



Acta Scientiarum. Technology

ISSN: 1806-2563

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Universidade Estadual de Maringá
Brasil

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Acta Scientiarum. Technology, vol. 34, núm. 2, abril-junio, 2012, pp. 177-183

Universidade Estadual de Maringá
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Single or paired increase of total alkalinity and hardness of water for cultivation of Nile tilapia juveniles, *Oreochromis niloticus*

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ABSTRACT. The present work aimed at evaluating the effects of single or paired increase of water's total alkalinity (TA) and total hardness (TH) on the performance of Nile tilapia juveniles' growth and culture water quality. Twenty five 25-L outdoor polyethylene aquaria were used to hold experimental fish (0.82 ± 0.06 g; 10 fish per aquarium) for 6 weeks. There were two conditions of TA (low or high) and of TH (moderate or high) in the culture water, obtained by the application of different salts (CaCO_3 , Na_2CO_3 and CaSO_4) upon a previously acidified water, all at the same rate. Water quality and growth performance variables were observed in each replicate. The acidification of the supply water with HCl has resulted in significantly lower final body weight ($p < 0.05$). Except for the Na_2CO_3 , growth performance of tilapia has improved significantly after CaCO_3 liming or CaSO_4 application ($p < 0.05$) and no significant difference was detected between these last two fish groups ($p > 0.05$). It was concluded that beyond a minimum level of TA ($\geq 20 \text{ mg L}^{-1} \text{ CaCO}_3$) and TH ($\geq 20 \text{ mg L}^{-1} \text{ CaCO}_3$), it is also important that fish culture waters have a TH/TA ratio higher than 1.

Keywords: calcium carbonate, calcium sulfate, fish culture, liming, sodium carbonate.

Aumento isolado ou combinado da alcalinidade e dureza da água de cultivo de juvenis de tilápia do Nilo, *Oreochromis niloticus*

RESUMO. O presente trabalho teve como objetivo avaliar os efeitos do aumento isolado ou combinado da alcalinidade total (AT) e da dureza total (DT) da água no desempenho produtivo de juvenis de tilápia do Nilo e na qualidade da água de cultivo. Vinte e cinco aquários de polietileno de 25 litros foram utilizados, cada um contendo dez juvenis de tilápia ($0,82 \pm 0,06$ g), que foram cultivados durante o período de seis semanas. Havia duas condições de AT (baixa ou alta) e duas de DT (moderada ou alta) que foram obtidas a partir da aplicação de diferentes sais (CaCO_3 , Na_2CO_3 and CaSO_4) em água previamente acidificada com HCl, todos na mesma taxa. Após seis semanas, os peixes mantidos em água acidificada apresentaram peso corporal significativamente menor que o observado no grupo controle ($p < 0,05$). Exceto pelo Na_2CO_3 , o desempenho produtivo da tilápia melhorou significativamente após a aplicação de CaCO_3 ou CaSO_4 na água ($p < 0,05$), não havendo diferença significativa entre esses dois últimos grupos ($p > 0,05$). Concluiu-se que além de níveis mínimos de AT ($\geq 20 \text{ mg L}^{-1} \text{ CaCO}_3$) e DT ($\geq 20 \text{ mg L}^{-1} \text{ CaCO}_3$), é também importante que a água de cultivo de peixes apresente relação DT/AT maior que um.

Palavras-chave: carbonato de cálcio, sulfato de cálcio, piscicultura, calagem, carbonato de sódio.

Introduction

The total alkalinity of the water comprises mainly its content of bicarbonates and carbonates. This is an important water quality indicator because reflects the buffering capacity of water to pH changes. Waters with moderate to high alkalinity are better for fish culture because they suffer minor changes of water pH over the day and over the entire production cycle. In that condition, fish are not frequently exposed to acidic or alkaline stress which may impair its growth rate and make it susceptible to disease (NTENGWE; EDEMA, 2008).

The total hardness of water refers to its content of soluble Ca^{2+} and Mg^{2+} . These ions are important for ionic regulation of freshwater fish because they affect the permeability of biological membranes and may lead to significant losses of water and ions when at inadequate concentrations in the body. Besides that, several other important physiological functions require Ca^{2+} for normal development. Although some Ca^{2+} can be obtained from the diet, the direct absorption by the gills from the water is the main source of Ca^{2+} for fish (SILVA et al., 2003).

