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FLESH QUALITY AND STRESS RESPONSES OF *Piaractus mesopotamicus* AFTER EXPOSURE TO SUBLETHAL LEVELS OF AMMONIA AND SUBSEQUENT RECOVERY

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ABSTRACT

This study aimed to evaluate de effect of the exposure to sub-lethal levels of ammonia on blood parameters and flesh quality of juvenile pacu (*Piaractus mesopotamicus*), including assessment of such parameters post recovery in ammonia-free water. Juveniles (27.1 ± 5.4 g) were exposed to concentrations of ammonia at 0.0 (control); 0.5; and 1.0 mg $N\text{-}NH_3$ L^{-1} for 10 days followed by the same period of recovery in ammonia-free water. On the 10th day post exposure and after a recovery period, samples of blood were taken for glucose, lactate analyses and evaluation of haematocrit. To evaluate lipid peroxidation, proximate composition and sensory analysis, samples of muscle/fillets were also obtained. Exposure to ammonia caused alterations in haematological response and negatively affected sensory analysis of pacu fillet. However, proximate composition was unchanged and lipid peroxidation process was not intensified in muscle. In conclusion, exposure to sublethal levels of ammonia induces secondary stress responses and altered the organoleptic characteristics of pacu flesh. Nevertheless, a recovery period of 10 days was sufficient to allow for a complete restoration of the homeostasis and organoleptic characteristics of the fillet.

Key words: freshwater fish; toxicity; proximate composition; lactate; lipid peroxidation; sensory analysis.

QUALIDADE DA CARNE E RESPOSTAS DE ESTRESSE EM *Piaractus mesopotamicus* APÓS EXPOSIÇÃO À NÍVEIS SUBLETAIS DE AMÔNIA E SUBSEQUENTE RECUPERAÇÃO

RESUMO

O objetivo do presente estudo foi avaliar os efeitos da exposição a níveis subletais de amônia nos parâmetros sanguíneos e qualidade da carne de juvenis de pacu (*Piaractus mesopotamicus*). Além disso, também foi realizada a avaliação desses parâmetros pós-recuperação em água livre de amônia. Os peixes (27,1 ± 5,4 g) foram expostos a 0,0 (controle); 0,5 e 1,0 mg $N\text{-}NH_3$ L^{-1} por 10 dias seguidos pelo mesmo tempo de recuperação em água livre de amônia. Ao final dos 10 dias de exposição e após o período de recuperação foram coletadas amostras de sangue para determinação dos níveis de glicose, lactato e dos valores de hematócrito. Para avaliação dos níveis de peroxidação lipídica, da composição proximal e realização da análise sensorial, amostras de músculo/filé também foram coletadas. A exposição à amônia ocasionou alterações nos parâmetros sanguíneos e afetou negativamente a análise sensorial dos filés de pacu. Entretanto, a composição proximal e o grau de peroxidação lipídica não foram alterados. Concluindo, a exposição a níveis subletais de amônia induziu respostas secundárias de estresse em juvenis de pacu e alterou características organolépticas do filé. Um período de recuperação de 10 dias foi suficiente para restaurar a homeostase e a qualidade do filé.

Palavras-chave: peixe de água doce; toxicidade; composição proximal; lactato; peroxidação lipídica; análise sensorial.

INTRODUCTION

In intensive aquaculture systems, ammonia-nitrogen build-up is a limiting factor due to its high toxicity for aquatic organisms (Abreu et al., 2012). Ammonia is a by-product of protein catabolism (Dabrowski, 1986) being the main excretion product of teleost fish, representing 70 to 90% of the total nitrogen input to the farming system (Randall and Tsui, 2002). In aquatic environments, ammonia exists in ionized (NH_4^+) and unionized

(NH_3) forms, being the latter a hydrophilic compound, which can easily diffuse through gill membranes (Liew et al., 2013). Thus, high concentrations of NH_3 in the water impair its excretion through the gills because the gradient is decreased, which in turn leads to ammonia accumulation in tissues and blood plasma (Haywood, 1983; Wilkie and Wood, 1996).

A primary cause of ammonia toxicity is the depolarising effect of the ion NH_4^+ on neurons and white muscle, displacing K^+ and causing activation of NMDA-type (N-methyl-D-aspartate) glutamate receptor, leading to an influx of excessive Ca^{2+} and subsequent cell death (Randall and Tsui, 2002). Effects of ammonia exposure vary depending on the duration and severity of the exposure, leading to physiological (Liew et al., 2013; Maltez et al., 2017), morphological (Rodrigues et al., 2014) and behavioural (Pinto et al., 2016) disturbances, which can negatively affect fish growth (Paust et al., 2011), immune system (Chen et al., 2011) and cause death in some cases (Medeiros et al., 2016). Studies have reported that ammonia toxic effects can alter the homeostasis, which could be reflected by blood parameters such as glycemia, lactate and haematocrit, commonly used as secondary stress markers (Li et al., 2013; Baldisserotto et al., 2014; Rama and Manjabhat, 2014).

Physiological responses of fish to stressors, such as ammonia exposure, range from increases in plasma catecholamine to intensification in the mobilization of energy substrates, which hastens *rigor mortis* and the deterioration of flesh quality (Sigholt et al., 1997). Fillet is the main edible part of fish to be marketed and is expected to please consumers as to the characteristics of colour, texture and good appearance. However, many factors can influence nutritional quality and sensory analysis of muscle, *e.g.* season of the year, feeding, pre-slaughter stress, inadequate rearing conditions and chemical composition (Dal Bosco et al., 2012; Saidi et al., 2010; Ribas et al., 2007). Proximate composition in fish depends on intrinsic and extrinsic characteristics that affect sensory analysis of the final product (Contreras-Guzmán, 1994).

Proximate compositions in dourado (*Salminus brasiliensis*) (Veeck et al., 2013) and darkbarbel catfish (*Pelteobagrus vachelli*) (Li et al., 2013) flesh were altered after ammonia exposure. Moreover, ammonia can induce oxidative stress by enhancing Reactive Oxygen Species (ROS) production (Sinha et al., 2014) and/or by decreasing antioxidant defences (Li et al., 2016; Maltez et al., 2017), resulting in greater oxidation of lipids (Hegazi et al., 2010), and consequently, loss of food quality (Zhang et al., 2016). Off-flavour, unpleasant odour, alterations in colour, texture and nutritional status, can subject the product to potential rejection by the consumers (Veeck et al., 2013). In this context, sensory analysis is an auxiliary tool for the assessment of the organoleptic properties and characterization of endpoints that will indicate acceptability levels of a given product by consumers (Dutcosky, 2007).

