidae, subfamily Myleinae. It is typical from Pantanal, the Amazon, and La Plata river basin (Borges, Conte-Junior, Franco, & Freitas, 2013). In nature, they feed mainly on fruits and seeds (Ferreira & Silva, 2012). In the national aquaculture, it is one of the most produced native species. Their positive characteristics include fast growth, omnivore feeding behavior, easy adaptation to artificial feeding, besides being a richly flavored fish (Borges et al., 2013). However, despite its commercial importance for Brazilian aquaculture, studies on stunning methods concerning fish behavior and meat quality are scarce in literature. Therefore, given the importance of this species for aquaculture production in Brazil, the present study evaluated the effect of electrical stunning parameters on fish behavior and meat quality.

Material and methods

Thirty six live pacus (964 ± 186 g) were purchased from a commercial fish farm, transported to the laboratory and housed in 6 plastic boxes of 300 L (6 fish per box) with open water circulation and water quality parameters appropriate for the species (pH = 5.4 ± 0.2, dissolved oxygen = 4.8 ± 0.0 mg L^{-1}, temperature = 27.9 ± 0.1ºC). Fish remained in the boxes for about 48 hours before the experiment.

Different stunning voltages (100, 150 and 200 V) of alternating current (AC) or direct current (DC) and frequency of 50 Hz were evaluated (6 treatments, n = 6 per treatment, total = 36) on pacus behavioral aspects and meat quality. For each treatment, three fish at a time were transferred rapidly from the storage containers, and housed in plastic boxes of 120 L water with salinity adjusted to contain approximately 700 $\mu S\ cm^{-1}$ conductivity (pH = 5.7 ± 0.4, water conductivity $\mu S\ cm^{-1}$ = 739.2 ± 22.2; dissolved oxygen = 4.7 ± 0.1 mg L^{-1}, temperature = 28.2 ± 0.5ºC). Therefore, for each treatment, slaughter was repeated 2 times. Two aluminum plates (65 x 35 cm wide) were placed close to the walls at a distance of 49 cm between them, and used for the formation of electric field inside the box. An electrode connected to a device developed specifically for this purpose was attached in each plate. Fish were subjected to application of electrical current for 180 seconds. The time of electrical current was determinated according to Scherer et al. (2005) and Vargas, Oliveira Filho, Natori, Lima, & Viegas (2013). Fish were removed from water, and housed in three other plastic boxes containing 120 L water to evaluate fish behavior. After, fish were subjected to slaughtering by cutting the gill arches and bleeding in running water for about 3 minutes. Then, the fish fillets were manually removed and kept refrigerated at 4ºC for instrumental color analysis and sensory evaluation.

Behavioural assessment

Fish behavior was assessed during electrical stunning to observe possible signs of stress in an attempt to escape, and the time required for the apparent stunning. After placing fish into the boxes containing 120 L water, the behavior was evaluated over 10 min. at 2 min. intervals, using a protocol developed by Kestin, van de Vis, & Robb (2002). Fish own behavior (swimming and equilibrium), response to stimuli (handling and needle-stick), and clinical reflexes (eye rotation and opercular movement) were evaluated using a 3-point scale of sensitivity, where 2 corresponds to full sensitivity, 1 corresponds to partial sensitivity, and 0 corresponds to lack of sensitivity.

Sensory evaluation

Sensory evaluation of fish fillets was performed in the laboratory under white fluorescent light on the same day of slaughtering. Sorting test (Friedman test) was used according to the methodology described by Meilgaard, Civille, & Carr (1999). The sensory panel was composed by eight experienced panelists in sensory evaluation of fish. Six fillets from each treatment and an evaluation form were presented to each assessor on coded trays identified by a three digit number in a balanced order. The assessors classified all fillets sorting in increasing order of 'less bleeding', corresponding to position 1 and value 1, to 'heavy bleeding', corresponding to position 6 and value 6. The scores given by the panelists were totaled and will be presented on this sum form.

Instrumental color

The instrumental color was determined on the day following slaughter using six pacu fillets, stored at 4°C. For each fillet, 3 color measurements in the region above the lateral line were made with the aid of a portable colorimeter (Miniscan XE, HunterLab, Reston, USA), previously calibrated with black and white standard, operating with D65 illuminant and 10° standard observer, and measuring area of 30 mm diameter. The results were expressed in terms of L^* (lightness), a^* (red-green), b^* (yellow-blue), in the CIELab color system – 'Commission Internationale de L'Eclairage'.