In units with low water exchange, liming can be successfully carried out to increase water pH and

alkalinity. Moreover, when liming is performed by using limestone (calcium carbonate) it also increases the calcium concentration of water (calcium hardness). Calcium is an essential element for fish growth and health, and a minimum concentration in water is desirable to get good growth performance indices. In general, culture waters with pH below 7.0 and total alkalinity below 50 mg L⁻¹ will get benefits by liming (QUEIROZ et al., 2004).

Currently, the standard material to lime fish ponds is agricultural limestone (CaCO₃) (THUNJAI et al., 2004). However, as CaCO₃ increases the water alkalinity and hardness simultaneously, it is not possible to know which factor was responsible for the observed results, if alkalinity, hardness or both. This knowledge is important because it would allow more precise corrections of alkalinity and/or hardness of fish culture water and, as a result, better growth performance results. Therefore, the present study aimed to evaluate the effects of single or paired increase of water total alkalinity and hardness on the growth performance of juveniles of Nile tilapia, *Oreochromis niloticus*, and culture water quality.

Material and methods

Fish and experimental system

One thousand sex reversed Nile tilapia, *Oreochromis niloticus*, fingerlings (0.55 ± 0.04 g) were obtained from the Centro de Pesquisas em Aquicultura of the Departamento Nacional de Obras Contra as Secas (DNOCS) in Pentecoste, Ceará State, Brazil (3°47'40.49"S; 39°16'36.07"W). Fish were transported in one plastic bag with water (1/3) and pure oxygen (2/3), taken to the Laboratório de Ciência e Tecnologia Aquícola – LCTA (Departamento de Engenharia de Pesca, Centro de Ciências Agrárias, Universidade Federal do Ceará, Fortaleza, Ceará, Brazil). In the lab, fish was initially stocked in one 1000-L fiberglass tank (reception tank) which was served by 24-h forced air through small air pumps, silicon pipes and air diffusers.

Fish stayed in the reception tank for one week, in order to acclimatize to the laboratory conditions. Forty eight hours after stocking, fish were treated with analytical grade potassium permanganate at 4 mg L⁻¹ for 48 hours to prevent bacterial infestation. After this period, analytical grade sodium thiosulfate was used at 4 mg L⁻¹ to neutralize the residual effect of potassium permanganate. Over the acclimatization period, fish fed a 45% protein commercial diet in four daily meals at 8 a.m., 11 a.m., 1 p.m. and 4 p.m. The daily feeding rate was equal to 10% of the stock biomass.

Twenty five polyethylene aquaria of 25 L were used to hold experimental fish. The aquaria were set in the lab's outdoor area which exposes the aquaria directly to the sun. The aquaria were served by non-stop aeration and had a cotton mesh cover to prevent fish escape. At the onset of the experiment, ten fingerlings (0.82 ± 0.06 g; 3.6 ± 0.52 cm) were stocked in each 25-L polyethylene aquarium. Pooled fish body weight was recorded for each aquarium as well as the total body length of ten randomly selected individuals. During the first week, dead fish found in aquaria were replaced for others from the reception tank with similar body weight and length. Fish were maintained in the experimental systems for 6 weeks.

Experimental design and husbandry

In the present work, two experimental factors were evaluated simultaneously in a 2 x 2 factorial design. There were two conditions of TA_ total alkalinity (low or high) and two of TH_ total hardness (moderate or high) in the culture water. Tap water was used as supply water after its residual chlorine had been removed by aeration and resting. Firstly, the supply water in four 500-L tanks was acidified with concentrated analytical grade HCl at 1.5 mL per 20 L. After achieving chemical equilibrium, 48h later, the acidified water in three of the four 500-L tanks was treated with one of the following analytical grade salts: CaCO₃, Na₂CO₃ and CaSO₄. The same application rate (2 g per 10 L) was used for all products. Depending on the product used, there was or not an increase of water TA and TH. The ratio between the TH and TA of water was calculated (TH/TA) (Table 1).