Fish reared in intensive aquaculture systems that have been exposed to oscillations in nitrogen compounds should undergo an adequate recovery period in ammonia-free water, in order to minimize the risk of cumulative stress (Wedemeyer, 2012) and to decrease impairment of end-products due to pre-slaughter stress (Terlouw et al., 2008). However, studies investigating the necessary recovery period in ammonia-free water, which can allow for resumption of homeostasis in fish exposed to ammonia, are fairly rare (Gisbert et al., 2004; Maltez et al., 2017) and still unknown for pacu.

Pacu fish *Piaractus mesopotamicus* is a native species to Plata basin (Godoy, 1975) and has a good market value, mainly in South America (Povh et al., 2009). It is an omnivorous species (Urbinati et al., 2010) and well accepted by the consumers due to the quality of its flesh (Jomori et al., 2003). Pacu stands out for its rusticity, presents good growth rates and fecundity, being well adapted to intensive rearing conditions (Bittencourt et al., 2010; Urbinati et al., 2010).

The aim of this study was to evaluate the effects of exposure to sublethal concentrations of un-ionized ammonia and recovery in ammonia-free water on flesh quality and blood parameters of juvenile pacu (*Piaractus mesopotamicus*).

METHODS

Experimental conditions

A total of 117 pacu juveniles (27.1±5.4 g) were used and obtained from a commercial fish farm. Fish were transported on paved road and upon arrival were acclimated in nine 250 L aerated tanks (13 fish per tank) for 15 days in a semi-static system. The experiments were approved by the Ethics and Animal Welfare Committee of the Federal University of Rio Grande – FURG (# 23116.004750/2015-43).

After the acclimation period, fish were exposed to total ammonia nitrogen (TAN) concentrations at 0.33±0.04 (control), 18.01±1.32 and 32.90±0.98 mg of TAN L^{-1} and concentrations of un-ionized ammonia were estimated at 0.01±0.01 (control); 0.5±0.02 and 0.97±0.04 mg $N\text{-}NH_3$ L^{-1}, respectively. For the presentation of results and discussion purposes, nominal concentrations of un-ionized ammonia (0.0 or control, 0.5 and 1.0 mg $N\text{-}NH_3$ L^{-1}) were used. The experimental design was completely randomized, with three treatments and three replicates.

The desired unionized ammonia concentrations were obtained by adding ammonium chloride (NH_3Cl_4) (Synth, Brazil) to the water or by performing partial water changes when necessary. Concentrations of un-ionized ammonia used herein were chosen based on the findings of Abreu et al. (2012), followed by pre-tests in which concentrations of 1.5 mg $N\text{-}NH_3$ L^{-1} or bellow did not determine mortality in pacu.

At the end of the exposure period and in order to evaluate recovery, water was thoroughly renewed and fish were maintained for 10 additional days in the experimental units with zero or minimal un-ionized ammonia concentration (<0.05 mg $N\text{-}NH_3$ L^{-1}).

Commercial feed was used (Supra Acqua *line*®) during acclimation and experimental periods. Composition of feed was 32% crude protein, 12% moisture, 3000 kcal kg^{-1} digestible energy, 5% ether extract, 2.5% calcium, 1% phosphorus and 300 mg kg^{-1} of vitamin C offered *ad libitum* (at 9:00 and 16:00h). Feed consumption was registered throughout the experimental period and feed provided was quantified daily. Weighing of the feed before and after feeding allowed for the control of feed consumption. The photoperiod was fixed at 12-h light:12-h dark.

Water parameters: temperature and dissolved oxygen (oxygen meter, EcoSense DO 200A), pH (pH meter, HANNA HI 8424), total ammonia (UNESCO, 1983), un-ionized ammonia (Colt, 2002),

nitrite (Bendschneider and Robinson, 1952) and total alkalinity (Eaton et al., 1995) were monitored daily, in the morning and prior to feeding. The parameters were maintained as follows: temperature 25.7±0.05 °C, pH 7.51±0.04, dissolved oxygen 7.63±0.06 mg L^{-1}, nitrite <0.05 mg $N\text{-}NO_2$ L^{-1} and alkalinity 48.3±2.88 mg $CaCO_3$ L^{-1}.

Sampling procedures

Blood and tissue samplings were carried out (nine fish/sampling period/treatment) on the 10th day of exposure and after recovery. Fish were captured from the tanks with a dip net and anaesthetized with hydrochloride benzocaine (50 mg L^{-1}). Blood was taken from caudal vasculature using heparin-coated syringes. Immediately after sampling, animals were killed by exposure to baths of hydrochloride benzocaine at 500 mg L^{-1} and liver was collected and individually weighed for the evaluation of the hepatosomatic index (HI). To evaluate lipid peroxidation, proximate composition and sensory analysis, samples of muscle were collected. For thiobarbituric acid-reactive substances (TBARS) and proximate composition evaluations, samples were stored in a ultrafreezer at - 80 ºC for further analysis. After filleting of the dorsal muscle, samples were stored in Styrofoam boxes filled with ice and used immediately for sensory analysis of the flesh on the 10th day and after recovery.

Blood variables analyses

Blood samples were used for the analyses of glucose (Accu-Chek Performa/Roche®, Germany) and lactate concentration (Accutrend Plus Cobas /Roche®, Germany). Haematocrit analysis was performed according to Goldenfarb et al. (1971), through centrifugation of 2/3 blood-filled heparinized micro capillary sealed tubes (12,000 rpm for 5 min), which were subsequently read on a micro-haematocrit reader card and expressed as a percentage of red blood cells in relation to total blood.