Experimental design and statistical analysis

A completely randomized experimental design was used, with 6 treatments and 6 repetitions (6 X 6). The results of fish behavior were described as an explanatory manner based on calculations of means, dispersion (e.g. standard deviation), and of proportion (percentage) of conscious fishes (sensitivity grades equal or higher than one). For the sensory analysis, the Friedman test was applied, and data were compared by Newell and Mac Farlane table at 5% significance level. The results of instrumental color were subjected to analysis of variance (ANOVA), and Tukey multiple comparison test for differences ($p < 0.05$) among average of treatments, using the statistical program Sigma Stat 3.5®.

Results and discussion

Behavioral assessment

No adverse reactions in an attempt to escape, neither signs of pain or stress were observed during stunning of pacus subjected to direct current (DC) in the range of 100 to 200 V for 3 min. Almost instantaneously with the start of application of electrical current, fish emerged immobile, with color change after a few seconds, showing more whitish appearance. Water was fairly turbid and foaming on the surface. For stunning with alternating current (AC) in the range of 100 to 200 V, pacus also exhibited no sign of stress, remaining in the same position throughout the time of application of electrical current. In this case, water was not turbid, remaining transparent without foaming. This characteristic may be related to the type of electrical current in combination with the salt water, once the continuous passage of electrical current leads to chemical reactions making water turbid and foaming. Kestin et al. (2002) also observed that in trout (*Oncorhynchus mykiss)*, Atlantic salmon, sea bream (*Sparus aurata)*, and eel (*Anguilla anguilla*) all behaviors and responses to stimuli were lost immediately when using electrical stunning. In sea bass (*Dicentrarchus labrax)* electricity caused fish immobility without any attempt to escape. Eyes and gills became clear after the use of electricity, while the mouth tended to be open (Knowles et al., 2007). According to Lines et al. (2003) when applying the electrical current in trout, fish were totally unconscious within 1 second. As reported by these authors, the mechanism of entry into unconsciousness is caused by the decrease in irrigated blood to the brain due to temporary stop of the heartbeat when electrical current is applied. In Atlantic salmon, the electrical stunning also stopped opercular movements (Robb & Roth, 2003), which may explain the immediate loss of consciousness of pacu after application of alternating (AC) or direct (DC) current. However, this event may not occur in all species, since in eels, subjected to a combination of 250 V for 10 s and 80 V by 7 min., it was observed that the application of electrical current with high voltage by a short period led to muscle contraction followed by an apparent relaxation, remaining immobile during the application of low electrical current for longer time (Morzel & van de Vis, 2003). In another study on eels, Robb, Wotton, & van de Vis (2002) observed that the application of electrical current also caused fish muscle contraction during stunning.

The analysis of spontaneous swimming capacity represents the variation in the normal swimming to total movement loss (Kestin et al., 2002). Most of pacus of the present study (66.7%) subjected to 100 DC presented signs of slow recovery (grade ≥ 1) after 4 min of observation (Table 1). After 8 min., the electrically 100 AC, 83.3% stunned pacus also exhibited slow swimming. For the other treatments, fish practically showed no swimming ability within 10 min. of recovery (grade < 1). This means that the application of electrical current up to 100 V caused loss of swimming ability in pacus, and the alternating current (AC) was more effective in this parameter. These results agree with Robb & Roth (2003), who reported that the application of direct current (DC) did not cause loss of consciousness in Atlantic salmon.

The assessment of the equilibrium of electrically stunned fish can be measured by the time to recover the normal equilibrium position, ranging from rapid recovery to total inability to return to normal position (Kestin et al., 2002). Slow recovery (grade ≥ 1) was observed after 4 min. in 100% fish subjected to 100 DC and 66.7% pacu subjected to 100 AC. At 8 min., 66.7% pacu subjected to 150 AC also showed slow recovery of equilibrium (Table 1).

Handling is verified by the escape ability of fish as a reflex to being caught by the tail (Kestin et al., 2002). After 4 min., only fish subjected to 100 DC (83.3%) exhibited slow response (grade ≥ 1) for human manipulation (handling), which was also observed after 6 minutes for 66.7% pacus subjected to 100 AC. For all treatments, no vigorous attempt to escape (grade 2) was observed (Table 1). According to Lambooij, Gerritze, Reimert, Burggraaf, & van de Vis (2008), no matter how small the voltage of alternating or direct current is, it is capable of causing confusion in fish, which may be the reason to why pacus were not able to recover quickly the escape attempt after application of electrical current.