Table 1. Total alkalinity (TA), total hardness (TH) and TH/TA ratio from the water for the cultivation of Nile tilapia fingerlings, *Oreochromis niloticus*, submitted to different managements. Fish (0.82 ± 0.06 g and 3.6 ± 0.52 cm) were stocked in 25-L plastic outdoor aquaria at 10 fish per aquarium for 6 weeks (mean ± s.d., n = 5).

| Water management ¹ | Total alkalinity (mg CaCO ₃ L ⁻¹) | Total hardness (mg CaCO ₃ L ⁻¹) | TH/TA ³ | Water classification |
|--|--|--|--------------------|------------------------------------|
| None | 30.8 ± 1.08 c ² | 54.2 ± 2.60 c | 1.76 | Control |
| HCl acidification | 9.2 ± 1.72 c | 58.7 ± 1.69 c | 6.38 | Low alkalinity, moderate hardness |
| CaCO ₃ liming | 73.0 ± 2.27 b | 117.2 ± 4.56 b | 1.61 | High alkalinity, high hardness |
| Na ₂ CO ₃ liming | 90.4 ± 2.65 a | 55.5 ± 1.60 c | 0.61 | High alkalinity, moderate hardness |
| CaSO ₄ application | 15.5 ± 1.46 d | 149.1 ± 4.67 a | 9.62 | Low alkalinity, high hardness |
| ANOVA ⁴ | < 0.001 | < 0.001 | | |

¹Dechlorinated tap water was initially acidified with concentrated analytical grade HCl at 1.5 mL per 20 L; afterwards, to the acidified water was applied one of the following analytical grade salts (CaCO₃, Na₂CO₃ or CaSO₄) at the same rate, i.e., 2 g per 10 L;

²Mean values in the same column which do not share a same letter are statistically different by the Tukey's test. ³Total hardness (TH): total alkalinity (TA) ratio.

No fertilization of water was performed in the tanks. Nevertheless, except for the aquaria treated with HCl, a significant development of phytoplankton was observed in aquaria over the experiment. Five aquaria were randomly designed to each control or treatment group. Initially, all aquaria were filled with tap water. In the second, third and fourth days after fish stocking, a specific volume of water in aquaria was replaced by the designed treated water at 1/3, 2/3 and 3/3, respectively. From the fourth day to the end, weekly exchanges of bottom water were carried out at 1/3 in order to clean the aquarium bottom and maintain the designed physical and chemical characteristics of the experimental waters. One phytoplankton inoculation was performed in all aquaria. For this purpose, two hundred and fifty milliliters of dark green water taken from fish tanks of a nearby fish culture station was poured into each aquarium at the 5th experimental day. It was noted that the outdoor waters got a strong green color just four days after inoculation.

All stocked fish were fed in the same frequency with the artificial diet used during the acclimatization period. The feeding rates employed were 15.0, 12.5 and 10.0% of the stocked biomass in the weeks 1-2, 3-4 and 5-6, respectively. The amount of feed allowed to each aquarium was adjusted fortnightly after fish body weightings.

Experimental variables and analytical procedures

Water quality and growth performance variables were examined in the present work. The water pH, TA, TH, free CO₂ and total ammonia were monitored weekly in all aquaria. Besides, water temperature, pH, TA and free CO₂ in the 24-h cycle were recorded hour-to-hour in two different days. The water pH was measured by using a portable pH meter (Tecnopon mPA210 P). The water temperature was observed by the use of a digital hand thermometer (Instrutherm TE-300). The analytical determinations of TA, TH, free CO₂ and total ammonia were carried out according to APHA (1999). Weekly, a 1 L-water sample was taken from each aquarium to perform the limnological analyses. Fish final body weight and length, survival, specific growth rate (SGR) and feed conversion rate (feed allowance/fish weight gain) were observed in all repetitions. SGR was calculated as follows: $[(\ln \text{ final body weight} - \ln \text{ initial body weight}) / \text{days of culture}] \times 100$.

Statistical analyses

The water quality and growth performance results were subjected to the one-way and two-way ANOVA, respectively, to detect if there were significant differences between the experimental groups. When the differences were significant, the

means were compared two-by-two using the Tukey's test. The statistical analyses were performed with the aid of the Sigma Stat 2.0 software (Jandel Statistics). The level of significance of 5% was adopted in all statistical analyses.

Results and discussion

Water quality

On average, the temperature of water in aquaria was $27.9 \pm 2.5^\circ\text{C}$, ranging between 24.6 and 35.0°C. No significant differences were detected between the treatments for water temperature ($p > 0.05$).

