

## Acute toxicity of non-ionized ammonia on tambacu (*Colossoma macropomum* x *Piaractus mesopotamicus*)<sup>1</sup>

Toxicidade aguda da amônia não ionizada sobre alevinos de tambacu (*Colossoma macropomum* x *Piaractus mesopotamicus*)

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**ABSTRACT** - Ammonia is a toxic compound to aquatic organisms and at high concentrations in water, it can cause various changes in the animal. Therefore, it becomes important to determine the tolerance of farmed aquatic animals for that substance. Thus, the aim of this study was to determine the mean lethal concentration (LC<sub>50-96h</sub>) of un-ionized ammonia (NH<sub>3</sub>) in tambacu fingerlings (*Colossoma macropomum* x *Piaractus mesopotamicus*) in acute toxicity test. The fingerlings were exposed to ammonia at concentrations of: 0.09 (control); 0.54; 1.23; 2.52; 3.44 and 3.66 mg L<sup>-1</sup> NH<sub>3</sub>, which were obtained from the application of NH<sub>4</sub>Cl. The test lasted 96 hours and mortalities were recorded over that period. The LC<sub>50</sub> was determined by the Trimmed Spearman Karber statistical method. The LC<sub>50-96h</sub> of non-ionized ammonia (NH<sub>3</sub>) for fingerlings of the tambacu hybrid was 1.63 mg L<sup>-1</sup>.

**Key words:** Aquaculture. Water quality. NH<sub>3</sub>.

**RESUMO** - A amônia é um composto tóxico para os organismos aquáticos e em elevadas concentrações na água pode causar diversas alterações no animal. Dessa forma, torna-se importante determinar o limite de tolerância dos animais aquáticos cultivados à essa substância. Sendo assim, o objetivo do trabalho foi determinar a concentração letal média (CL<sub>50-96h</sub>) da amônia não ionizada (NH<sub>3</sub>) em alevinos de tambacu (*Colossoma macropomum* x *Piaractus mesopotamicus*), em teste de toxicidade aguda. Os alevinos foram expostos à amônia, nas concentrações de: 0,09 (controle); 0,54; 1,23; 2,52; 3,44 e 3,66 mg L<sup>-1</sup> NH<sub>3</sub>, que foram obtidas a partir da aplicação de NH<sub>4</sub>Cl. O teste teve duração de 96 h e as mortalidades foram registradas ao longo desse período. A CL<sub>50</sub> foi determinada pelo método estatístico *Trimmed Spearman Karber*. A CL<sub>50-96h</sub> da amônia não ionizada (NH<sub>3</sub>) para alevinos do híbrido tambacu foi 1,63 mg L<sup>-1</sup>.

**Palavras-chave:** Aquicultura. Qualidade de água. NH<sub>3</sub>.

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## INTRODUCTION

Aquaculture has gained great value as a primary source of animal protein in many countries and has the potential to improve global food security (OTTINGER; CLAUSS; KUENZER, 2016). Due to the intensification of production, with the cultivation of fish at high stocking densities and/or little water renewal, a gradual accumulation of ammonia may occur in the water. Therefore, there has been increased concern about possible toxic effects of ammonia (NH<sub>3</sub>) in these cultivation systems (ZHOU; BOYD, 2015). Thus, it is important to determine the ammonia tolerance limit of cultivated aquatic animals.

The toxic effects of ammonia on aquatic organisms can be evaluated by toxicity tests. From the acute toxicity test carried out over a period of 24 to 96 hours, it is possible to determine the mean lethal concentration (LC<sub>50</sub>), which is the concentration of the substance that causes mortality of 50% of the organisms in the time of exposure and in the test conditions (COSTA *et al.*, 2008).

There are several studies on the acute and chronic toxicity of ammonia (NH<sub>3</sub>) for different species of fish (BENLI; KÖKSAL, 2005; HEGAZI; HASANEIN, 2010; ROUMIEH *et al.*, 2013; SEMRA, 2014; SILVA *et al.*, 2018; WANG *et al.*, 2017). However, studies on the toxicity of non-ionized ammonia in native species are still scarce.