The sensitivity to the needle stick on the sideline is measured by the response rate when a needle is passed on the lateral line of fish (Kestin et al., 2002). This parameter was found to be moderate (grade ≥ 1) after 4 min. most of pacu subjected to 150 DC (83.3%), 100 DC (100%), and 100 AC (66.7%). After 6 min., the pacus subjected to 200 DC, 200 AC, 150 AC, and 100 AC exhibited slow response. After 8 min., all treatments exhibited slow response for this parameter (grade ≥ 1), except fish subjected to 100 AC, which showed fast response (grade 2) (Table 1).

The rotation of fish eyes, vestibulo-ocular reflex (VOR) is measured by the eye movement when it is rotated by the shaft itself (Kestin et al., 2002). Kestin et al. (2002) have reported that along with breath, which is measured by the opercular movements, VOR is the last parameter to be lost and the first to return to the normal function when fish recover sensitivity, thus it is one of the most important behavioral parameters to be evaluated. The VOR was partial (grade ≥ 1) within only 2 min. of recovery in 83.3% pacus subjected to 100 DC and 150 DC. After 4 min., fish subjected to 200 DC, 150 AC, and 100 AC showed partial VOR. After 6 min., 100% fish submitted to 100 DC had already normal VOR (grade 2). After 2 min. of recovery, slow opercular movement (grade ≥ 1) was observed in 66.7% pacu subjected to 150 DC, and 100% pacu subjected to 100 DC. After 4 min., along with these fish, a slow movement was also observed in fish subjected to 200 DC, 200 AC, 150 AC, and 100 AC. After 6 min., fish subjected to 100 DC already exhibited rapid movement of the opercula (grade 2), which remained until 10 min. of recovery (Table 1). The faster recovery of consciousness in pacus subjected to application of continuous current is consistent with the findings of Nordgreen et al. (2008), who reported that the alternating current is more effective than the continuous current, with respect to the time that the electrically stunned fish become insensitive.

Unlike the results of the present study, sea bass subjected to electrical stunning at 40 to 120 V and 50 to 400 Hz for 10 s exhibited no recovery of consciousness after 20 min. (Zampacavallo et al., 2015). This may indicate that different species and resistance factors may lead or not to recovery of consciousness after application of electrical current.

In eels subjected to a combination of 250 V for 10 s, followed by 80 V for 7 min., moderate breathing or muscle activity was observed after fish were transferred to observation tanks, which may be a reflex activity rather than recovery of consciousness (Morzel & van de Vis, 2003). In another study using electrical current in eels, it was observed that in low-voltage current, fish showed vigorous movements shortly after the electrical current was removed. With the increase of DC voltage, the time of recovery to normal position took longer. However, exposure to low voltage for a long time resulted in death of half of the total fish studied (Robb et al., 2002).

Table 1. Scores (mean ± standard deviation) using a 3-point scale of sensitivity, where 2 corresponds to full sensitivity, 1 corresponds to partial sensitivity, and 0 corresponds to lack of sensitivity of the evaluation of parameters of own behavior (swimming and equilibrium), response to stimulus (handling and needle-stick) and clinical reflexes (eye rotation and opercular movement) of pacu (n = 6 per treatment) stunned by different types of electrical current (AC or DC) and voltage.