The acidification of the supply water with HCl has produced a significant drop in the pH of water and an increase in the concentration of free CO₂ ($p < 0.05$). When the different salts (CaCO₃, Na₂CO₃ and CaSO₄) were applied to the acidified water, the effect on pH and free CO₂ has varied significantly between the treatments (Table 2; $p < 0.05$). The application of Na₂CO₃ on the acidified water has produced the strongest effect on pH and free CO₂, followed by CaCO₃ and CaSO₄. Carbonates have an alkaline reaction with water ($\text{CO}_3^{2-} + \text{H}_2\text{O} \rightarrow \text{HCO}_3^- + \text{OH}^-$) which explains the pH increase. The reduction in the concentration of free CO₂ in water by CaCO₃ or Na₂CO₃ liming can be explained by the following reactions: $\text{Na}_2\text{CO}_3 + \text{CO}_2 + \text{H}_2\text{O} \rightarrow 2\text{Na}^+ + 2\text{HCO}_3^-$ and $\text{CaCO}_3 + \text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{Ca}^{2+} + 2\text{HCO}_3^-$ (BOYD, 1979).

Table 2. pH, free CO₂ and total ammonia from the water for the cultivation of Nile tilapia fingerlings, *Oreochromis niloticus*, subjected to different managements. Fish (0.82 ± 0.06 g and 3.6 ± 0.52 cm) were stocked in 25-L plastic outdoor aquaria at 10 fish per aquarium for 6 weeks (mean \pm s.d., $n = 5$).

| Water management ¹ | pH | Free CO ₂ (mg L ⁻¹) | NH ₃ -NH ₄ ⁺ (mg L ⁻¹) |
|--|-------------------------------|---|--|
| None | 7.5 ± 0.13 c ² | 3.2 ± 0.36 b | 0.40 ± 0.04 b |
| HCl acidification | 6.0 ± 0.11 e | 6.0 ± 1.92 a | 0.99 ± 0.09 a |
| CaCO ₃ liming | 7.8 ± 0.10 b | 1.7 ± 0.67 c | 0.52 ± 0.11 b |
| Na ₂ CO ₃ liming | 8.3 ± 0.03 a | 0.7 ± 0.30 d | 0.40 ± 0.14 b |
| CaSO ₄ application | 6.7 ± 0.14 d | 5.4 ± 0.99 a | 0.52 ± 0.13 b |
| ANOVA P | < 0.001 | < 0.001 | < 0.001 |

¹ Dechlorinated tap water was initially acidified with concentrated analytical grade HCl at 1.5 mL per 20 L; afterwards, one of the following analytical grade salts was applied on the previously acidified water at the same rate (2 g per 10 L): CaCO₃, Na₂CO₃ or CaSO₄; ² Mean values in the same column which do not share a same letter are statistically different by the Tukey's test ($p < 0.05$).

The stronger effect of Na₂CO₃ on alkalinity, pH and CO₂ might suggest that Na₂CO₃ is a better product than CaCO₃ to correct those variables of water quality. While Na₂CO₃ is soluble in water, CaCO₃ has a poor solubility (TEIR et al., 2007). However, CaCO₃ as agricultural limestone is the product most frequently used both for agriculture (PEIXOTO et al., 1998) and aquaculture liming (QUEIROZ et al., 2004). In aquaculture, the reasons for the use of agricultural limestone (CaCO₃) instead of Na₂CO₃ as the standard product

for liming are (1) the increase of both water alkalinity and hardness carried out by CaCO_3 liming and not by Na_2CO_3 and (2) the lower cost of CaCO_3 . When the alkalinity of water is much greater than hardness, as could be elicited by Na_2CO_3 liming, the pH of water in ponds with dense algal communities can reach 11 or more in the middle afternoon (BOYD, 1979). Therefore, although the application of Na_2CO_3 in water or sediments increases alkalinity and pH more rapidly, CaCO_3 is a safer product for liming fish ponds than Na_2CO_3 .

