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Imbalances in the hardness/alkalinity ratio of water and Nile tilapia's growth performance

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ABSTRACT. The present work determined the effects of water with unbalanced calcium hardness/total alkalinity (CH/TA) ratio on Nile tilapia growth performance. Two unbalanced CH/TA ratios were tested for 8 weeks using one indoor system with 30 circular polyethylene 100 L tanks and one outdoor system with 36 circular polyethylene 250 L tanks. The factors and their specific levels tested were the total alkalinity of water (TA; 50 and 100 mg L^{-1} $CaCO_3$) and the CH/TA ratio of water (CH/TA; 1/2, 1/1 and 5/1). In the indoor system, the 5/1 CH/TA ratio tanks had higher total ammonia nitrogen (TAN) than the 1/2 or 1/1 CH/TA ratio tanks. In the outdoor system, higher concentrations of TAN were found in the 1/2 CH/TA ratio tanks. The soil of the 5/1 CH/TA ratio indoor tanks had higher concentrations of organic carbon than the 1/2 or 1/1 CH/TA ratio tanks. The detrimental effects of the 5/1 CH/TA ratio on fish growth performance were stronger than those associated with the 1/2 CH/TA ratio. A significantly better food conversion ratio was observed in the 1/1 CH/TA ratio outdoor tanks regardless of the TA level of the water.

Keywords: fish culture, water quality, *Oreochromis niloticus.*

Desequilíbrios da relação dureza/alcalinidade da água e desempenho produtivo da tilápia do Nilo

RESUMO. O presente estudo determinou os efeitos de diferentes relações dureza cálcica/alcalinidade total (DC/AT) da água de cultivo sobre o desempenho produtivo da tilápia do Nilo. Duas relações DC/AT foram testadas durante oito semanas em dois sistemas de cultivo: interno, com a utilização de 30 tanques de 100 L e externo, com 36 tanques de 250 L. As variáveis testadas foram a alcalinidade total da água (AT; 50 e 100 mg L^{-1} $CaCO_3$) e a relação DC/AT da água (1/2, 1/1 e 5/1). No sistema interno, os tanques com relação DC/AT de 5/1 apresentaram maior concentração de nitrogênio amoniacal total (NAT) que os tanques com relações DC/AT de 1/2 ou 1/1. No sistema externo, as maiores concentrações de NAT foram observadas nos tanques com relação DC/AT de 1/2. O solo dos tanques internos com relação DC/AT de 5/1 apresentaram maiores concentrações de carbono orgânico. Os efeitos deletérios da relação DC/AT de 5/1 sobre o desempenho zootécnico dos peixes cultivados foram maiores que aqueles associados à relação DC/AT de 1/2. Fator de conversão alimentar significativamente melhor foi observado nos tanques externos com relação DC/AT de 1/1, independentemente do nível de alcalinidade total da água.

Palavras-chave: piscicultura, qualidade de água, *Oreochromis nilocitus*.

Introduction

The total alkalinity (TA) of water is defined as the concentration of titratable bases, mainly bicarbonates (HCO_3^-) and carbonates (CO_3^{-2}), expressed as $CaCO_3$ equivalents. Water used for aquaculture should have a TA $\geq$ 20 mg L^{-1} $CaCO_3$ (in freshwater) (ANDRADE et al., 2007; ROJAS; ROCHA 2004; WURTS; DURBOROW 1992) and TA $\geq$ 100 mg L^{-1} $CaCO_3$ (in saltwater) (FERREIRA et al., 2011) to achieve optimum fish or shrimp growth performance, respectively. Water with low TA is more susceptible to acidification than water with high TA. The TA of water can be increased by the addition of carbonated salts, such as limestone (calcium carbonate) (FURTADO et al., 2011).

The hardness of water is defined as the concentration of divalent cations, mainly calcium and magnesium, expressed as $CaCO_3$ equivalents. The total hardness of water includes calcium hardness (CH) and magnesium hardness. Usually only the CH of water is considered rather than its total hardness due to the higher importance of calcium to fish physiology. Since fish absorb calcium and magnesium directly from water, water hardness has practical importance for fish culture (SILVA et al., 2003). Water with a low hardness can impair fish growth due to calcium deficiency. In freshwater culture systems, it is advised that the water hardness be higher than 20 mg L^{-1} $CaCO_3$ to achieve optimum fish growth (BOYD, 1979). Contrarily, water hardness in saltwater culture systems is not a concern because the concentration of calcium in these systems is usually very high (PORTZ et al., 2006).