Tambacu is a hybrid resulting from the induced cross between tambaqui females (*Colossoma macropomum* CUVIER, 1818) and pacu males (*Piaractus mesopotamicus* HOLMBERG, 1887). It is a very popular species in Brazil, because it combines favorable characteristics of the two parental species, such as greater tolerance to low temperatures and rusticity of pacu and the higher growth rate of tambaqui (GOMES; SIMÕES; ARAÚJO-LIMA, 2010). In addition to its use in fish farming, the species is highly appreciated in sport fishing (VARANDAS *et al.*, 2013).

Thus, the objective of this work was to determine the mean lethal concentration (LC<sub>50-96 h</sub>) of non-ionized ammonia (NH<sub>3</sub>) for tambacu fingerlings (*Colossoma macropomum* x *Piaractus mesopotamicus*) in an acute toxicity test.

## MATERIAL AND METHODS

The experiment was carried out at the Indoor Cultivation Laboratory of the Center for Biotechnology Applied to Aquaculture (CEBIAQUA) 3°44'23.599" S, 38°34'27.482" W of the Department of Fisheries

Engineering, Center for Agricultural Sciences, Federal University of Ceará.

Tambacu fingerlings with average weight of  $0.72 \pm 0.19$  g and mean total length of  $3.7 \pm 0.28$  cm were obtained from a commercial fish farm in the municipality of Piracuruca-PI. For transport, the fish were placed in a 1000 L fish transport box with oxygen injected through bubbling during the entire journey.

Upon arriving at the laboratory facilities, the animals were kept in 3 (three) circular tanks of 1000 L with water previously dechlorinated and provided with constant aeration. The acclimatization time to laboratory conditions was two weeks. During this period, the fish were fed until apparent satiety, with balanced commercial feed for omnivorous fish, containing 35% crude protein, in four meals at 08:00, 11:00, 14:00 and 17:00 h.

The acute toxicity test was performed according to the methodology adapted from NBR 15088:2016 of the Brazilian Association of Technical Standards (2016). Initially, 120 fish were removed from stock and anesthetized in batches of 10 individuals in a solution with eugenol (30 mg L<sup>-1</sup>). Then, they were weighed and measured ( $2.18 \pm 0.69$  g and  $5.25 \pm 0.57$  cm) using a scale (MARK 2200, BEL) and caliper (Disma), respectively, and transferred to the experimental units. The experimental units consisted of twelve polypropylene monoblocks with a useful volume of 30 L, provided with constant aeration, at a density of 10 fish-aquarium<sup>-1</sup> ( $0.76 \pm 0.03$  g L<sup>-1</sup>). The animals remained under observation and acclimatization to the test conditions for 48 h. During this period and throughout the test, the fish were not fed. After 24 h of acclimatization to the test conditions, 100% of the water was changed and there was no further change during the test, thus configuring a static system.

The nominal concentrations of total ammonia tested were defined from a preliminary study and were as follows: 0 (control); 5; 15; 30; 45 and 60 mg L<sup>-1</sup> TAN. To obtain these concentrations, we added 15; 45; 90; 135 and 180 mL, respectively, of a stock solution of ammonium chloride (NH<sub>4</sub>Cl) with a concentration of 10000 mg L<sup>-1</sup> of total ammonia (TAN).

This solution was prepared from the dissolution of 38.158 g of NH<sub>4</sub>Cl in 1 liter of distilled water. The concentrations of TAN were determined by the method of Nessler and subsequently, the concentrations of non-ionized ammonia (NH<sub>3</sub>) were obtained from the application of the values of NAT, pH and water temperature to the Emerson Formula (EL-SHAFAI *et al.*, 2004).