Since CaSO_4 (agricultural gypsum) is more soluble in water than CaCO_3 (ERNANI et al., 2001), it increases water hardness more than CaCO_3 . However, CaSO_4 is at least two times more expensive than CaCO_3 . Thus, Na_2CO_3 was superior to CaCO_3 for raising the TA and pH of water and for lessening its concentration of free CO_2 ; and CaSO_4 was better than CaCO_3 for raising the TH of water. Perhaps a blend of Na_2CO_3 and CaSO_4 would be a very interesting product to lime fish ponds because it would combine the power of Na_2CO_3 to increase water alkalinity and the power of CaSO_4 to increase water hardness. Nevertheless, economic questions should also be considered to better evaluate this possibility because, as noted previously, Na_2CO_3 and CaSO_4 are products more expensive than CaCO_3 .

The application of CaCO_3 , Na_2CO_3 or CaSO_4 on the acidified water has reduced the concentration of total ammonia (Table 2). The higher concentration of total ammonia in the acidified water can be explained by its lower algal density. Beside the process of bacterial nitrification, the absorption of ammonia by microalgae is one of the main ammonia sinks in aquatic environments (BECKER, 1994). Therefore, the treatment of acidic waters with CaCO_3 , Na_2CO_3 or CaSO_4 has produced an indirect reduction in the concentration of total ammonia in water by creating a better environment for algal development. Another possibility is the co-precipitation of the organic matter to the aquaria bottom along with CaCO_3 , Na_2CO_3 or CaSO_4 . Lin and Yi (2003) have applied calcium hydroxide in Nile tilapia's ponds to precipitate phosphorus and organic matter to the sediments and, therefore, to clean the water from these pollutants.

Although the general pattern of alkalinity and pH variation over the 24-h cycle has been the same for the HCl, CaCO_3 , Na_2CO_3 and CaSO_4 aquaria, the ranges for these two variables were remarkably different. In the present work, the pH of the HCl aquaria ranged between 5.8 and 7.1 over the 24-h cycle. This was the highest variation of pH observed among the aquaria and reflects the lower alkalinity of the acidified water ($9.2 \pm 1.72 \text{ mg CaCO}_3 \text{ L}^{-1}$) when compared to the others treatments. Interestingly, the lowest variation of pH over the

24-h cycle was not observed in the aquaria with the highest mean TA (Na_2CO_3 aquaria; $\text{TA} = 90.4 \pm 2.65 \text{ mg CaCO}_3 \text{ L}^{-1}$), but in CaCO_3 aquaria. Among all treatments, Na_2CO_3 was the only one with TH lower than TA (TH/TA ratio = 0.61) (Table 1). The TH/TA ratio for CaCO_3 aquaria was 1.61. When the hardness of water is lower than alkalinity its pH may increase very much at the end of afternoon due to the lack of CaCO_3 precipitation. The free CO_3^{2-} in waters with TH/TA ratio lower than 1 can cause an expressive increase in the pH of water as follows: $\text{CO}_3^{2-} + \text{H}_2\text{O} \rightarrow \text{HCO}_3^- + \text{OH}^-$ (BOYD, 1979). Therefore, beside a minimum level of TA ($\geq 20 - 30 \text{ mg CaCO}_3 \text{ L}^{-1}$), it is also important that fish culture waters have TH/TA ratio higher than 1 to prevent great variations of pH over the 24-h cycle.

Fish growth performance

The final survival of fish was not significantly different between the treatments and the control water (Table 3; $p > 0.05$). On average, the final survival of tilapia was $88.3 \pm 9.63\%$.

The acidification of the supply water with HCl has resulted in significantly lower tilapia's final body weight, final body length and SGR (Table 3; $p < 0.05$). After acidification, the indicators of water quality have changed as follows: pH and TA have decreased from 7.5 ± 0.13 to 6.0 ± 0.11 and from 30.8 ± 1.08 to $9.2 \pm 1.72 \text{ mg CaCO}_3 \text{ L}^{-1}$, respectively; the concentrations of free CO_2 and total ammonia have increased from 3.2 ± 0.36 to $6.0 \pm 1.92 \text{ mg L}^{-1}$ and from 0.40 ± 0.04 to $0.99 \pm 0.09 \text{ mg L}^{-1}$, respectively. On the other hand, the TH of the supply water was not significantly affected by the acidification ($p > 0.05$). Therefore, the factors responsible for the poor growth of fish stocked in the HCl-aquaria might be the lower pH and TA of water, and/or higher concentrations of free CO_2 and total ammonia. Ammonia level was probably not the cause for fish mortality, because in low pH waters the major proportion of ammonia is in its non-toxic form NH_4^+ (MIRON et al., 2008). Similarly, although the mean concentration of free CO_2 in HCl-aquaria had been higher than the observed in the supply water, it is still acceptable for fish growth (BOYD; TUCKER, 1998). Thus, by exclusion, the causes for fish impairment after water acidification have probably been the low rearing water's pH and/or the alkalinity. As a matter of fact, the low alkalinity of the acidified water has produced the greatest variation of pH over the 24-h cycle (5.80 – 7.12), as noted before. Moreover, the mean pH of the acidified water (6.0 ± 0.11) is below the minimum appropriate level for aquaculture (6.5) (BOYD; TUCKER, 1998). Thus, fish in HCl-aquaria have been subjected to more stress by pH than the individuals in the other treatments.