In addition to the absolute values of TA and CH, it is also important to consider the CH/TA ratio of water. It is possible to have apparently adequate values of TA and CH but improper CH/TA ratios. The most common problem is when the TA of water is higher than its CH (CH/TA ratio < 1). Under these conditions, there can be an undesirable and dangerous increase in the pH of the culture water during late afternoon. On the other hand, it is assumed that hardness of water higher than its alkalinity (CH/TA ratio > 1) is harmless to fish growth (BOYD, 1979). Cavalcante et al. (2009) concluded that the culture water with a TA ≥ 50 mg L^{-1} $CaCO_3$ and a CH ≥ 140 mg L^{-1} $CaCO_3$ produced the best growth performance for 0.8 g Nile tilapia fingerlings. These values give a CH/TA ratio of water > 2.8. Recently, Cavalcante et al. (2012) observed that besides a minimum level of TA (≥ 20 mg L^{-1} $CaCO_3$) and total hardness (TH ≥ 20 mg L^{-1} $CaCO_3$), it is also important that Nile tilapia culture waters have a CH/TA ratio ≥ 1. Therefore, it seems that CH/TA ratios > 1 are more preferable than CH/TA ratios < 1. Generically, however, both cases can be referred to as unbalanced CH/TA ratios of water. At the present time, the effects of unbalanced CH/TA ratios of water have not been suitably tested with Nile tilapia.

The aim of this present work was to determine the effects of two unbalanced CH/TA ratios of culture water on Nile tilapia growth performance in outdoor experimental tanks.

Material and methods

Fish and experimental system

Two thousand sex-reversed Nile tilapia, *Oreochromis niloticus*, fingerlings (0.4 ± 0.05 g) were obtained from the Aquaculture Research Center of the Departamento Nacional de Obras Contra as Secas (DNOCS; Pentecoste, Ceará State, Brazil). Fingerlings were transported by road to the Laboratório de Ciência e Tecnologia Aquícola (Fortaleza, Ceará State, Brazil). In the lab, fish were acclimatized in one polyethylene circular 1,000 L tank with continuous mechanical aeration.

After 24h, potassium permanganate was added to the tank at 4 mg L^{-1} for 48h to prevent bacterial diseases in the acclimation tank (LASIER et al., 2000). After this period, sodium thiosulfate was used at 4 mg L^{-1} to neutralize the residual potassium permanganate. Over the acclimation period, fish were given a high protein commercial sinking diet (CP = 460 g kg^{-1}, Aquaxcel 4515 0.8 mm, Evialis Animal Nutrition, Paulínia, São Paulo State, Brazil). The ration was supplied to the fish four times daily at 8:00, 11:00 a.m., 13:00 and 14:00 p.m. hours at 15% of the stock biomass per day. The acclimation period lasted one week.

Culture systems and experimental design

The outdoor culture system includes 36 circular polyethylene 250 L tanks without any mechanical aeration. Six experimental groups were randomly distributed into the tanks in a 2 x 3 factorial design. Factors and levels tested were the total alkalinity of water (TA; 50 and 100 mg L^{-1} $CaCO_3$) and the calcium hardness/total alkalinity ratio of water (CH/TA; 1/2, 1/1 and 5/1; Table 1). These three levels of CH/TA ratio were selected to represent a downward unbalanced, a balanced and an upward unbalanced CH/TA ratio, respectively. Each treatment had six replications.

Table 1 Total alkalinity (TA; mg L^{-1} $CaCO_3$ eq.), total hardness (TH; mg L^{-1} $CaCO_3$ eq.), calcium hardness (CH; mg L^{-1} $CaCO_3$ eq.) and CH/TA ratio of water for juvenile Nile tilapia culture in experimental 250-L outdoor tanks over 8 weeks (mean ± S.D.; n = 6).