Proximate composition and Lipid peroxidation level analyses

Chemical proximate composition analysis of fillet was carried out according to the methodology of AOAC (1995). Muscle tissue (fillet sample) was homogenized at a ratio of 1:5 (w/v) in buffer (Tris-HCl – 100 mM; EDTA – 2 mM; and $MgCl_2.6H_2O$ – 5mM) (Rocha et al., 2009). The supernatants resulting from the centrifugation of the homogenates (10,000 x*g*, 20 minutes, 4ºC) were used for analyses. After homogenization, total protein content was determined through the Biuret method (Doles®). Lipid peroxidation was measured using the methodology described by Oakes and Van Der Kraak (2003). This method quantifies malondialdehyde (MDA) levels, a by-product of lipid peroxidation, by measuring TBARS. The fluorescence (excitation: 520 nm; emission: 580 nm) readings were performed in a spectrofluorimeter (Victor 2, Perkin Elmer, MA, USA) and the results were expressed as nmol MDA mg $protein^{-1}$.

Sensory tests

Samples of pacu flesh were submitted to sensory analysis whereby a group of 30 non-trained individuals, comprised of 48 and 52% of female and male tasters, respectively, and ranging between 23 and 45 years old was established. The same panel of evaluators carried out the tests right after the collection of flesh post-exposure and after the recovery period. Sensory tests were individually conducted, under white light. Each taster received an evaluation form for the assessment and ranking of colour, texture, odour and overall appearance, using an appropriate 9-point hedonic scale according to the methodology of Stone and Sidel (2004) as follows: 1 - dislike extremely; 2 - dislike very much; 3 - dislike moderately; 4 - dislike slightly; 5 - neither like nor dislike; 6 - like slightly; 7 - like moderately; 8 - like very much and 9 - like extremely.

Statistical analysis

Values were expressed as means ± standard deviation. Data were submitted to non-parametric analyses using Kruskall-Wallis and Mann-Whitney tests. The minimum significance level was set at 5% ($p<0.05$) in all cases (Zar, 1996).

RESULTS

Blood parameters, hepatosomatic index and feed consumption

No mortality occurred in any group and the final weights on the 10th and 20th days were 27.4 ± 3.2 g and 30.6 ± 3.8 g, respectively. After exposure, fish submitted to 0.5 mg $N\text{-}NH_3$ L^{-1} had higher glucose levels compared to control and fish exposed to 1.0 mg $N\text{-}NH_3$ L^{-1} and fish from treatment 1.0 mg $N\text{-}NH_3$ L^{-1} had lower haematocrit compared to control and treatment 0.5 mg $N\text{-}NH_3$ L^{-1}. Fish exposed to 0.5 and 1.0 mg $N\text{-}NH_3$ L^{-1} treatment had lower lactate levels, HI and daily feed consumption compared to control. No differences in blood parameters, HI and daily feed consumption were observed among treatments after recovery (Table 1).

Different superscripts indicate significant differences among treatments within the exposure or recovery period after Kruskal-Wallis and Mann-Witney's tests ($p<0.05$).

Proximate composition, lipid peroxidation in muscle and sensory analysis of the flesh

Moisture, protein, ashes, lipid content, and lipid peroxidation levels were not significantly different in muscle regardless of treatment throughout the experiment. Sensory tests on fillet samples generally scored between 5 and 7 in the hedonic scale, which indicates that tasters' opinions varied between "neither like nor dislike" and "like moderately". Scores for colour and overall appearance of fillets were significantly lower in treatment 1.0 mg $N\text{-}NH_3$ L^{-1} after exposure. No differences ($p>0.05$) were observed among treatments after recovery (Table 2).

Table 1. Blood parameters, hepatosomatic index and feed consumption (mean ± SD) of juvenile pacu *Piaractus mesopotamicus* exposed to sublethal levels of un-ionized ammonia and after recovery in ammonia-free water.

Parameter	Treatment	Exposure (10th day)	Recovery (20th day)
Glucose (mg dL^{-1})	C	76.1±6.2[b]	65.4±18.9[a]
	0.5	113. 9±13.1[a]	72.7±12.7[a]
	1.0	59.1±29.7[b]	76.2±11.9[a]
Haematocrit (%)	C	18.7±4.6[a]	24.2±2.3[a]
	0.5	21.1±7.6[a]	25.6±2.6[a]
	1.0	12.9±2.5[b]	26.22±2.6[a]
Lactate (mmol L^{-1})	C	6.2±1.1[a]	7.3±1.4[a]
	0.5	3.9±1.6[b]	7.4±2.9[a]
	1.0	3.3±0.5[b]	6.6±4.0[a]
Hepatosomatic Index (HI)	C	0.012±0.004[a]	0.012±0.001[a]
	0.5	0.008±0.002[b]	0.011±0.002[a]
	1.0	0.008±0.001[b]	0.011±0.001[a]
Feed Consumption (mg day^{-1})	C	1868.8±384.7[a]	1484.8±607.5[a]
	0.5	859.6±483.8[b]	1427.8±510.1[a]
	1.0	599.4±544.8[b]	1343.6±652.6[a]

Table 2. Proximate composition, sensory analysis and lipid peroxidation levels (TBARS) (mean±SD) of juvenile pacu *Piaractus mesopotamicus* exposed to sublethal levels of un-ionized ammonia and after recovery in ammonia-free water.

Parameter		Treatment	Exposure	Recovery
Proximate composition	Moisture (%)	C	78.2±0.4[a]	79.0±0.1[a]
		0.5	78.6±0.1[a]	79.1±0.2[a]
		1.0	78.3±01[a]	78.7±0.3[a]
	Protein (%)	C	16.3±1.6[a]	16.2±1.6[a]
		0.5	15.1±3.3[a]	13.9±4.5[a]
		1.0	17.6±1.5[a]	18.0±0.1[a]
	Ashes (%)	C	1.2±0.03[a]	1.3±0.05[a]
		0.5	1.1±0.04[a]	1.4±0.01[a]
		1.0	1.3±0.01[a]	1.3±0.01[a]
	Lipid (%)	C	4.3±1.2[a]	3.5±1.4[a]
		0.5	6.0±3.1[a]	5.6±4.4[a]
		1.0	2.8±1.5[a]	2.0±0.4[a]
Sensory analysis	Colour	C	6.9±1.8[a]	6.8±1.7[a]
		0.5	7.0±1.9[a]	6.7±1.6[a]
		1.0	5.6±2.0[b]	7.1±1.7[a]
	Odour	C	6.7±2.0[a]	6.3±2.0[a]
		0.5	6.7±1.9[a]	6.8±1.6[a]
		1.0	6.3±1.7[a]	6.6±1.8[a]
	Texture	C	6.5±1.8[a]	6.7±1.8[a]
		0.5	6.6±1.6[a]	6.4±1.8[a]
		1.0	6.4±1.8[a]	6.76±1.9[a]
	Overall appearance	C	7.0±1.8[a]	6.7±1.8[a]
		0.5	7.1±1.6[a]	6.5±1.7[a]
		1.0	5.8±1.7[b]	7.0±1.5[a]
Oxidative stress	TBARS (nmol MDA mg $protein^{-1}$)	C	1.0±0.4[a]	2.7±1.7[a]
		0.5	1.2±0.4[a]	1.8±1.0[a]
		1.0	1.3±0.8[a]	1.9±1.0[a]

