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High levels of total ammonia nitrogen as NH_4^+ are stressful and harmful to the growth of Nile tilapia juveniles

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ABSTRACT. This study determined whether high levels of total ammonia nitrogen (TAN) as NH_4^+ are harmful to the growth performance of Nile tilapia juveniles. Fingerlings (0.31 ± 0.04 g) were assigned to 30 polyethylene 100-L tanks in a roofed room for 12 rearing weeks. There were increasing levels of TAN by increased NH_4Cl application rates (0.0; 0.25 and 0.50 g tank^{-1} week^{-1}) at three conditions of water pH (acidic, 6.2 ± 0.5; neutral, 7.2 ± 0.8 and alkaline, 8.8 ± 0.3). The application of HCl to acidic tanks caused 100% of TAN to be converted into NH_4^+. The poorest growth performance results were observed for the alkaline tanks subjected to the highest application of NH_4Cl. In acidic tanks, fish survival has dropped in those tanks under the highest application rate of NH_4Cl. Tilapia growth was lower in neutral tanks when the NH_4Cl application rate increased to 0.50 g tank^{-1} week^{-1}. It was concluded that waterborne ionized ammonia (NH_4^+) is indirectly toxic to tilapia due to the harmful metabolites derived from it, such as nitrite and chloramines as well as due to water acidification.

Keywords: non-ionized ammonia, ionized ammonia, fish culture, water quality.

Elevados níveis de nitrogênio amoniacal total como NH_4^+ são estressantes e danosos ao crescimento de juvenis de tilápia nilótica

RESUMO. O objetivo do presente trabalho foi determinar se níveis elevados de nitrogênio amoniacal total (NAT) como NH_4^+ são prejudiciais ao desempenho produtivo de juvenis de tilápia do Nilo. Os alevinos (0.31 ± 0.04 g) foram distribuídos em 30 tanques de polietileno de 100 L, localizados em sala coberta, sendo mantidos nesse sistema durante doze semanas. Estabeleceram-se níveis crescentes de NAT na água pela aplicação de taxas crescentes de NH_4Cl (0,0; 0,25 e 0,50 g tanque^{-1} semana^{-1}), em três condições de pH da água (ácido, 6,2 ± 0,5; neutro, 7,2 ± 0,8 e alcalino, 8,8 ± 0,3). A aplicação de HCl nos tanques ácidos fez com que 100% do NAT estivesse na forma de NH_4^+. Os piores resultados de desempenho produtivo foram observados nos tanques alcalinos submetidos à maior taxa de aplicação de NH_4Cl. Nos tanques ácidos, a sobrevivência dos peixes caiu naqueles tanques submetidos à maior taxa de aplicação de NH_4Cl. O crescimento das tilápias foi pior nos tanques neutros quando a aplicação de NH_4Cl foi elevada para 0,50 g tanque^{-1} semana^{-1}. Concluiu-se que NH_4^+ é indiretamente tóxico para tilápia por produzir metabólitos tóxicos, tais como nitritos e cloraminas, assim como por acidificar a água de cultivo.

Palavras-chave: amônia não ionizada, amônia ionizada, piscicultura, qualidade de água.

Introduction

Ammonia arises in water as an end product of the protein catabolism by living organisms. The total ammonia nitrogen (TAN) comprises two distinct forms: NH_3 or non-ionized ammonia and NH_4^+ or ionized ammonia. Only the NH_3 form goes freely through fish gills and it is therefore considered the TAN's most toxic form. Lemarié et al. (2004), citing Haywood (1983), stated that NH_3 is 300 - 400 times more toxic than NH_4^+ to fish. The pH and temperature of water are the main factors that affect the proportion between NH_3 and NH_4^+ (PARRA; YUFERA, 1999).

Excretion of ammonia to the water by fish is carried out mainly through their gills by simple diffusion as NH_3. Therefore, increased concentrations of NH_3 in water can interfere with NH_3 excretion by fish and cause toxicity. Hence, the ideal water quality for an efficient NH_3 excretion is low TAN and low pH (< 8). Under those conditions the concentrations of NH_3 in water are very low, which ensure a rapid diffusion of blood NH_3 to water. Typically, low water pH occurs at night in fish ponds, making that daytime as the most suitable for NH_3 excretion by fish. Contrarily, the regularly higher values of water pH and temperature in the afternoons lessen the NH_3 excretion by fish. Consequently, afternoon is the most critical period of the day for NH_3 toxicity (HARGREAVES; KUCUK, 2001).