Treatment[1]		TA	TH	CH	CH/TA
TA	CH/TA				
	1/2	53 ± 5	58 ± 5	28 ± 5	0.5 ± 0.04
50	1/1	56 ± 3	97 ± 5	48 ± 2	0.8 ± 0.02
	5/1	55 ± 4	277 ± 9	231 ± 10	4.1 ± 0.23
	1/2	105 ± 5	94 ± 5	50 ± 3	0.5 ± 0.02
100	1/1	105 ± 5	147 ± 6	99 ± 3	0.9 ± 0.01
	5/1	104 ± 5	529 ± 22	482 ± 24	4.7 ± 0.11

[1]Depending on the treatment, the initial water (tap water) was manipulated with analytical grade EDTA to reduce water hardness, with $CaCl_2$ to increase water hardness without alkalinity change and with Na_2CO_3 to increase total alkalinity without hardness change.

At the start of the experiment, six Nile tilapia juveniles were stocked in each outdoor tank (0.47 ± 0.03 g) for eight weeks. No water exchange was performed in the culture system and new water was added only to compensate for evaporation loss from the tanks.

Analytical grade EDTA, sodium carbonate (Na_2CO_3) and calcium chloride ($CaCl_2$) were used to manipulate the TA and CH/TA ratio of water to reach the desired values (Table 1). After the initial setup, the desired water quality in each experimental tank was maintained by fortnightly applications of Na_2CO_3 or $CaCl_2$. Over the trials, fish were fed the same commercial diet used during the acclimation period four times daily (8:00, 11:00 a.m., 13:00, and 16:00 p.m. hours), with feeding rates based on the stock biomass.

Experimental variables

The water quality variables monitored were temperature, pH, electrical conductivity (EC), total ammonia nitrogen (TAN), nitrite and reactive phosphorus. The first three variables (temperature, pH and EC) were measured twice daily at 8:00 a.m. and 16:00 p.m. hours; the last three variables (TAN, nitrite and reactive phosphorus) were measured fortnightly between 9:00 and 10:00 a.m. hours. Besides, it was also monitored the water dissolved oxygen twice per week at 08:00 hour. For this, the Winkler method with azide modification was used following APHA (1999).

Electronic equipments were used to monitor water temperature, pH and EC (Instrutherm digital thermometer, Marconi MA522 pH-meter, Lutron CD-4301 water conductivity meter, respectively). TAN (indophenol method), reactive phosphorus (ammonium molybdate method) and nitrite (diazotizing and coupling method) were analyzed according to standard methods (APHA, 1999). Growth performance variables observed were fish survival, final body weight, specific growth rate, yield, and feed conversion ratio.

Water quality and growth performance variables were analyzed by two-way ANOVA with TA (50 and 100 mg L^{-1} $CaCO_3$) and CH/TA ratio (1/2, 1/1 and 5/1) as the main factors. Tukey's test was used for pairwise comparisons of means.

The assumptions of normal distributions 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

Water quality

The TA and CH/TA ratio of the culture water have significantly affected the water quality (Table 2; $p < 0.05$).

The significantly higher value of water pH observed in the 1/2 CH/TA ratio tanks at 16h confirmed that water with TA > hardness can be stressful for fish culture if pH values higher than 9 are reached. It is generally accepted that freshwater fish such as tilapia grows better at waters with pH between 6.0 and 9.0 (BOYD; TUCKER, 1998).

Table 2. Water quality of 250 L polyethylene outdoor tanks stocked with six Nile tilapia juveniles exposed to different values of total alkalinity (TA) and calcium hardness (CH)/TA ratios for 8 weeks (mean ± S.D.; n = 6).