Different superscripts indicate significant differences among treatments within the exposure or recovery period after Kruskal-Wallis and Mann-Witney tests ($p<0.05$).

DISCUSSION

Exposure to distinct sub-lethal levels of ammonia (0.5 and 1.0 mg N-NH_3 L^{-1}) altered blood parameters of juvenile pacu in this study. The overall elevations in glycemia were likely a response to circulating catecholamine during the exposure period, and hyperglycemia in fish is often used as a reliable secondary stress response marker (Tavares-Dias et al., 2001). Increased glycemia is also a cortisol-related condition, in which cortisol activates gluconeogenesis and regulates glucose demand in peripheral circulation (Barcellos et al., 2000). Glycemia in pacu was higher in treatments 0.5 N-NH_3 L^{-1}, and this increment was also reported in the same species submitted to 3.0 mg N-NH_3 L^{-1} for 24h (Abreu et al., 2012). Other species such as the African catfish (*Clarias gariepinus*) exposed to 15.2 mg N-NH_3 L^{-1} (Schram et al., 2010), silver catfish (*Rhamdia quelen*) exposed to 0.5 mg N-NH_3 L^{-1} (Baldisserotto et al., 2014) and pirarucu (*Arapaima gigas*) exposed to 2.0 mg N-NH_3 L^{-1} (Cavero et al., 2004) showed the same pattern with elevations in plasma glucose after exposure to un-ionized ammonia. After exposure, blood glucose in treatment 1.0 mg N-NH_3 L^{-1} was undistinguishable from that of control fish, which could indicate energy depletion from hepatic glycogen reserves. In fact, increased glucose concentration in blood is related to the availability of glycogen and glucose in the liver (Baldisserotto et al., 2014).

Haematocrit may be affected in different ways after a stressor is applied. Haemoconcentration or haemodilution may occur as a consequence of osmoregulatory disturbances (Takahashi et al., 2006) that may take place during exposure to ammonia. Moreover, oscillations in the number of blood cells are likely to occur as well (Yang et al., 2010; Li et al., 2013; Li et al., 2014). Haematocrit decreased in pacu after exposure to ammonia at the concentration of 1.0 mg N-NH_3 L^{-1}. Similarly, the same response was observed when the species was exposed for 12 h to 3.0 mg N-NH_3 L^{-1}, and the decreased haematocrit observed was caused by a reduction in the volume of erythrocytes led by ion losses which were evinced by a reduction in blood osmolality (Abreu et al., 2012). Haematocrit also decreased in darkbarbel catfish (*Pelteobagrus vachelli*) exposed to 0.12 mg N-NH_3 L^{-1} of ammonia for 60 days (Li et al., 2013). However, perch (*Sander lucioperca*) exposed to concentrations of 0.05 and 0.26 mg N-NH_3 L^{-1} of unionized ammonia for 42 days did not present significant differences in haematocrit (Schram et al., 2014).

Maintenance of homeostasis under stressful conditions requires higher energy supply, in which anaerobic pathways are activated for the utilization of glycogen reserves, leading to gluconeogenesis-induced elevations in lactate (Wendelaar Bonga, 1997; Miron et al., 2008). Lactate was reduced for both treatments exposed to ammonia and could be linked to the lethargic behaviour of fish thoughout exposure, similarly to what has been described for pirarucu (*Arapaima gigas*) exposed to 20 mg TAN L^{-1} for 24 h. (Brandão et al., 2006).

The hepatosomatic index (HI) is frequently used as a biomarker for contaminants and to provide insight into the nutritional status of fish (Narra et al., 2015). After exposure, HI decreased in either treatments, which is consistent with the reductions observed in feed consumption for both treatments after the same period. Such conditions are in line with a possible depletion of hepatic reserves for the supply of energy (Souza et al., 2002). The reduction in feed consumption observed herein is also similar to those observed for other species exposed to ammonia such as wolffish *Anarhichas minor*, 0.13; 0.25 and 0.39 mg N-NH_3 L^{-1} (Foss et al., 2003), perch *Sander lucioperca*, 0.26 mg N-NH_3 L^{-1} (Schram et al., 2014) and African catfish *Clarias gariepinus*, 15.2 mg N-NH_3 L^{-1} (Schram et al., 2010).

Our findings for flesh moisture (78-79%) protein (13-18%), ashes (1.1-1.4%) and lipids (2-6%) corroborate previous investigations on the same species (Ramos Filho et al., 2008; Tanamati et al., 2009; Freitas et al., 2011; Oliveira et al., 2014). Proximate composition was not affected by any of the treatments, comparable to that observed in yellow catfish (*Pelteobagrus fulvidraco*) exposed to chronic levels of ammonia (3.36; 6.76; 13.44 and 26.88 mg TAN/L) for 56 days (Li et al., 2011), in which proximate composition was also unchanged. However, dourado (*Salminus brasiliensis*) and darkbarbel catfish (*Pelteobagrus vachelli*) exposed to ammonia for 15 (0.1 mg N-NH_3 L^{-1}) (Veeck et al., 2013) and 60 (0.12 mg N-NH_3 L^{-1}) (Li et al., 2013) days, respectively, had significant reductions in lipid content, which is presumably a consequence of depletion in energy reserves.