Besides the passive diffusion of NH_3 to water, there is also the active excretion of NH_4^+ as an additional excretion pathway in fishes. Nevertheless, the NH_4^+ excretion is a less-efficient process than the NH_3 excretion and it depends on the concentration of Na^+ ions in water (CARNEIRO et al., 2009). As a consequence, ammonia tends to increase in fish blood during the afternoons (WOOD, 1993; ZIMMER et al., 2010).

Despite its safer status, it is speculated that high levels of NH_4^+ in water could also impair fish growth. Willingham et al. (1979) have already pointed out that regardless of the lower toxicity of NH_4^+ to fish compared with NH_3, the concentrations of the former compound in water are usually well above those observed for the latter one. Consequently, those authors concluded, NH_4^+ can also cause considerable toxicity to fish and other aquatic animals. The aim of this study was to determine whether high levels of TAN as NH_4^+ are stressful and harmful to the growth performance of Nile tilapia juveniles.

Material and methods

One thousand male-reversed Nile tilapia, *Oreochromis niloticus*, fingerlings were obtained from DNOCS - *Departamento Nacional de Obras Contra as Secas* (Pentecoste, Ceará State, Brazil) and transported by road to the LCTA – *Laboratório de Ciência e Tecnologia Aquícola* (Fortaleza, Ceará State, Brazil). After acclimation, fish (0.31 ± 0.04 g) were assigned to 30 round polyethylene 100-L tanks in a roofed room. The fish culture was carried out in clear water, without plankton. The culture tanks were served by mechanical aeration for 24h provided by one 2.5 hp blower. At the onset, three tilapia fingerlings were stocked in each tank for twelve rearing weeks.

Over the entire experiment, fish fed a commercial extruded 55%-CP diet split into four meals at 8 and 11 a.m.; 1 and 4 p.m. The daily feeding rate was fixed at 10% of the stocked biomass. There was no water exchange throughout the culture. New water was added just to replenish the water lost by evaporation or samplings. Tap water was used to fill up the tanks.

Eight different experimental groups were established in the present work by the application of three rates of analytical grade NH_4Cl (0.00; 0.25 and 0.50 g tank^{-1} week^{-1}) at three levels of water pH (moderately acidic, neutral and moderately alkaline; Table 1).

Out of the possible combinations between those two factors (ammonia and pH), only the crossing between none application of NH_4Cl and neutral pH of water was not accomplished due to unavailability of tanks. There were increasing levels of total ammonia nitrogen (TAN) at each condition of water pH (acidic, 6.2 ± 0.5; neutral, 7.2 ± 0.8 and alkaline, 8.8 ± 0.3). Under those different values of water pH, the increasing levels of TAN brought about different concentrations of non-ionized ammonia (NH_3; 0.00 - 0.15 mg L^{-1}) and ionized ammonia in water (NH_4^+; 0.15 - 2.52 mg L^{-1}; Table 1).

There were four repetitions for the acidic and alkaline tanks and three repetitions for the neutral tanks.

Table 1. Concentrations of total ammonia nitrogen (TAN), non-ionized ammonia (NH_3) and ionized ammonia (NH_4^+; mg L^{-1}) in Nile tilapia 100-L indoor tanks over 12 weeks (mean ± S.D.; n = 4 or 3). Tanks were subjected to different rates of analytical grade NH_4Cl and values of pH. Acidic, neutral and alkaline pH of water were 6.2 ± 0.5; 7.2 ± 0.8 and 8.8 ± 0.3, respectively. The water temperature of 28°C was used in the calculations of NH_3 concentrations.