Variable	CH/TA ratio of water	TA (mg L^{-1} $CaCO_3$ eq.)	
		50	100
	1/2	6.97 ± 0.29 Aa[1]	7.30 ± 0.15 Ab
8 hour pH	1/1	6.98 ± 0.13 Aa	7.35 ± 0.12 Ab
	5/1	6.94 ± 0.16 Aa	7.18 ± 0.09 Bb
	1/2	7.47 ± 0.27 Aa	7.60 ± 0.20 Ab
16 hour pH	1/1	7.32 ± 0.17 Ba	7.49 ± 0.21 Bb
	5/1	7.29 ± 0.20 Ba	7.43 ± 0.16 Bb
	1/2	539 ± 44.5 Ba	676 ± 31.7 Cb
EC[2] (μS cm^{-1})	1/1	555 ± 33.6 Ba	753 ± 51.1 Bb
	5/1	783 ± 20.1 Aa	1567 ± 86.9 Ab
	1/2	0.664 ± 0.39 A	0.691 ± 0.40 A
Total ammonia N (mg L^{-1})	1/1	0.602 ± 0.39 B	0.544 ± 0.37 B
	5/1	0.601 ± 0.42 B	0.661 ± 0.47 AB
	1/2	0.334 ± 0.12 A	0.310 ± 0.11 A
NO2- (mg L^{-1})	1/1	0.285 ± 0.10 B	0.289 ± 0.10 B
	5/1	0.319 ± 0.11 A	0.320 ± 0.10 A
	1/2	0.166 ± 0.02 A	0.165 ± 0.02 B
Reactive phosphorus (mg L^{-1})	1/1	0.166 ± 0.02 A	0.163 ± 0.02 B
	5/1	0.170 ± 0.02 A	0.178 ± 0.02 A

Two-way ANOVA P						
Factor	8 a.m. pH	4 p.m. pH	8 a.m. EC	TAN	NO_2^-	Reactive P
Total alkalinity	<0.001	<0.001	<0.001	ns	ns	ns
H/A ratio	<0.001	<0.001	<0.001	0.009	0.005	<0.001
Alkalinity vs. H/A	0.003	ns[3]	<0.001	<0.05	ns	0.006

[1]For each variable, mean values in the same row or column that do not share the same lower case or capital letter, respectively, are statistically different (Tukey's test; $p < 0.05$); absence of letters indicates no statistical significance ($p > 0.05$). [2]Electrical conductivity at 8h; [3]Non-significant ($p > 0.05$).

When the TA of culture water is higher than its hardness, it is assumed that the lower concentration of soluble calcium relative to free carbonate ions in water prevents a major precipitation of $CaCO_3$ to the tank bottom caused by algal photosynthesis. In the present work, the color of the culture water in the outdoor tanks has become progressively greener till the end of the experiment. Calcium carbonate precipitation would be favorable to the culture environment because it would have prevented the pH from reaching high and dangerous levels at afternoons (LI et al., 2007).

In the present study, the maximum pH of water during the afternoon did not reach even higher values once the imbalance between the TA and CH was small. Furthermore, there were several cloudy days throughout the experimental period. In the 5/1 CH/TA ratio tanks, probably the excessive calcium had partially precipitated to the tank bottoms as calcium carbonate. This would have reduced the concentration of soluble carbonate in water and prevented the alkaline reaction between carbonate and water as follows: $CO_3^- + H_2O \rightarrow HCO_3^- + OH^-$ (BOYD, 2012).

The electrical conductivity (EC) of water has significantly increased as more $CaCl_2$ was added to increase the CH/TA ratio of water from 1/2 to 5/1. The EC of water can be used as an eutrophication index since nutrient-rich waters have higher concentrations of cations and anions, such as Ca^{2+}, Mg^{2+}, K^+, SO_4^{-2} and NO_3^- (AKKOYUNLU; AKINER, 2012). It is assumed that pond waters with EC up to 1000 μS cm^{-1} are suitable for fish culture (BOYD; TUCKER, 1998). Therefore, just the 5/1 CH/TA ratio tanks have exceeded this limit in the present work and a suitable environment (sufficient light) can develop undesirable algal blooms. Thus, an additional cause for the decline in fish growth observed in the 5/1 tanks could be the dissolved oxygen shortage overnight. The average dissolved oxygen concentration at 8:00h in the outdoor tanks was 3.5 ± 2.1 mg L^{-1} ranging between 6.5 mg L^{-1}, in the first experimental week, and 1.0 mg L^{-1}, in the last experimental week.