Exposure to ammonia induced lipid peroxidation in different tissues of fish, such as brain (Ching et al., 2009), gills, liver (Maltez et al., 2017) and muscle (Hegazi et al., 2010). However, after 10 days of ammonia exposure (0.5 and 1.0 mg N-NH_3 L^{-1}), no alterations were observed in fresh fillet of pacu in this study, since TBARS concentrations were unchanged regardless of treatment used. These results resemble those reported for dourado (*Salminus brasiliensis*) in which no significant differences were observed for TBARS after 12 h of ammonia (0.1 mg N-NH_3 L^{-1}) exposure prior to slaughter and after a period of 15 days. Notwithstanding, fish exposed for 12 h to ammonia, prior to being slaughtered, were more susceptible to lipid peroxidation of fillet during the freezing process (Veeck et al., 2013).

Although low scores were obtained for pacu flesh during sensory analysis using a 9-point hedonic scale, similar results were observed for the same species (*P. mesopotamicus*), tambaqui (*Colossoma macropomum*) and the hybrid tambacu (*Colossoma macropomum* x *Piaractus mesopotamicus*) stored for different times, namely 19 days in the former two species and 16 days in the latter. Scores ranged from 5 to 7 (neither like nor dislike to like moderately) for pacu in this study whereas 5 and 6 (neither like nor dislike to like slightly) and 6 (like slightly) were observed for tambaqui (Borges et al., 2014), which mean good acceptability by the tasters. Similar results were observed for tinned Nile tilapia (*Oreochromis niloticus*) (5-10 cm, total length) with scores above 7 (like moderately), which indicates good acceptability of the product (Pizato et al., 2012).

Pre-slaughter stress may influence the quality of flesh (Kristoffersen et al., 2006; Ribas et al., 2007; Daniel et al., 2014), which can be also negatively affected by stress conditions occurring in earlier stages of fish farming. Severe stress conditions impact the organoleptic and nutritional characteristics of the final product to be stored (Poli et al., 2005). Ammonia-related stress

may accelerate the onset of *rigor mortis*. After death, oxygen supply to the muscle is discontinued and anaerobic metabolism takes over, muscle glycogen is consumed and the flesh quality is compromised (Pottinger, 2001).

Overall sensory analysis of fillet post ammonia exposure ranged from "indifferent" to "like moderately". Treatment exposed to 1.0 mg $N\text{-}NH_3$ L^{-1} had "indifferent" and "like slightly" correspondent scores, which is similar to another study on pacu flesh stored in ice for 10 days with no previous ammonia exposure (Borges et al., 2014). On the basis of these results, our study shows that ammonia exposure altered the freshness of pacu flesh, since scores for sensory analysis were virtually identical to that of pacu flesh stored in ice for 10 days as reported by the aforementioned study.

Homeostasis resumption depends on the length and intensity of the exposure to a given stressor). (Barton, 2002 In fact, 10 days of exposure to ammonia disrupted physiology in pacu to some extent, however, after 10 days of allocation of fish to ammonia-free water, resumption of homeostasis was observed on the basis of the parameters considered herein, which returned to baseline levels regardless of the concentration tested. During recovery, feed consumption was resumed and HI returned to normal, which demonstrate that once the stressor is removed, the energy storage capacity can be easily recovered in pacu.

As for the sensory analysis after recovery from ammonia exposure, scores denoted "like slightly" and "like moderately", which indicate a better evaluation compared to the sensory analysis of fish carried out immediately after the exposure period. Thus, provided there is an adequate recovery period in good water quality, albeit a stress challenge has been previously imposed, our study has demonstrated that the organoleptic properties of the flesh will not be significantly affected even after a 10-day period of ammonia exposure.

CONCLUSION

Exposure to sublethal concentrations of un-ionized ammonia for 10 days induced secondary stress responses and negatively affected sensory analysis in pacu flesh. Notwithstanding, a recovery period of 10 days was sufficient to allow for a complete restoration of the metabolic parameters evaluated and the organoleptic characteristics of the fillet.

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REFERENCES

Abreu, J.S.; Esteves, F.R.; Urbinati, E.C. 2012. Stress in pacu exposed to ammonia in water. Revista Brasileira de Zootecnia, 41(7): 1555-1560.

AOAC 1995. Official methods and recommended practices of the American Oil Chemist's Society, Ed. D. Feistane, Washington D.C.

Baldisserotto, B.; Martos-Sitcha, J.A.; Menezes, C.C.; Toni, C.; Prati, R.L.; Garcia, L.O.; Salbego, J.; Mancera, J.M.; Martínez-Rodríguez, G. 2014. The effects of ammonia and water hardness on the hormonal, osmoregulatory and metabolic responses of the freshwater silver catfish *Rhamdia quelen*. Aquatic Toxicology (Amsterdam, Netherlands), 152(1): 341-352.

Barcellos, L.J.G.; Souza, S.M.G.; Woehl, V.M. 2000. Estresse em peixes: Fisiologia da Resposta ao Estresse, Causas e Consequências (Revisão). Boletim do Instituto de Pesca, 26(1): 99-111.

Barton, B.A. 2002. Stress in Fishes: A Diversity of Responses with Particular Reference to Changes in Circulating Corticosteroids. Integrative and Comparative Biology, 42(3): 517-525.

Bendschneider, K.; Robinson, R.J. 1952. A new spectrophotometric method for the determination of nitrite in sea water. Journal of Marine Research, 11(1): 87-96.

Bittencourt, F.; Feiden, A.; Signor, A.A.; Boscolo, W.R.; Lorenz, E.K.; Maluf, M.L.F. 2010. Densidade de estocagem e parâmetros eritrocitários de pacus criados em tanques-rede. Revista Brasileira de Zootecnia, 39(11): 2323-2329.

Borges, A.; Medina, B.G.; Conte-Junior, C.A.; Freitas, M.Q. 2014. Aceitação sensorial e perfil de textura instrumental da carne cozida do pacu (*Piaractus mesopotamicus)*, do tambaqui (*Colossoma macropomum*) e do seu hibrido tambacu eviscerados e estocados em gelo. Revista Brasileira de Ciência Veterinária, 20(3): 160-165.

Brandão, F.R.; Gomes, L.C.; Chagas, E.C. 2006. Respostas de estresse em pirarucu (*Arapaima gigas*) durante práticas de rotina em piscicultura. Acta Amazonica, 36(3): 349-356.