NH_4Cl application rate (g tank^{-1} week^{-1})	Variable	pH of water[1]		
		Acidic	Neutral	Alkaline
0.00	TAN	1.17 ± 0.15	-[3]	0.21 ± 0.09
	NH_3	-[2]	-	0.06 ± 0.03
	NH_4^+	1.17 ± 0.15 4	-	0.15 ± 0.06
	% TAN as NH_4^+	100.0	-	71.4
0.25	TAN	2.16 ± 0.07	0.67 ± 0.02	0.37 ± 0.10
	NH_3	-	0.01 ± 0.001	0.12 ± 0.03
	NH_4^+	2.16 ± 0.07	0.66 ± 0.02	0.26 ± 0.07
	% TAN as NH_4^+	100.0	98.5	70.3
0.50	TAN	2.52 ± 0.15	1.47 ± 0.27	0.49 ± 0.05
	NH_3	-	0.02 ± 0.003	0.15 ± 0.02
	NH_4^+	2.52 ± 0.15	1.45 ± 0.27	0.34 ± 0.03
	% TAN as NH_4^+	100.0	98.6	69.4

[1]The pH of supply water was handled with HCl (acidic), NaOH (alkaline) or none (neutral); [2]There is an insignificant concentration of NH_3 when the pH of water is lower than 7 (BOYD, 1979); [3]Not done; [4]Calculated as the difference between TAN and NH_3.

A 10.8 N HCl solution was directly applied into the culture tanks to lower their pH to the desired value (6.2). The pH lowering was monitored *in situ* with a portable pH-meter. Besides, the acidic solution was supplied slowly to allow fish adaptation. The same procedure was done in the alkaline tanks at which a 1 M NaOH solution was applied to their water to reach a water pH near 9.2. In the neutral tanks, no product was applied to the tank water, and pH remained unchanged. At each two-day interval, the pH of water of all tanks were recorded and adjusted if they were far from the designed values of 6.2 (acidic tanks) and 9.2 (alkaline tanks). Despite our best effort, the average pH of water in the alkaline tanks was 8.8 ± 0.3 and not 9.2, as initially desired.

Depending on the treatment, analytical grade NH_4Cl was applied to the culture tanks. In the 0.0-g NH_4Cl tanks, no addition of NH_4Cl was made over the entire experimental period. In the 0.25 and 0.50-g NH_4Cl tanks, there were applications of 0.25 and 0.50 g NH_4Cl tank^{-1} week^{-1} from the beginning until the end, respectively.

Water quality and growth performance were observed in the present study. Daily, at 8 a.m. and 4 p.m., the water temperature, pH and electrical conductivity (EC) were recorded in each tank using portable equipments. Fortnightly, water samples were taken from all tanks to perform the analyses of free CO_2 (sodium carbonate titration), dissolved oxygen (Winkler's method), nitrite (diazotization method) and reactive phosphorus (molybdenum blue method), following the guidelines presented by APHA (1999). Total ammonia nitrogen in water samples were determined weekly by the indophenol method (BOYD, 1979).

The following growth performance variables were monitored and calculated out of the experimental treatments: fish survival, final body weight, specific growth rate [(ln final body weight – ln initial body weight)/days of rearing] x 100 and fish yield.

Water quality and growth performance variables were analyzed by one-way ANOVA. The significantly different means were compared pairwise with the Tukey's test. The assumptions of normal distribution and homogeneity of variances were checked before analysis. Percentage and ratio data were analyzed using arcsine-transformed data. All ANOVA analyses were carried out at 5% level of significance using SigmaStat for Windows 2.0 (Jandel Statistics).

Results and discussion

As expected, the different application rates of NH_4Cl carried out in the present work have produced increasing levels of TAN in the tank water. On the other hand, there was a surprisingly drop of TAN in the alkaline tanks for a same application rate of NH_4Cl (Table 1). For that, the following explanation is proposed. At alkaline waters, the proportion of TAN as non-ionized gaseous ammonia (NH_3) increases when compared to neutral or acidic waters (BOYD, 1979; DIANA et al., 1997). In the present work, all experimental tanks were continuously served by mechanical aeration supplied by one 2.5-hp blower connected to silicon hoses and air stones. Therefore, it is speculated that gaseous NH_3 was partially released from the alkaline tanks to the atmosphere due to the mechanical aeration of the water, lowering thus the concentrations of TAN in those tanks. This is considered an important finding because it might have practical applications to the water quality management of fish ponds. In fish ponds, a greater proportion of toxic but gaseous NH_3 in water occurs in the afternoon due to the higher pH and temperature of water (BOYD, 1998). Hence, afternoon seems to be the most suitable period to remove gaseous NH_3 from fish ponds through mechanical aeration of water. The amount of gaseous NH_3 lost to atmosphere can be even higher by the conversion of NH_4^+ into NH_3 as the pH of water increases as CO_2 is simultaneously released to the air by aeration (TREASURER, 2010).