Behavior	Treatment	Recovery Time (min.)				
		2	4	6	8	10
Swimming	200 DC	0.0 ± 0.0 0%[1]	0.0 ± 0.0 0%	0.2 ± 0.4 16.7%	0.3 ± 0.5 33.3%	0.2 ± 0.4 16.7%
	150 DC	0.0 ± 0.0 0%	0.3 ± 0.5 33.3%	0.3 ± 0.5 33.3%	0.3 ± 0.5 33.3%	0.7 ± 0.8 50%
	100 DC	0.2 ± 0.4 16.7%	0.8 ± 0.8 66.7%	1.0 ± 0.6 83.3%	1.0 ± 0.6 83.3%	1.2 ± 0.4 100%
	200 AC	0.0 ± 0.0 0%	0.0 ± 0.0 0%	0.0 ± 0.0 0%	0.2 ± 0.4 16.7%	0.3 ± 0.5 33.3%
	150 AC	0.0 ± 0.0 0%	0.3 ± 0.5 33.3%	0.3 ± 0.5 33.3%	0.5 ± 0.5 50%	0.5 ± 0.5 50%
	100 AC	0.0 ± 0.0 0%	0.2 ± 0.4 16.7%	0.5 ± 0.5 50%	1.2 ± 0.8 83.3%	1.3 ± 0.8 83.3%
Equilibrium	200 DC	0.0 ± 0.0 0%	0.3 ± 0.5 33.3%	0.2 ± 0.4 16.7%	0.0 ± 0.0 0%	0.3 ± 0.5 33.3%
	150 DC	0.0 ± 0.0 0%	0.3 ± 0.5 33.3%	0.5 ± 0.5 50%	0.5 ± 0.5 50%	0.7 ± 0.5 66.7%
	100 DC	0.5 ± 0.8 33.3%	1.3 ± 0.5 100%	1.5 ± 0.5 100%	1.7 ± 0.5 100%	2.0 ± 0.0 100%
	200 AC	0.0 ± 0.0 0%	0.0 ± 0.0 0%	0.2 ± 0.4 16.7%	0.3 ± 0.5 33.3%	0.5 ± 0.5 50%
	150 AC	0.2 ± 0.4 16.7%	0.3 ± 0.5 33.3%	0.7 ± 0.8 50%	0.8 ± 0.8 66.7%	1.3 ± 0.8 83.3%
	100 AC	0.0 ± 0.0 0%	0.7 ± 0.5 66.7%	1.3 ± 0.8 83.3%	1.8 ± 0.4 100%	1.8 ± 0.4 100%
Handling	200 DC	0.0 ± 0.0 0%	0.0 ± 0.0 0%	0.2 ± 0.4 16.7%	0.2 ± 0.4 16.7%	0.2 ± 0.4 16.7%
	150 DC	0.0 ± 0.0 0%	0.3 ± 0.5 33.3%	0.5 ± 0.5 50%	0.8 ± 0.8 66.7%	0.3 ± 0.8 16.7%
	100 DC	0.3 ± 0.5 33.3%	1.2 ± 0.8 83.3%	1.7 ± 0.5 100%	1.7 ± 0.5 100%	1.7 ± 0.5 100%
	200 AC	0.0 ± 0.0 0%	0.0 ± 0.0 0%	0.0 ± 0.0 0%	0.2 ± 0.4 16.7%	0.3 ± 0.5 33.3%
	150 AC	0.0 ± 0.0 0%	0.3 ± 0.5 33.3%	0.5 ± 0.8 33.3%	0.7 ± 0.5 66.7%	0.7 ± 0.5 66.7%
	100 AC	0.0 ± 0.0 0%	0.0 ± 0.0 0%	0.8 ± 0.8 66.7%	1.7 ± 0.5 100%	1.8 ± 0.4 100%
Needle-stick	200 DC	0.0 ± 0.0 0%	0.7 ± 0.8 50%	1.0 ± 0.9 66.7%	1.0 ± 0.9 66.7%	0.8 ± 0.8 66.7%
	150 DC	0.0 ± 0.0 0%	0.8 ± 0.4 83.3%	1.2 ± 0.4 100%	1.2 ± 0.4 100%	1.2 ± 0.4 100%
	100 DC	0.3 ± 0.8 16.7%	1.5 ± 0.5 100%	1.3 ± 0.8 83.3%	1.5 ± 0.5 100%	1.5 ± 0.5 100%
	200 AC	0.0 ± 0.0 0%	0.5 ± 0.5 50%	0.7 ± 0.5 66.7%	1.0 ± 0.6 83.3%	1.0 ± 0.6 83.3%
	150 AC	0.5 ± 0.5 33.3%	0.5 ± 0.8 33.3%	1.0 ± 0.6 83.3%	1.3 ± 0.8 83.3%	1.3 ± 0.8 83.3%
	100 AC	0.2 ± 0.4 16.7%	0.8 ± 0.8 66.7%	1.7 ± 0.5 100%	2.0 ± 0.0 100%	1.7 ± 0.5 100%
Eye rotation	200 DC	0.0 ± 0.0 0%	1.0 ± 0.6 83.3%	1.3 ± 0.5 100%	1.5 ± 0.5 100%	1.2 ± 0.4 100%
	150 DC	1.5 ± 0.8 83.3%	0.7 ± 0.8 50%	1.5 ± 0.5 100%	1.7 ± 0.5 100%	1.7 ± 0.5 100%
	100 DC	1.5 ± 0.8 83.3%	1.8 ± 0.4 100%	2.0 ± 0.0 100%	2.0 ± 0.0 100%	2.0 ± 0.0 100%
	200 AC	0.0 ± 0.0 0%	0.5 ± 0.5 50%	1.2 ± 0.8 83.3%	1.2 ± 0.8 83.3%	1.3 ± 0.8 83.3%
	150 AC	0.3 ± 0.5 33.3%	1.2 ± 0.8 83.3%	1.5 ± 0.8 83.3%	1.5 ± 0.8 83.3%	1.8 ± 0.4 100%
	100 AC	0.2 ± 0.4 16.7%	1.5 ± 0.8 83.3%	1.8 ± 0.4 100%	1.8 ± 0.4 100%	1.7 ± 0.5 100%
Opercular movements	200 DC	0.3 ± 0.5 33.3%	0.7 ± 0.5 66.7%	1.2 ± 0.4 100%	1.2 ± 0.4 100%	1.2 ± 0.4 100%
	150 DC	0.7 ± 0.5 66.7%	1.2 ± 0.4 100%	1.2 ± 0.4 100%	1.3 ± 0.5 100%	1.3 ± 0.5 100%
	100 DC	1.2 ± 0.4 100%	1.8 ± 0.4 100%	2.0 ± 0.0 100%	2.0 ± 0.0 100%	2.0 ± 0.0 100%
	200 AC	0.2 ± 0.4 16.7%	0.8 ± 0.4 83.3%	0.8 ± 0.4 83.3%	0.7 ± 0.5 66.7%	0.5 ± 0.5 50%
	150 AC	0.3 ± 0.5 33.3%	0.7 ± 0.5 66.7%	1.2 ± 0.8 83.3%	1.2 ± 0.4 100%	1.3 ± 0.8 83.3%
	100 AC	0.3 ± 0.5 33.3%	0.8 ± 0.4 83.3%	1.5 ± 0.5 100%	2.0 ± 0.0 100%	2.0 ± 0.0 100%