The test lasted 96 h and was conducted in duplicate, with a photoperiod of 10 L:14 D. Mortalities were recorded during the 96 h of exposure and the

criterion of death adopted was the absence of movement or reaction to mechanical stimuli. The dead animals were removed from the aquariums as soon as this condition was verified. In addition, possible patterns of individuals' behavioral changes were observed during the test.

Temperature ( $^{\circ}\text{C}$ ), dissolved oxygen ( $\text{mg L}^{-1}$ ) and pH were measured after 30 (thirty) minutes of the addition of  $\text{NH}_4\text{Cl}$  and every 24 h, using a portable digital oximeter (MO-900, INSTRUTHERM) and portable digital pH meter (pH-1700, INSTRUTHERM). Determinations of NAT concentrations ( $\text{mg L}^{-1}$ ) were performed at the beginning of the test and after 48 h and 96 h, using the Nessler method, with a spectrophotometer (DR 2700, HACH). The concentrations of nitrite and nitrate ( $\text{mg L}^{-1}$ ) were determined at 48 h and 96 h, using the diazotation and cadmium reduction methods, respectively, with a spectrophotometer (DR 2700, HACH). The hardness and total alkalinity of the water ( $\text{mg L}^{-1} \text{CaCO}_3$ ) were determined by titration at 48 h and 96 h from the start of the test.

Data on water quality parameters were subjected to analysis of variance (ANOVA). When there was a significant difference between treatments, means were compared two by two using the Tukey test. A significance level of 5% was adopted. Statistical analyses were performed with the aid of BioEstat 5.0 and Excel 2013 (Microsoft Corp.). With the mortality data at the end of the experimental period, the mean lethal concentration  $\text{LC}_{50}$  was determined for 24, 48, 72 and 96 h using the Trimmed Spearman Karber statistical method (HAMILTON; RUSSO; THURSTON, 1977).

## RESULT AND DISCUSSION

The parameters temperature, dissolved oxygen ( $\text{DO}_2$ ), nitrite, nitrate and water hardness (Table 1) did

not show statistically significant differences between the different concentrations of  $\text{NH}_3$  tested. The pH and alkalinity showed statistically significant differences ( $p \leq 0,05$ ) between the different concentrations of  $\text{NH}_3$  tested. However, all parameters remained close to each other and within the levels considered ideal for the cultivation of fish. Thus, it is understood that they did not interfere in the result of the mean lethal concentration of  $\text{NH}_3$  ( $\text{LC}_{50-96 \text{ h}}$ ), that is, the mortality of the animals was not a consequence of changes in these water quality parameters.

The concentrations of total ammoniacal nitrogen (TAN) and non-ionized ammonia ( $\text{NH}_3$ ) observed in the water of the acute toxicity test are presented in Table 2. It is possible to notice that there was divergence between the desired concentrations and those observed by TAN. This divergence of values occurred because it was not possible to precisely manipulate the concentrations of TAN in the water, since there are biological reactions (excretion and absorption of ammonia by fish and nitrification), physical and chemical (volatilization) of ammonia in water (SÁ, 2012). In addition, in some experimental units, rest of feces was observed, a sign that the period without feeding was insufficient, with decomposition of organic matter and release of ammonia into water. However, this fact did not affect the test results.

As the objective of the test was to determine the average lethal concentration ( $\text{LC}_{50-96 \text{ h}}$ ) of non-ionized ammonia ( $\text{NH}_3$ ) in tambacu, from now on all results will be presented as a function of these concentrations.