The application of HCl to acidic tanks caused 100% of TAN to be in the form of NH_4^+ (Table 1). Moreover, very little NH_3 was observed in neutral tanks (≤ 0.02 mg L^{-1}). Hence, acidification of water is a possible management to control NH_3 toxicity in fish tanks. However, Boyd and Tucker (1998) have recommended it only for emergencies.

As expected, the highest concentrations of NH_3 were found in the alkaline tanks (0.06 - 0.15 mg L^{-1}). Pillay (1992), Boyd and Tucker (1998), and El-Shafai et al. (2004) have proposed that NH_3 levels below 0.1 mg L^{-1} are safe for aquaculture. Salmonid aquaculture, in particular, has even narrower safety limits for NH_3, lower than 0.01 mg L^{-1} (PERSON-LE RUYET et al., 1997). Therefore, only the two higher application rates of NH_4Cl (0.25 and 0.50 mg L^{-1}) in the alkaline tanks exceeded the 0.1 mg L^{-1} critical NH_3 level in the present work (Table 1). Consequently, poor results of fish growth performance would be expected only for those treatments.

Values of average water temperature in the experimental tanks were 26.5 ± 0.6°C and 26.9 ±

0.8°C at 8 a.m. and 4 p.m., respectively. The minimum and maximum temperatures observed over the experiment were 22.9°C and 27.9°C, respectively. The average concentration of dissolved oxygen in water was 6.5 ± 1.4 mg L^{-1} (82% saturation) and no significant differences were observed between the tanks for those variables ($p > 0.05$).

There was a decrease in the total alkalinity of water in the alkaline tanks as the NH_4Cl application rate was increased from 0.0 to 0.5 g $tank^{-1}$ $week^{-1}$ (Figure 1). Toxic chloramines (NH_2Cl) can be produced in water by the reaction between non-ionized ammonia and hypochlorous acid ($NH_3 + HClO \rightarrow NH_2Cl + H_2O$). Next, the increase of chloramines leads to water acidification through the following reaction: $2NH_2Cl + HOCl \rightarrow N_2 + 3H^+ + 3Cl^- + H_2O$ (WAN et al., 2000). Therefore, it is supposed that besides NH_3 there was also toxic NH_2Cl in the alkaline tanks subjected to the highest application rate of NH_4Cl. Additionally, the nitrification process itself also acidifies the water ($NH_4^+ + 1½\ O_2 \rightarrow NO_2^- + 2\ H^+ + H_2O$; HARGREAVES, 1998).

Alkaline tanks presented significantly higher EC than neutral and acidic tanks due to the Na^+ and OH^- input by the NaOH application in the first tanks. Besides that, the EC readings in the acidic tanks were significantly higher than those in neutral tanks due to the H^+ and Cl^- input by the HCl application in the first tanks. The application rate of NH_4Cl in water had not significantly affected the EC regardless the water pH (acidic, neutral or alkaline; Figure 1). In general, all tanks presented EC values below the upper limit suitable for aquaculture of 1000 μS cm^{-1} (BOYD; TUCKER 1998). The EC of water, specifically by its Na^+ concentration, can affect ammonia excretion by fish because ionized ammonia (NH_4^+) can be actively excreted by fish in the exchange for Na^+ (HARGREAVES; KUCUK 2001). Therefore, waters with higher salinities (EC) make easier the ammonia excretion by fish.