Cavero, B.A.S.; Pereira-Filho, M.; Bordinhon, A.M.; Fonseca, F.A.L.; Ituassú, D.R.; Roubach, R.; Ono, E.A. 2004. Tolerância de juvenis de pirarucu ao aumento da concentração de amônia em ambiente confinado. Pesquisa Agropecuária Brasileira, 39(5): 513-516.

Chen, J.Z.; Zhang, X.L.; Hu, G.D.; Qu, J.H.; Fan, L.M.; Song, C. 2011. The immune response of GIFT *Oreochromis niloticus* and its susceptibility to Streptococcus iniae under stress in different ammonia. Journal of Ecology and Environmental Sciences, 20(6): 629-634.

Ching, B.; Chew, S.F.; Wong, W.P.; Ip, Y.K. 2009. Environmental ammonia exposure induces oxidative stress in gills and brain of *Boleophthalmus boddarti* (mudskipper). Aquatic Toxicology (Amsterdam, Netherlands), 95(3): 203-212.

Colt, J. 2002. List of spreadsheets prepared as a complement. In: Wedemeyer, G.A. (Ed.). Fish hatchery management. Washington: American Fisheries Society. p. 91–186.

Contreras-Guzmán, E.S. 1994. Bioquímica de Pescados e Derivados. Jaboticabal: FUNEP. 409p.

Dabrowski, K.R. 1986. Active metabolism larval and juvenile fish: ontogenetic changes, effect of water temperature and fasting. Fish Physiology and Biochemistry, 1(3): 125-144.

Dal Bosco, A.; Mugnai, C.; Mourvaki, E.; Castellini, C. 2012. Seasonal changes in the fillet fatty acid profile and nutritional characteristics of wild Trasimeno Lake goldfish (*Carassius auratus* L.). Food Chemistry, 132(2): 830-834.

Daniel, A.P.; Veeck, A.P.L.; Klein, B.; Ferreira, L.F.; Cunha, M.A.; Parodi, T.V.; Zeppenfeld, C.C.; Schmidt, D.; Caron, B.O.; Heinzmann, B.M.; Baldisserotto, B.; Emanuelli, T. 2014. Using the Essential Oil of *Aloysia triphylla* (L'Her.) Britton to Sedate Silver Catfish (*Rhamdia quelen*) during Transport Improved the Chemical and Sensory Qualities of the Fish during Storage in Ice. Journal of Food Science, 79(6): 1205-1211.

Dutcosky, S.D. 2007. Análise Sensorial de Alimentos. 2ª ed. Curitiba: Champagnat. 246p.

Eaton, A.D.; Clesceri, L.S.; Greenberg, A.E. 1995. Standard methods for the examination of water and wastewater. 19ª ed. Baltimore: United Book Press, Inc.

Foss, A.; Evensen, T.H.; Vollen, T.; Øiestad, V. 2003. Effects of chronic ammonia exposure on growth and food conversion efficiency in juvenile spotted wolffish. Aquaculture (Amsterdam, Netherlands), 228(3-4): 215-224.

Freitas, J.M.A.; Higuchi, L.H.; Feiden, A.; Maluf, M.L.F.; Dallagnol, J.M.; Boscolo, W.R. 2011. Salga seca e úmida de filés de pacu (*Piaractus mesopotamicus*). Semina: Ciências Agrárias, 32(2): 613-620.

Gisbert, E.; Rodriguez, A.; Cardona, L.; Huertas, M.; Gallardo, M.A.; Sarasquete, C.; Sala-Rabanal, M.; Ibarz, A.; Sánchez, J.; Castello-Orvay, F. 2004. Recovery of Siberian sturgeon yearlings after an acute exposure to environmental nitrite: changes in the plasmatic ionic balance, Na+–K+ ATPase activity, and gill histology. Aquaculture (Amsterdam, Netherlands), 239(1-4): 141-154.

Godoy, M.P. 1975. Peixes do Brasil: subordem *Characoidei*: bacia do rio Mogi-Guassu. v.1-4. Piracicaba: Franciscana, 216p.

Goldenfarb, P.B.; Bowyer, F.P.; Hall, E.; Brosious, E. 1971. Reproducibility in the hematology laboratory: the microhematocrit determination. American Journal of Clinical Pathology, 56(1): 35-39.

Haywood, G.P. 1983. Ammonia toxicity in teleost fishes: A review. Canadian Technical Report of Fisheries and Aquatic Sciences, 1(4): 35p.

Hegazi, M.M.; Attia, Z.I.; Ashour, O.A. 2010. Oxidative stress and antioxidant enzymes in liver and white muscle of Nile tilapia juveniles in chronic ammonia exposure. Aquatic Toxicology (Amsterdam, Netherlands), 99(2): 118-125.

Jomori, R.K.; Carneiro, D.J.; Malheiros, E.B.; Portella, M.C. 2003. Growth and survival of pacu *Piaractus mesopotamicus* (Holmberg, 1887) juveniles reared in ponds or at different initial larviculture periods indoors. Aquaculture (Amsterdam, Netherlands), 22(1-4): 277-287.

Kristoffersen, S.; Tobiassen, T.; Steinsund, V.; Olsen, R.L. 2006. Slaughter stress, postmortem muscle pH and rigor development in farmed Atlantic cod (*Gadus morhua L.*). International Journal of Food Science & Technology, 41(7): 861-864.

Li, B.; Fan, Q.; Yang, K.; Zhang, L.; Guo, H.; Wang, Q.; Gao, Y.; Zhu, S.; Fan, W. 2011. Effects of chronic ammonia stress on foraging, growth, and haematological parameters of Yellow Catfish (*Pelteobagrus fulvidraco*) juveniles. Chinese Journal of Applied and Environmental Biology, 6(1): 824-829.

Li, M.; Chen, L.Q.; Qin, J.G.; Li, E.C.; Yu, N.; Du, Z.Y. 2013. Growth performance, antioxidant status and immune response in darkbarbel catfish *Pelteobagrus vachelli* fed different PUFA/vitamin E dietary levels and exposed to high or low ammonia. Aquaculture (Amsterdam, Netherlands), 406-407(1): 18-27.