Some behavioral changes (clinical signs) of the fish were observed throughout the exposure period. In the first hours, animals exposed to the highest concentration of ammonia ( $3.60 \text{ mg L}^{-1}$ ) presented hyperactivity, body darkening, seizures and loss of balance, followed by death, all of which died during the first 24 h of the test (Table 3). Over the course of 96 h, these signs, as well as

**Table 1** - Water quality parameters of the acute non-ionized ammonia ( $\text{NH}_3$ ) toxicity test on tambacu fingerlings (mean  $\pm$  SD)

Parameters	Concentration of non-ionized ammonia ( $\text{NH}_3$ ) ( $\text{mg L}^{-1}$ )					
	0.09	0.54	1.23	2.52	3.44	3.60
pH	8.3 $\pm$ 0.2 a <sup>1</sup>	8.2 $\pm$ 0.2 ab	8.2 $\pm$ 0.1 ab	8.1 $\pm$ 0.1 b	8.1 $\pm$ 0.1 b	8.1 $\pm$ 0.1 b
Temp ( $^{\circ}\text{C}$ )	27.7 $\pm$ 0.3	27.6 $\pm$ 0.3	27.8 $\pm$ 0.3	27.7 $\pm$ 0.3	27.8 $\pm$ 0.4	27.9 $\pm$ 0.4
$\text{DO}_2$ ( $\text{mg L}^{-1}$ )	6.4 $\pm$ 0.4	6.3 $\pm$ 0.2	6.5 $\pm$ 0.5	6.6 $\pm$ 0.4	6.4 $\pm$ 0.2	6.3 $\pm$ 0.2
$\text{NO}_2$ ( $\text{mg L}^{-1}$ )	0.013 $\pm$ 0.009	0.019 $\pm$ 0.008	0.033 $\pm$ 0.023	0.008 $\pm$ 0.004	0.022 $\pm$ 0.016	0.043 $\pm$ 0.036
$\text{NO}_3$ ( $\text{mg L}^{-1}$ )	0.5 $\pm$ 0.1	0.6 $\pm$ 0.1	0.4 $\pm$ 0.1	0.5 $\pm$ 0.1	0.7 $\pm$ 0.4	1.0 $\pm$ 0.4
Alkalinity ( $\text{mg L}^{-1} \text{CaCO}_3$ )	116.79 $\pm$ 2.43 a	115.01 $\pm$ 2.55 ac	106.59 $\pm$ 5.10 ab	105.06 $\pm$ 6.71 bc	101.75 $\pm$ 5.09 b	95.37 $\pm$ 6.89 b
Hardness ( $\text{mg L}^{-1} \text{CaCO}_3$ )	113.88 $\pm$ 3.14	112.45 $\pm$ 2.73	110.79 $\pm$ 5.18	111.74 $\pm$ 4.94	115.54 $\pm$ 1.82	113.64 $\pm$ 3.52

<sup>1</sup>Different letters on the same line indicate statistically significant differences between the means by the Tukey test ( $p \leq 0,05$ )

**Table 2** - Mean and standard deviation of total ammoniacal nitrogen (TAN) and non-ionized ammonia (NH<sub>3</sub>) concentrations in the water of the acute toxicity test with tambacu fingerlings

Observed concentration	TAN nominal (mg L <sup>-1</sup> )					
	0	5	15	30	45	60
TAN (mg L <sup>-1</sup> )	0.63 ± 0.14	5.08 ± 0.19	12.09 ± 0.45	35.10 ± 6.36	51.73 ± 1.98	58.38 ± 0.18
NH <sub>3</sub> (mg L <sup>-1</sup> )	0.09 ± 0.02	0.54 ± 0.07	1.23 ± 0.06	2.52 ± 0.37	3.44 ± 0.20	3.60 ± 0.01

**Table 3** - Cumulative percentage of mortality in tambacu as a function of time, in the acute toxicity test of non-ionized ammonia (NH<sub>3</sub>), in the two repetitions