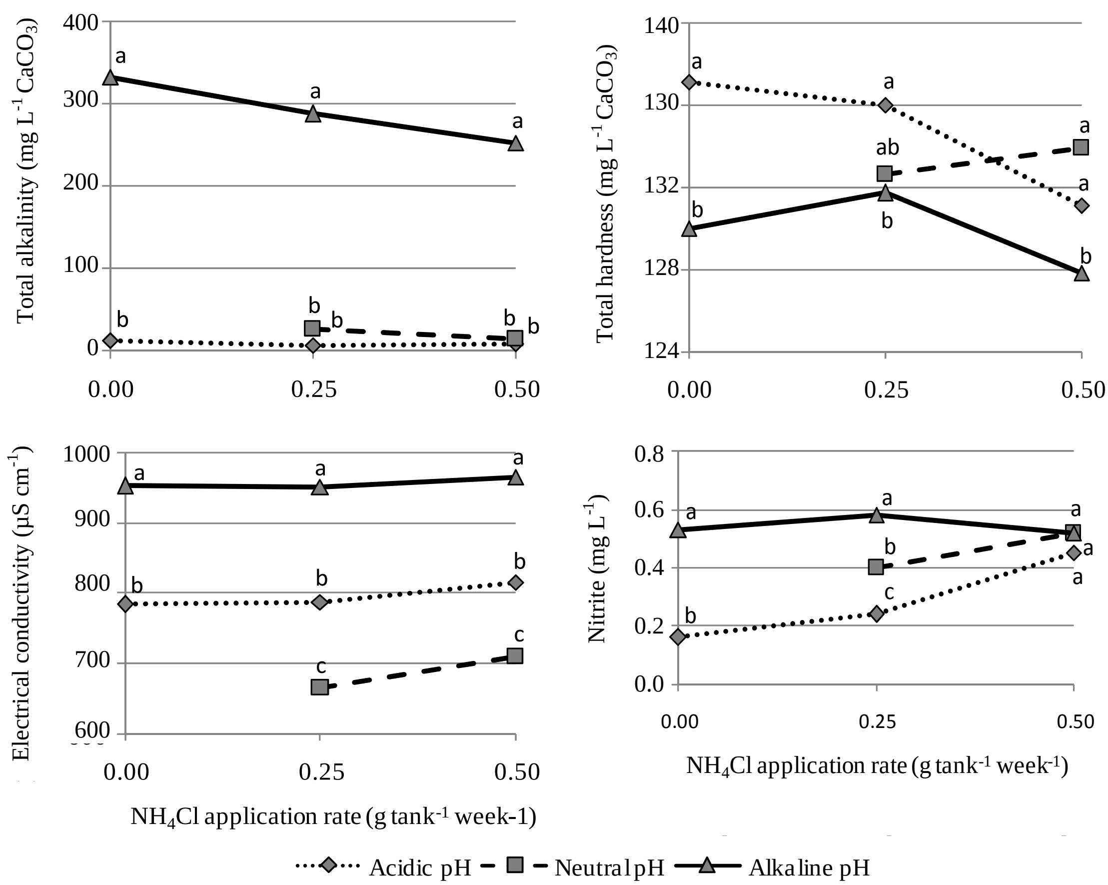

Figure 1. Water quality of 100-L tanks, for Nile tilapia rearing, subjected to different concentrations of total ammonia nitrogen and water pH over 12 weeks (three fish per tank). Acidic, neutral and alkaline pH of water were 6.2 ± 0.5; 7.2 ± 0.8 and 8.8 ± 0.3, respectively. Each symbol represents the average of three (neutral pH) or four (acidic and alkaline pH) repetitions. For a same application rate of NH_4Cl, means not sharing a same letter are statistically different by Tukey's test (upright comparisons; $p < 0.05$).

At the NH_4Cl application rates of 0.0 and 0.25 g $tank^{-1}$ $week^{-1}$, the concentrations of nitrite in water were significantly higher in alkaline than in acidic or neutral tanks. However, at the highest NH_4Cl application rate (0.5 g $tank^{-1}$ $week^{-1}$), no significant differences were detected between all tanks for nitrite (Figure 1). Probably, the higher pH of water in alkaline tanks (8.8 ± 0.3) when compared to those for the neutral (7.2 ± 0.8) and acidic (6.2 ± 0.5) tanks have favored the bacterial nitrification process, which has produced more nitrite from ammonia (SINHA; ANNACHHATRE, 2007). In acidic and neutral tanks, the concentrations of nitrite in water have increased when more NH_4Cl was applied to the tanks (Figure 1). As ammonia is the precursor of nitrite, it is expected more nitrite when there is more ammonia in water. Again the higher total alkalinity in alkaline tanks has probably aided the conversion of nitrite into nitrate by *Nitrobacter* that not allowed the increase of nitrite in those tanks.