Li, M.; Yu, N.; Qin, J.G.; Li, E.; Du, Z.; Chen, L. 2014. Effects of ammonia stress, dietary linseed oil and *Edwardsiella ictaluri* challenge on juvenile darkbarbel catfish *Pelteobagrus vachelli*. Fish & Shellfish Immunology, 38(1): 158-165.

Li, M.; Gong, S.; Li, Q.; Yuan, L.; Meng, F.; Wang, R. 2016. Ammonia toxicity induces glutamine accumulation, oxidative stress and immunosuppression in juvenile yellow catfish *Pelteobagrus fulvidraco*. Comparative Biochemistry and Physiology, 183-184(Part C): 1-6.

Liew, H.J.; Sinha, A.K.; Nawata, C.M.; Blust, R.; Wood, C.M.; Boeck, G.D. 2013. Differential responses in ammonia excretion, sodium fluxes and gill permeability explain different sensitivities to acute high environmental ammonia in three freshwater teleosts. Aquatic Toxicology (Amsterdam, Netherlands), 126(1): 63-76.

Maltez, L.C.; Stringhetta, G.R.; Enamorado, A.D.; Okamoto, M.H.; Romano, L.A.; Monserrat, J.M.; Sampaio, L.A.; Garcia, L. 2017. Ammonia exposure and subsequent recovery trigger oxidative stress responses in juveniles of Brazilian flounder *Paralichthys orbignyanus*. Fish Physiology and Biochemistry, 43(6): 1747-1759.

Medeiros, R.S.; Lopez, B.A.; Sampaio, L.A.; Romano, L.A.; Rodrigues, R.V. 2016. Ammonia and nitrite toxicity to false clownfish *Amphiprion ocellaris*. Aquaculture International, 24(4): 1-9.

Miron, D.S.; Moraes, B.; Becker, A.G.; Crestani, M.; Spanevello, R.; Loro, V.L.; Baldisserotto, B. 2008. Ammonia and pH effects on some metabolic parameters and gill histology of silver catfish, *Rhamdia quelen* (Heptapteridae). Aquaculture (Amsterdam, Netherlands), 277(3-4): 192-196.

Narra, M.R.; Rajender, K.; Reddy, R.R.V.; Rao, J.; Begum, G. 2015. The role of vitamin C as antioxidant in protection of biochemical and haematological stress induced by chlorpyrifos in freshwater fish *Clarias batrachus*. Chemosphere, 132(1): 172-178.

Oakes, K.D.; Van Der Kraak, G.J. 2003. Utility of the TBARS assay in detecting oxidative stress in white sucker (*Catostomus commersoni*) populations. Aquatic Toxicology (Amsterdam, Netherlands), 63(4): 447-463.

Oliveira, L.M.F.S.; Leal, R.S.; Mesquita, T.C.; Pimenta, M.E.S.G.; Zangeronimo, M.G.; Sousa, R.V.; Alvarenga, R.R. 2014. Effect of ractopamine on the chemical and physical characteristics of pacu (*Piaractus mesopotamicus*) steaks. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 66(1): 185-194.

Paust, L.O.; Foss, A.; Imsland, A.K. 2011. Effects of chronic and periodic exposure to ammonia on growth, food conversion efficiency and blood physiology in juvenile Atlantic halibut (*Hippoglossus hippoglossus* L.). Aquaculture (Amsterdam, Netherlands), 315(3-4): 400-406.

Pinto, D.S.B.; Maltez, L.C.; Stringhetta, G.R.; Pellegrin, L.; Nitz, L.F.; Figueiredo, M.R.C.; Garcia, L.O. 2016. Ammonia and nitrite acute toxicity in juvenile piavuçu *Leporinus macrocephalus* (Actinopterygii, Anostomidae). Pan-American Journal of Aquatic Sciences, 11(4): 292-300.

Pizato, S.; Kraieski, J.; Sarmento, C.; Prentice, C. 2012. Avaliação da qualidade tecnológica apresentada por tilápia do Nilo (*Oreochromis niloticus)* enlatada. Semina: Ciências Agrárias, 33(2): 667-674.

Poli, B.M.; Parisi, G.; Scappini, F.; Zampacavallo, G. 2005. Fish welfare and quality as affected by pre-slaughter and slaughter management. Aquaculture International, 13(1-2): 29-49.

Pottinger, T.G. 2001. Effects of husbandry stress on flesh quality indicators in fish. In: Kestin, S.; Warriss, P. (Eds.). Farmed fish quality. Blackwell Science: Bristol, p.145-161.

Povh, J.A.; Ribeiro, R.P.; Lopera-Barrero, N.M.; Gomes, P.C.; Blanck, D.V.; Vargas, L.; Jacometo, C.B.; Lopes, T.S. 2009. Monitoramento da variabilidade genética do pacu, *Piaractus mesopotamicus*, do programa de aumento do estoque do rio Paranapanema. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 61(5): 1191-1195.

Rama, S.; Manjabhat, S.N. 2014. Protective effect of shrimp carotenoids against ammonia stress in common carp, *Cyprinus carpio*. Ecotoxicology and Environmental Safety, 107(1): 207-213.

Ramos Filho, M.M.; Ramos, M.I.L.; Hiane, P.A.; Souza, E.M.T. 2008. Perfil lipídico de quatro espécies de peixes da região pantaneira de Mato Grosso do Sul. Food Science and Technology (Campinas), 28(2): 361-365.

Randall, D.J.; Tsui, T.K.N. 2002. Ammonia toxicity in fish. Marine Pollution Bulletin, 45(1-12): 17-23.

Ribas, L.; Flos, R.; Reig, L.; Mackenzie, S.; Barton, B.A.; Tort, L. 2007. Comparison of methods for anaesthetizing Senegal sole (*Solea senegalensis*) before slaughter: Stress responses and final product quality. Aquaculture (Amsterdam, Netherlands), 269(1-4): 250-258.

Rocha, A.M.; Salomão De Freitas, D.P.; Burns, M.; Vieira, J.P.; De La Torre, F.R.; Monserrat, J.M. 2009. Seasonal and organ variations in antioxidant capacity, detoxifying competence and oxidative damage in freshwater and estuarine fishes from Southern Brazil. Comparative Biochemistry and Physiology Part C, 150(4): 512-520.