NH <sub>3</sub> (mg L <sup>-1</sup> )	Time (hours)							
	24		48		72		96	
	I	II	I	II	I	II	I	II
0.09 (control)	-	-	-	-	-	-	-	-
0.54	-	-	-	-	-	-	-	-
1.23	10	-	-	-	20	-	-	-
2.52	50	-	100	80	-	90	-	100
3.44	80	90	100	100	-	-	-	-
3.60	100	100	-	-	-	-	-	-

lethargy and swimming on the water surface, were also observed in fish exposed to intermediate concentrations (1.23; 2.52 and 3.44 mg L<sup>-1</sup> NH<sub>3</sub>). In this case, 95% and 100% of the animals exposed to 2.52 and 3.44 mg L<sup>-1</sup> NH<sub>3</sub>, respectively, were dead after 48 h of exposure. At the concentration 1.23 mg L<sup>-1</sup> NH<sub>3</sub>, 10% of the animals died in 24 h and 10% in 72 h. No mortality and abnormal behavior was observed in animals exposed to 0,09 (control) and 0.54 mg L<sup>-1</sup> NH<sub>3</sub>.

Similar behavioral changes have been reported for freshwater species exposed to ammonia, such as Nile tilapia (*Oreochromis niloticus*) (BENLI; KÖKSAL, 2005) and yellow catfish (*Pelteobagrus fulvidraco*) (ZHANG *et al.*, 2012).

According to Hanna *et al.* (2013), behavioral changes in fish exposed to high concentrations of non-

ionized ammonia in water may be consequences of histopathological changes in the gills and brains.

The mean lethal concentrations (LC<sub>50</sub>) of NAT and NH<sub>3</sub> in tambacu fingerlings decreased over the 96 h exposure (Table 4). These results show that, over time, the animals' tolerance to ammonia decreased. The LC<sub>50-96 h</sub> of non-ionized ammonia for tambacu fingerlings was 1,63 mg L<sup>-1</sup>.

Barbieri and Bondioli (2015), obtained LC<sub>50-96 h</sub> equal to 0,014 mg L<sup>-1</sup> NH<sub>3</sub> for juvenile pacu with average weight 1,2 g ± 0,3 g in water with pH 6,98, temperature 25 °C and hardness 40 mg L<sup>-1</sup> CaCO<sub>3</sub>. Therefore, the results of the present study reveal a greater tolerance of tambacu to ammonia compared to its parent species.

There are some species that are tolerant of high concentrations of ammonia in the water and use different

**Table 4** - Variation of LC<sub>50</sub> in tambacu fingerlings exposed to total ammoniacal nitrogen (TAN) and non-ionized ammonia (NH<sub>3</sub>) for 96 h

Time (hours)	TAN (mg L <sup>-1</sup> )	NH <sub>3</sub> (mg L <sup>-1</sup> )
24	35.15 (29.54 - 41.84)	2.56 (2.25 - 2.91)
48	21.11 (18.43 - 24.17)	1.78 (1.61 - 1.97)
72	19.39 (16.73 - 22.48)	1.67 (1.49 - 1.88)
96	18.70 (16.43 - 21.29)	1.63 (1.47 - 1.81)

strategies to reduce ammonia toxicity. The main strategies are: conversion of ammonia to other less toxic substances, such as glutamine, for example, which can be accumulated in the brain and tissues, reduction of ammonia through the regulation of amino acid catabolism and maintenance of ammonia excretion through active transport of  $\text{NH}_4^+$  (IP; CHEW, 2010; SINHA *et al.*, 2013). In addition, recent studies have reported that taurine can mitigate the toxic effects of ammonia in fish, although the protective mechanisms have not been fully elucidated (REN *et al.*, 2016; ZHANG *et al.*, 2018).

The higher tolerance of tambacu to ammonia compared to pacu and tambaqui suggests that hybridization may confer some strategy to reduce ammonia toxicity. From this perspective, more studies should be conducted to understand the biology and physiology of this hybrid.

On the other hand, the higher tolerance of tambacu to ammonia may have been influenced by the hardness of the water, which varied from 110.79 to 115.78  $\text{mg L}^{-1}$   $\text{CaCO}_3$  in the experimental units. Total hardness is defined as the sum of the concentrations of calcium ( $\text{Ca}^{2+}$ ) and

magnesium ( $\text{Mg}^{2+}$ ) soluble in water (SÁ, 2012). It is known that  $\text{Ca}^{2+}$  plays an important role in the ionic regulation of freshwater fish and can alter the toxicity of ammonia in these animals. However, it is not yet known how the  $\text{Ca}^{2+}$  ion eases the stress associated with high concentrations of  $\text{NH}_3$  (BALDISSEROTTO, 2009).