The CH/TA ratio has significantly affected the concentration of TAN. Significantly higher concentrations of TAN were recorded in the 1/2 CH/TA ratio tanks (Table 2). No significant differences in TAN were detected between the 1/1 and 5/1 CH/TA ratio tanks ($p > 0.05$). These results suggested that culture waters with unbalanced CH/TA ratios, such as 1/2 and 5/1, lead to higher TAN concentration relative to culture waters with a balanced CH/TA ratio (1/1). Under TAN stress, fish do not eat and digest feed well (SKOV et al., 2011). As a consequence, more feed is wasted in the tank or environment as non-fed ration and fish feces, and the increased amount of decaying organic matter in water raises the TAN concentration. Thus, adjusting the hardness/alkalinity ratio of water equal to one is an important tool for water quality management in order to indirectly control the levels of ammonia in the water.

Fish performance

The results of water quality presented above indicated that the 5/1 CH/TA ratio tanks had lower water pH and higher levels of EC relative to the corresponding results observed in the 1/2 CH/TA ratio tanks (Table 2). These adverse conditions probably worked additively to decrease fish growth in the 5/1 CH/TA ratio tanks as compared to the 1/2 CH/TA ratio tanks. In fact, the imbalance between hardness and alkalinity was much greater in the 5/1 CH/TA ratio tanks than in the 1/2 CH/TA ratio tanks. A better comparison between these two unbalanced CH/TA ratios of water would be 2/1 CH/TA ratio vs. 1/2 CH/TA ratio, for example. Therefore, minor imbalances of the CH/TA ratio of culture water upward or downward may not impact significantly the fish growth performance. On the other hand, major imbalances of the CH/TA ratio of culture water can cause severe negative effects on fish growth and feed efficiency.

In the present work, final body weight along with the specific growth rate (SGR) and yield results have clearly shown that the detrimental effects of the 5/1 CH/TA ratio of water (excessive calcium) on fish growth performance were stronger than those from the 1/2 CH/TA ratio (Table 3). Wurts and Stickney (1989) showed that calcium-activated proteins in fish gills control important passive and energy-dependent processes related to ionic metabolism (osmoregulation) in those animals. Townsend and Baldisserotto (2001) observed greater survival of silver catfish, *Rhamdia quelen*, fingerlings submitted to stressful water pH (acidic or alkaline) when the water hardness increases to 150 mg L^{-1} $CaCO_3$. Therefore, low waterborne calcium can impair fish osmoregulation and, consequently, growth performance, and harder waters may exert some protection against acidity and alkalinity in fish. On the other hand, excessive (and toxic) waterborne calcium seems to be also associated with osmotic impairment in fish (TOWNSEND et al., 2003). It is noteworthy that the calcium hardness of water increased close to 500 mg L^{-1} $CaCO_3$ in the present work, more than three times higher than the upper suitable limit for aquaculture (BOYD; TUCKER, 1998). Molokwu and Okpokwasili (2002) found that very soft (hardness < 10 mg L^{-1} $CaCO_3$) and very hard waters (hardness > 300 mg L^{-1} $CaCO_3$) impair *Clarias gariepinus*' egg incubation and larval rearing. The same response pattern was observed by Silva et al. (2003) in a study on *Rhamdia quelen*.

Table 3 Growth performance of Nile tilapia juveniles exposed to different values of total alkalinity (TA) and calcium hardness (CH)/TA ratios for 8 weeks. Six fish 250 L outdoor tank^{-1}. Initial body weight = 0.47 ± 0.03 g (mean ± S.D.; n = 6).

Variable	CH/TA ratio	Total alkalinity (mg L^{-1} $CaCO_3$ eq.) 50		Total alkalinity 100	
Survival (%)	1/2	86.1 ± 6.8		83.3 ± 14.9	
	1/1	88.9 ± 8.6		83.3 ± 0.0	
	5/1	86.1 ± 6.8		80.6 ± 6.8	
Final body weight (g)	1/2	11.02 ± 0.69 B[1]		11.15 ± 0.63 B	
	1/1	12.62 ± 0.48 A		12.88 ± 1.04 A	
	5/1	9.95 ± 0.83 C		9.81 ± 0.42 C	
SGR[2] (% BW day^{-1})	1/2	5.62 ± 0.13 B		5.70 ± 0.15 B	
	1/1	5.83 ± 0.12 A		5.93 ± 0.21 A	
	5/1	5.46 ± 0.21 C		5.40 ± 0.14 C	
Yield (g m^{-3})	1/2	227.4 ± 17.8 B		221.6 ± 33.1 B	
	1/1	268.9 ± 23.2 A		257.5 ± 20.8 A	
	5/1	205.3 ± 18.4 C		189.6 ± 17.9 C	
FCR[3]	1/2	1.49 ± 0.13 B		1.48 ± 0.18 B	
	1/1	1.27 ± 0.08 A		1.26 ± 0.13 A	
	5/1	1.51 ± 0.23 B		1.55 ± 0.15 B	
Two-way ANOVA P					
Factor	Surv	FBW	SGR	Yield	FCR
Total alkalinity	ns[4]	ns	ns	ns	ns
H/A ratio	ns	<0.001	<0.001	<0.001	<0.001
Alkalinity vs. H/A	ns	ns	ns	ns	ns