Rodrigues, R.V.; Romano, L.A.; Schwarz, M.H.; Delbos, B.; Sampaio, L.A. 2014. Acute tolerance and histopathological effects of ammonia on juvenile maroon clownfish *Premnas biaculeatus* (Block 1790). Aquaculture Research, 45(7): 1133-1139.

Saidi, S.A.; Azaza, M.S.; Abdelmouleh, A.; Pelt, J.V.; Kraiem, M.M.; El-Feki, A. 2010. The use of tuna industry waste in the practical diets of juvenile Nile tilapia (*Oreochromis niloticus*, L.): effect on growth performance, nutrient digestibility and oxidative status. Aquaculture Research, 41(12): 1875-1886.

Sigholt, T.; Erikson, U.; Rustad, T.; Johansen, S.; Nordtvedt, T.S.; Seland, A. 1997. Handling stress and storage temperature affect meat quality of farmed-raised Atlantic salmon (*Salmo salar*). Journal of Food Science, 62(4): 898-905.

Sinha, A.K.; Abdelgawad, H.; Giblen, T.; Zinta, G.; Rop, M.; Asard, H.; Blust, R.; Boeck, G. 2014. Anti-Oxidative defences are modulated differentially in three freshwater teleosts in response to ammonia-induced oxidative stress. PLoS One, 9(4): e95319.

Schram, E.; Roques, J.A.C.; Abbink, W.; Spanings, T.; Vries, P.; Bierman, S.; Vis, H.V.; Flik, G. 2010. The impact of elevated water ammonia concentration on physiology, growth and feed intake of African catfish (*Clarias gariepinus*). Aquaculture (Amsterdam, Netherlands), 306(1-4): 108-115.

Schram, E.; Roques, J.A.C.; Kuijk, T.V.; Abbink, W.; Heul, J.V.; Vries, P.; Bierman, S.; Vis, H.V.; Flik, G. 2014. The impact of elevated water ammonia and nitrate concentrations on physiology, growth and feed intake of pikeperch (*Sander lucioperca*). Aquaculture (Amsterdam, Netherlands), 420–421(1): 95-104.

Souza, V.L.; Urbinati, E.C.; Gonçalves, D.C.; Silva, P.C. 2002. Composição corporal e índices biométricos do pacu, *Piaractus mesopotamicus* Holmberg, 1887 (Osteichthyes, Characidae) submetido a ciclos alternados de restrição alimentar e realimentação. Acta Scientiarum, 24(2): 533-540.

Stone, H.; Sidel, J.L. 2004. Sensory evaluation practices. Academic Press: London. 311p.

Tavares-Dias, M.; Sandrim, E.F.S.; Moraes, F.R.; Carneiro, P.C.F. 2001. Physiological Responses of "Tambaqui" *Colossoma macropomum* (Characidae) to acute stress. Boletim do Instituto de Pesca, 27(1): 43-48.

Takahashi, L.S.; Abreu, J.S.; Biller, J.D.; Urbinati, E.C. 2006. Efeito do ambiente pós-transporte na recuperação dos indicadores de estresse de pacus juvenis, *Piaractus mesopotamicus*. Acta Scientiarum. Animal Sciences, 28(4): 469-475.

Tanamati, A.; Stevanato, F.B.; Visentainer, J.E.L.; Matsushita, M.; Souza, N.E.; Visentainer, J.V. 2009. Fatty acid composition in wild and cultivated pacu and pintado fish. European Journal of Lipid Science and Technology, 111(2): 183-187.

Terlouw, E.M.C.; Arnould, C.; Auperin, B.; Berri, C.; Le Bihan-Duval, E.; Deiss, V.; Lefèvre, F.; Lensink, B.J.; Mounier, L. 2008. Pre-slaughter conditions, animal stress and welfare: current status and possible future research. Animal, 2(10): 1501-1517.

UNESCO – Organização das Nações Unidas para a Educação, a Ciência e a Cultura. 1983. Chemical methods for use in marine environmental monitoring. Paris: Intergovernamental Oceanographic Commissiony. 53p. (Manual and Guides 12).

Urbinati, E.C.; Goncalves, F.D.; Takahashi, L.S. 2010. Pacu *Piaractus mesopotamicus*. In: Baldisseroto B; Gomes, L.C. (Org.). Espécies nativas para piscicultura no Brasil. 2ª ed. Santa Maria: UFSM. p. 1-606.

Veeck, A.P.L.; Garcia, L.O.; Baldisserotto, B.; Zaniboni Filho, E.; Emanuelli, T. 2013. Proximate composition and lipid stability of dourado (*Salminus brasilensis*, Cuvier, 1817) fillets exposed to different levels of ammonia and oxygen *in vivo*. Journal of the Science of Food and Agriculture, 93(10): 2590-2595.

Wedemeyer, G. 2012. Physiology of fish in intensive culture systems. Dordrecht: Springer Science & Business Media. 232p.

Wendelaar Bonga, S.E. 1997. The stress response in fish. Physiological Reviews, 77(3): 591-625.

Wilkie, M.P.; Wood, C.M. 1996. The adaptations of fish to extremely alkaline environments. Comparative Biochemistry and Physiology Part B, 113(4): 665-673.

Yang, W.; Xiang, F.; Sun, H.; Chen, Y.; Minter, E.; Yang, Z. 2010. Changes in the selected hematological parameters and gill Na+/K+ ATPase activity of juvenile crucian carp *Carassius auratus* during elevated ammonia exposure and the post-exposure recovery. Biochemical Systematics and Ecology, 38(4): 557-562.

Zar, J.H. 1996. Biostatistical Analysis. 3rd ed. New Jersey: Prentice-Hall. 662p.

Zhang, L.; Feng, L.; Jiang, W.D.; Liu, Y.; Jiang, J.; Li, S.H.; Tang, L.; Kuang, S.Y.; Zhou, X.Q. 2016. The impaired flesh quality by iron deficiency and excess is associated with increasing oxidative damage and decreasing antioxidant capacity in the muscle of young grass carp (*Ctenopharyngodon idellus*). Aquaculture Nutrition, 22(1): 191-201.