At the application rate of 0.25 g NH_4Cl $tank^{-1}$ $week^{-1}$, fish survival in the alkaline tanks was significantly lower than in acidic and neutral tanks (Figure 2; $p < 0.05$). In the 0.25-g NH_4Cl alkaline tanks, there was 0.12 ± 0.03 mg NH_3 L^{-1} while in the 0.25-g NH_4Cl acidic and neutral tanks there were none or just 0.01 ± 0.001 mg NH_3 L^{-1}, respectively. Moreover, there was significantly more toxic nitrite in the alkaline tanks. In the acidic tanks, fish survival has dropped only in those tanks subjected to the highest NH_4Cl application. In the acidic tanks, as TAN increased at the higher NH_4Cl application rate, the concentrations of nitrite in water have increased accordingly (Figure 1). Hence, the probable cause of fish mortality at the 0.5-g NH_4Cl acidic tanks was nitrite toxicity. No fish mortalities were registered in the neutral tanks despite the NH_4Cl application rate had been performed (0.25 or 0.50 g $tank^{-1}$ $week^{-1}$; Figure 2). This result suggests that there is an interaction between the water pH and nitrite in regards to nitrite toxicity to fish. It seems that a nitrite level toxic to fish in an acidic pH can be harmless in a neutral pH. Probably, those two stressors, low pH of water and high nitrite, have acted synergistically to affect fish survival.