Table 3. Growth performance of Nile tilapia fingerlings, *Oreochromis niloticus*, reared in waters with different values of total alkalinity and total hardness. Fish (0.82 ± 0.06 g and 3.6 ± 0.52 cm) were stocked in 25-L plastic outdoor aquaria at 10 fish per aquarium for 6 weeks (mean \pm s.d.; n = 5).

| Variable | Experimental water ¹ | | | Control water ⁴ | P of Student's <i>t</i> test ⁵ |
|---|---------------------------------|-----------------|---------------------------|----------------------------|---|
| | TA ² | TH ³ | | | |
| | | Moderate | High | | |
| Final body weight (g) | Low | 3.7 ± 0.24 a* | 4.7 ± 0.21 b ⁶ | 4.7 ± 0.31 | < 0.001 |
| | High | 3.8 ± 0.07 a* | 4.8 ± 0.21 b | | |
| Final body length (cm) | Low | 5.4 ± 0.14 Aa* | 6.6 ± 0.32 Ab | 6.4 ± 0.34 | < 0.001 |
| | High | 6.3 ± 0.27 Ba | 6.6 ± 0.11 Aa | | |
| Survival (%) | Low | 87.5 ± 4.33 | 88.0 ± 8.37 | 88.0 ± 13.04 | ns |
| | High | 82.0 ± 10.95 | 96.0 ± 5.48 | | |
| SGR ⁷ (% BW day ⁻¹) | Low | 3.61 ± 0.30 a* | 4.20 ± 0.15 b | 4.12 ± 0.14 | < 0.001 |
| | High | 3.70 ± 0.14 a* | 4.20 ± 0.26 b | | |
| FCR ⁸ | Low | 3.17 ± 1.26 a | 2.05 ± 0.13 b | 2.03 ± 0.15 | 0.012 |
| | High | 2.64 ± 0.27 a* | 1.88 ± 0.15 b | | |
| <i>Two-way ANOVA P</i> | | | | | |
| Factor | Weight | Length | Survival | SGR | FCR |
| Alkalinity | ns ⁹ | < 0.001 | ns | ns | ns |
| Hardness | < 0.001 | < 0.001 | 0.052 | < 0.001 | 0.005 |
| Alk x hard | ns | < 0.001 | ns | ns | ns |

¹ Low alkalinity and moderate hardness water: dechlorinated tap water acidified with concentrated analytical grade HCl at 1.5 mL per 20 L; low alkalinity and high hardness water: previously acidified water treated with analytical grade CaSO₄ at 2 g per 10 L; high alkalinity and moderate hardness water: acidified water limed with analytical grade Na₂CO₃ at 2 g per 10 L; high alkalinity and high hardness water: acidified water limed with analytical grade CaCO₃ at 2 g per 10 L; ² TA = total alkalinity; "Low alkalinity" stands for waters with TA ranging from 9.2 up to 15.5 mg CaCO₃ L⁻¹; "high alkalinity" stands for waters with TA ranging from 73.0 up to 90.4 mg CaCO₃ L⁻¹; ³ TH = total hardness; "Moderate hardness" stands for waters with TH ranging from 55.5 up to 58.7 mg CaCO₃ L⁻¹; "high hardness" stands for waters with TH ranging from 117.2 up to 149.1 mg CaCO₃ L⁻¹; ⁴ Dechlorinated tap water (TA = 30.8 ± 1.08 mg CaCO₃ L⁻¹ and TH = 54.2 ± 2.60 mg CaCO₃ L⁻¹); ⁵ Refers to the comparisons between the experimental waters and the control water; ⁶ For a same variable, mean values not sharing a same lower case or capital letter in a row and column, respectively, are significantly different by the Tukey's test ($p < 0.05$); mean values with an asterisk are significantly different from the control water by the Student's *t* test; ⁷ Specific growth rate (SGR) = $[(\ln \text{final body weight} - \ln \text{initial body weight}) / n^{\circ} \text{ of days}] \times 100$; ⁸ Feed conversion rate = feed allowance (g)/fish weight gain (g); ⁹ Non significant ($p > 0.05$).