Tomasso *et al.* (1980), studying the effects of pH and calcium on ammonia toxicity in canal catfish (*Ictalurus punctatus*) observed that an increase in water hardness from 40 to 440  $\text{mg L}^{-1}$   $\text{CaCO}_3$  (pH 7, temperature 21-25 °C) resulted in an increase in the value of  $\text{LC}_{50-24\text{ h}}$  of non-ionized ammonia from  $1.39 \pm 0.06$  to  $1.79 \pm 0.07$   $\text{mg L}^{-1}$   $\text{NH}_3$ . Ferreira, Cunha and Baldisserotto (2013) did not observe a significant effect of water hardness on the  $\text{LC}_{50-96\text{ h}}$  of non-ionized ammonia in jundiá juveniles (*Rhamdia quelen*) and reported that, apparently, the effect of water hardness or  $\text{Ca}^{2+}$  ions on the acute toxicity of  $\text{NH}_3$  is species specific.

It is notable, therefore, that the toxicity of non-ionized ammonia in fish depends on some intrinsic factors, such as: species, weight, size and age and extrinsic, such as: pH, temperature and water hardness (Table 5).

**Table 5** -  $\text{LC}_{50}$  values of non-ionized ammonia ( $\text{NH}_3$ ) in different species of fish

Species	$\text{LC}_{50}$ ( $\text{mg L}^{-1}$ )	Weight (g)	pH	Hardness ( $\text{mg L}^{-1}$ $\text{CaCO}_3$ )	Time of exposure (hours)	References
<i>Piaractus mesopotamicus</i>	0.014	1.2	6.98	40	96	Barbieri e Bondioli (2015)
<i>Hypophthalmichthys molitrix</i>	0.35	Larvae	8.63	-	96	Wang <i>et al.</i> (2017)
<i>Aristichthys nobilis</i>	0.33	Larvae	8.85	-	96	Wang <i>et al.</i> (2017)
<i>Carassius gibelio</i>	0.73	1.96	7.18	-	96	Wang <i>et al.</i> (2017)
<i>Oreochromis niloticus</i>	7.4	10.1	8.0	-	48	Benli e Köksal (2005)
<i>Oreochromis aureus</i> <sup>1</sup>	2.83	3.19	7.94	4.4	24	Semra (2014)
<i>Oreochromis aureus</i> <sup>2</sup>	3.14	3.36	8.15	732.5	24	Semra (2014)
<i>Ictalurus punctatus</i>	1.39	-	7.0	40.0	24	Tomasso <i>et al.</i> (1980)
<i>Ictalurus punctatus</i>	1.79	-	7.0	440.0	24	Tomasso <i>et al.</i> (1980)
<i>Rhamdia quelen</i>	1.45	11.04	7.5	20.0	96	Miron <i>et al.</i> (2008)
<i>Carassius auratus</i>	4.68	0.51	-	-	96	Yang <i>et al.</i> (2010)
<i>Pelteobagrus fulvidraco</i>	0.65	3.52	7.42	101.4	96	Zhang <i>et al.</i> (2012)
<i>Lophiosilurus alexandri</i>	3.66	33.87	8.62	-	96	Silva <i>et al.</i> (2018)

<sup>1</sup>Test performed in salinity 1; <sup>2</sup>Test performed in salinity 12

## CONCLUSION

The mean lethal concentration ( $\text{LC}_{50-96\text{ h}}$ ) of non-ionized ammonia ( $\text{NH}_3$ ) for tambacu hybrid fingerlings (*Colossoma macropomum* x *Piaractus mesopotamicus*) was  $1.63 \text{ mg L}^{-1}$ , revealing a higher tolerance of tambacu to ammonia compared to their parental species.

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