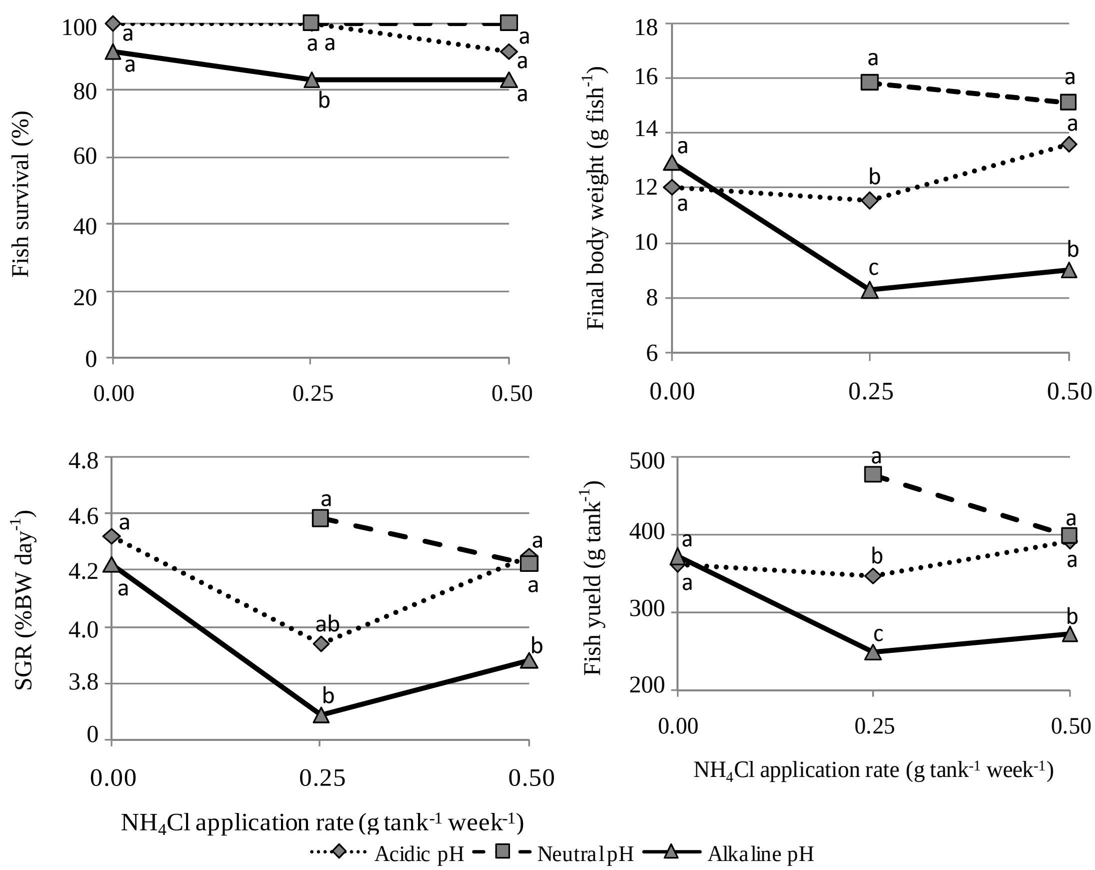

Figure 2. Growth performance of Nile tilapia juveniles stocked in 100-L tanks subjected to different concentrations of total ammonia nitrogen and water pH over 12 weeks (three fish per tank). Acidic, neutral and alkaline pH of water were 6.2 ± 0.5; 7.2 ± 0.8 and 8.8 ± 0.3, respectively. Initial body weight = 0.31 ± 0.04 g. Each symbol represents the average of three (neutral pH) or four (acidic and alkaline pH) repetitions. For a same pH and application rate of NH_4Cl, means not sharing a same letter are statistically different by Tukey's test (upright comparisons; $p < 0.05$).

Although there was much more TAN in the 0.0-g NH_4Cl acidic tanks (1.17 ± 0.15 mg L^{-1}) than in the 0.0-g NH_4Cl alkaline tanks (0.21 ± 0.09 mg L^{-1}), the concentrations of non-ionized ammonia (NH_3) were higher in the latter tanks (0.0 versus 0.06 ± 0.03 mg L^{-1}, respectively; Table 1). However, that value (0.06 mg TAN L^{-1}) is still below the critical 0.1 mg NH_3 L^{-1} level for aquaculture (BOYD; TUCKER, 1998). As a consequence, no significant differences for fish growth performance were observed between the 0.0-g NH_4Cl acidic tanks and the 0.0-g NH_4Cl alkaline tanks (Figure 2). On the other hand, the nitrite level in the 0.0-g NH_4Cl alkaline tanks was significantly higher than in the 0.0-g NH_4Cl acidic tanks (Figure 1). Possibly higher levels of total alkalinity and/or EC (Na^+) in the first tanks have somehow protected fish against nitrite toxicity. Some works have shown that increased salinity of water is capable to minimize the toxic effects of NH_3 and nitrite on fishes (SAMPAIO et al., 2002; WEIRICH; RICHE, 2006a and b). Tomasso et al. (1980) and Twitchen and Eddy (1994) have observed that channel catfish and rainbow trout exposed to high levels of NH_3 increased their Na^+ efflux to the water. As previously said, the concentrations of NH_3 in water exceeded the critical level of 0.1 mg L^{-1} only in alkaline tanks for the NH_4Cl application rates of 0.25 or 0.50 g $tank^{-1}$ $week^{-1}$ (Table 1). Consequently, those tanks have shown significantly lower final body weight, SGR and yield of fish than acidic and neutral tanks (Figure 2). Wilkie and Wood (1996) have stated that fish present increased plasma and tissue ammonia in alkaline environments.

The final body weight and yield of fish in the 0.25-g NH_4Cl neutral tanks were significantly higher than for the 0.25-g NH_4Cl acidic tanks (Figure 2; $p < 0.05$). This result suggests that a harmless value of water pH to fish in a low-TAN environment could be deleterious in a high-ammonia environment and vice-versa. However, no significant differences were verified for tilapia growth performance between the 0.5-g NH_4Cl neutral tanks and the 0.5-g NH_4Cl acidic tanks. Except for survival, tilapia growth was lower in the neutral tanks when the NH_4Cl application rate was increased to 0.50 g $tank^{-1}$ $week^{-1}$. This was due probably to nitrite toxicity, which has increased in neutral tanks when more NH_4Cl was applied.

Conclusion

As well as non-ionized ammonia (NH_3), ionized ammonia (NH_4^+) can also be harmful to fish due to the toxic metabolites derived from it, such as nitrite and chloramines, and through water acidification. Furthermore, a high but harmless level of NH_4^+ will be converted into a high and harmful NH_3 level when the pH of water rises. In fish ponds, increase of water pH is routinely observed in the late afternoons, day after day. Therefore, fish farmers should be aware not only about the NH_3 level of the water but also on the TAN level (NH_3 + NH_4^+).

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