[1] Proportion (percentage) of conscious fishes (sensitivity grades equal or higher than one).

Sensory evaluation

Sensory evaluation was performed to elucidate if the assessors were able to distinguish possible bleeding in the fillets, caused by the use of electricity as stunning method. According to the results, the treatments with 200 and 150 Volts (DC) received higher scores (48 and 40 points, respectively, Table 2), with increased bleeding in the fillets. Studies on other fish species also reported increase in bleeding when electrical current was applied for desensitization (Lambooij et al., 2008; Vargas et al., 2013). The presence of hemorrhage may compromise the meat quality, once consumers prefer to buy fish fillets with a light color or natural color characteristic of the species (Pullela et al., 2000), otherwise it may indicate failures in fish processing or storage. Pacus have light colored meat (Pullela et al., 2000), but the natural color characteristics were not observed in the treatments at 200 and 150 Volts DC.

In contrast, the stunned pacu fillets subjected to 100 volts AC or DC exhibited less bleeding, probably due to the lower voltage. However, the period of unconsciousness must also be taken into account, since the consciousness recovery was very fast in these treatments, as observed by the behavioral analysis (Table 1). The 200 V AC treatment also stood out, once the stunned fillets presented lower bleeding, despite the higher voltage used in the study. In addition, it is worth noting that pacus remained unconscious for a longer period after the application of electrical stunning under these conditions. These findings emphasize the differences between the electrical currents applied to pacus, once according to the sensory evaluation the continuous electrical current caused higher bleeding. Another negative fact is that higher bleeding may impair the shelf life of frozen fillets, because blood contains iron, which is one of the catalysts of lipid oxidation in fish meat (Sánchez-Alonso & Borderías, 2008).

Table 2. Sum of the results from the sensory analysis (Sorting test)[a] of pacu fillets after stunning by different types of electrical current (AC or DC) and voltage[b].

Treatments	Sum
200 DC	48 a
150 DC	40 ab
150 AC	32 abc
200 AC	23 bcd
100 DC	17 cd
100 AC	8 d

[a]All fillets sorting in increasing order of 'less bleeding', corresponding to value 1, to 'heavy bleeding', corresponding to value 6. The scores given sum of eight panelists. [b]Different letters indicate minimum numerical difference between the sums ($22 = p < 0.05$).