[1]For each variable, mean values in the same row or column that do not share the same lower case or capital letter, respectively, are statistically different (Tukey's test; $p < 0.05$); absence of letters indicates no statistical difference ($p > 0.05$). [2]Specific growth rate (% body weight per day) = [(ln final body weight - ln initial body weight)/rearing days] x 100. [3]Feed conversion ratio = weight of feed offered (g)/fish weight gain (g). [4]Non-significant ($p > 0.05$).

In closed and semi-closed aquaculture systems, such as the ones used in the present study, alkalinity tends to decrease over time whereas hardness tends to increase over time. In the first case, oxidative chemical and biological processes that occur in water, such as bacterial nitrification, release H^+ ions to water decreasing thereby its alkalinity. In the latter case, the continuous input of calcium through the supply of feeds to fish increases the hardness of water (HARGREAVES, 2006; POLEO et al., 2011). Therefore, the combined effect of alkalinity decrease and hardness increase over time results in a progressive increase of the CH/TA ratio. As a result, CH/TA ratios of water < 1 tend to become more balanced. On the other hand, CH/TA ratios > 1 tend to become even more unbalanced over time. Depending on the degree of alkalinity removal and calcium input, those effects could significantly change the initial CH/TA ratio of water and affect fish growth performance.

Significantly better food conversion ratio (FCR) results were seen in the 1/1 CH/TA ratio tanks regardless of the level of TA (50 and 100 mg L^{-1} $CaCO_3$; Table 3). Andrade et al. (2007) have not found clear differences on silver catfish growth performance when the juveniles were reared at 30, 80 or 130 mg L^{-1} $CaCO_3$. Contrarily, *Prochilodus lineatus* and Nile tilapia larvae have grown more when subjected to 32 mg L^{-1} $CaCO_3$ than to 15 or 55 mg L^{-1} $CaCO_3$ (ROJAS et al., 2001; ROJAS; ROCHA, 2004). The differences between those researches could be explained by the fish age. The present work and that from Andrade et al. (2007) have employed juvenile fish; those from Rojas et al. (2001) and Rojas and Rocha (2004) have employed fish larvae. Therefore, fish seems to be sensitive to water alkalinity only during larval phase. Afterwards, water hardness is probably more important than water alkalinity in producing significant effects on fish growth. Nevertheless, fish can also modulate the mechanism of calcium uptake to achieve a normal status of calcium in the body (HWANG et al., 1996). Hence, significant effects of water hardness on fish growth would be seen only in very soft or very hard waters.

The differences for FCR between fish in the 1/2 and 5/1 CH/TA ratio tanks were not significant ($p > 0.05$). Therefore, aside from assessment of which unbalanced CH/TA ratio of water was worse (1/2 or 5/1), our results evidenced that the best CH/TA ratio of water was 1/1. It was also clear that the 5/1 CH/TA ratio (calcium hardness between 250 - 500 mg L^{-1} $CaCO_3$ and TA between 50 - 100 mg L^{-1} $CaCO_3$) had negatively impacted fish growth and feed efficiency.

Conclusion

In summary, the 1/1 CH/TA ratio of water has simultaneously prevented the afternoon pH increase caused by CH/TA ratios < 1 and probably caused no osmotic stress to the fish, as elicited by CH/TA ratios > 1. These data also indicated the importance of regulating absolute values of water hardness and alkalinity between 20 - 200 mg L^{-1} $CaCO_3$ eq.

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