Instrumental color

Color of fish meat is a major quality parameter evaluated by consumers. There is evidence that electrical stimulation causes changes in meat color due to muscle contraction, thus damaging skeleton and veins, which will later be visualized in the fillets (Morzel & van de Vis, 2003; Scherer et al., 2005; Knowles et al., 2008). In the present study, fillet color varied according to the voltage and type of current used in the stunning method (Table 3). Fish subjected to 150 AC, 150 DC, and 100 DC had higher ($p < 0.05$) L^* values (lightness), followed by the treatments 200 AC and 100 AC, while the fillets subjected to 200 DC presented lower L^* values (Table 3). Lightness of fish fillets is an attractive attribute when buying fish. In fresh fish, under correct stunning and slaughter procedures, fillets are bright. However, stressful practices during the processing stages prior to fish death can make the fillets more opaque (lower L^* value) (Roth et al., 2010). In this context, with respect to the L^* parameter, treatments at 150 AC, 150 DC, and 100 DC presented better results, whereas the treatment at 200 DC was not suitable for pacus stunning.

A significant ($p < 0.05$) increase in red color was observed in pacu fillets subjected to 200 DC and 150 DC (higher a^* values), while fish subjected to 200 AC and 150 AC exhibited moderate redness (a^*), and those subjected to 100 DC and 100 AC presented less intense red color. Lower L^* values and higher a^* values are observed when bleeding occurs in fish, caused by electrical stunning methods (Morzel & van de Vis, 2003).

The b^* value (yellow intensity) was significantly ($p < 0.05$) higher in the fillets subjected to 150 DC, while lower values were found in the fillets subjected to 100 AC. The variation in yellow color (b^*) of the fillets is a parameter poorly understood. However, some authors argue that the lower intensity of yellow color may be due to animal stress during stunning procedures (Roth et al., 2010; Digre et al., 2011). It may occur because of the rapid decrease in post-mortem pH causing protein denaturation and water loss, resulting in reflected light changes of b^* parameter (Scherer et al., 2005). Therefore, it is assumed that the voltage and type of electrical current for desensitization of pacus should provide the best combination to avoid damage on meat quality and excessive animal stress prior to slaughtering.

When comparing the electrical stunning with other stunning and slaughter methods on the effect of color of fish meat, the results are divergent. In Nile tilapia (*Oreochromis niloticus*) the electric shock led to higher a^* values (redness) and lower L^* values (lightness) in relation to fish slaughtered by gill arch-cutting (Lambooij et al., 2008). In a study of eels, higher a^* values and lower L★ values were observed in fish subjected to electrical stunning as compared to salt bath (Morzel & van de Vis, 2003). In contrast, a study on grass carp (*Ctenopharyngodon idella)* found no color changes when electrical stunning was compared to thermal shock in water and ice (Scherer et al., 2005). In turbots (*Psetta maxima*) electricity was used as an alternative method to thermal shock in ice and water, and no significant changes were observed for L★, a★, and b★ parameters. Furthermore, the authors found no presence of hemorrhage in the fillets (Knowles et al., 2008), which evidenced that electrical stunning causes no damage in meat color if properly applied. More studies are needed to investigate other electrical stunning parameters such as wavelength, duration of electrical current, and water conductivity, aimed to increase stunning time prior to slaughter, without compromising the quality of pacu fillets.

Table 3. Mean ± standard deviation of the color parameters L★ (lightness), a★ (green-red intensity), b★ (yellow-blue intensity) of pacu fillets stunned by different types of electrical current (AC or DC) and voltage[a].

Treatment	Color		
	L★	a★	b★
200 DC	34.3 ± 2.3 b	1.8 ± 1.9 a	6.5 ± 1.2 ab
200 AC	36.9 ± 3.3 ab	0.4 ± 2.2 ab	6.7 ± 1.6 ab
150 DC	39.0 ± 1.1 a	1.6 ± 1.3 a	8.1 ± 1.1 a
150 AC	39.2 ± 2.7 a	-0.4 ± 1.1 ab	6.2 ± 2.1 ab
100 DC	38.5 ± 2.7 a	-1.3 ± 1.3 b	5.3 ± 2.3 ab
100 AC	36.1 ± 1.1 ab	-2.1 ± 0.6 b	3.8 ± 1.5 b

[a] Different letters on the same column indicate significant differences ($p < 0.05$).

Conclusion

The application of alternating-current (AC) as a stunning method is more suitable for pacus when compared to the application of direct current (DC), once AC led to immediate loss of consciousness without signs of pain or stress, thus allowing fish to remain apparently unconscious for a longer period before bleeding, with low hemorrhage in the fillets. The alternating current of 200V kept pacus for up to four minutes in apparent unconsciousness, and caused little bleeding in the fillets, thus it is a potentially recommended combination for pacu processing industry.

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