When CaCO₃ was applied on the acidified water, the mean pH and TA of water have increased to 7.8 ± 0.10 and 73.0 ± 2.27 mg CaCO₃ L⁻¹ respectively. Besides, the water's TH and TH/TA ratio have changed from 58.7 ± 1.69 to 117.2 ± 4.56 mg CaCO₃ L⁻¹; and from 6.38 to 1.61, respectively (Table 1). In this case, the fish's final body weight, SGR and FCR have been significantly improved (Table 3; $p < 0.05$). The same positive effect of CaCO₃ (in fact CaMgCO₃) liming on fish's FCR was also observed by Gomes and Silva (2009) in a study on tambaqui (*Colossoma macropomum*). Additionally, the CaCO₃ liming has turned the pH adequate for aquaculture. Besides, the alkalinity of water after CaCO₃ liming has allowed the lowest variation of pH over the 24-h cycle.

The liming of the acidic water with Na₂CO₃ has produced a strong increase in pH and alkalinity to 8.3 ± 0.03 and 90.4 ± 2.65 mg CaCO₃ L⁻¹, respectively. On the other hand, the TH has been insignificantly changed after Na₂CO₃ liming from 58.7 ± 1.69 to 55.5 ± 1.60 mg CaCO₃ L⁻¹ ($p < 0.05$). The TH/TA ratio of water has decreased from 6.38 to 0.61 (Table 1). Nevertheless, the final body weight, SGR and FCR of fish have not been significantly affected by Na₂CO₃ liming, remaining reduced as well as those results observed in the HCl-aquaria (Table 3; $p > 0.05$). In this case, although the pH and TA of water had been corrected to more adequate levels for fish culture, the TH/TA ratio of the water turned to be lower than 1. In this situation, the pH may rise rapidly in the late afternoon due to photosynthesis because a

greater amount of free CO₃²⁻ remains in solution when the TH/TA ratio of water is below 1 (BOYD, 1979). The consequence is a high release of OH⁻ to water. Conversely, when the TH/TA ratio of water is above 1, part of the CO₃²⁻ is precipitated to the bottom and does not form OH⁻. This fact is intensified by Na₂CO₃ due to its high water solubility. Therefore, as important as the absolute values of TA and TH of water for fish culture, it is also critical to consider the TH/TA ratio of water. This ratio must be equal or higher than 1 to achieve good results of fish growth performance. On the other hand, considering only the TH/TA ratio of water is also inadequate because the HCl-aquaria had a TH/TA ratio of 6.38 and the tilapia's growth had been poor. In this way, the following factors should be considered together to correctly evaluate the quality of water for fish culture: pH, alkalinity, hardness and the TH/TA ratio of water.

The difference in the growth performance between tilapia reared in CaSO₄-aquaria or CaCO₃-aquaria was not statistically significant (Table 3; $p > 0.05$). Among the four variables of water quality focused in the present work, i.e., pH, alkalinity, hardness and TH/TA of water, only the TA of CaSO₄-aquaria (15.5 ± 1.46 mg CaCO₃ L⁻¹) has remained outside the appropriate range for aquaculture (20 – 150 mg CaCO₃ L⁻¹) (BOYD; TUCKER, 1998). In spite of that, tilapia stocked in the CaSO₄-aquaria had grown well and reached, as noted previously, results similar to those found in the CaCO₃-aquaria. In this last case, the TA of water was 73.0 ± 2.27 mg CaCO₃ L⁻¹. Therefore, Nile

tilapia juveniles seem to tolerate well rearing waters with lower TAs ($> 15.0 \text{ mg CaCO}_3 \text{ L}^{-1}$).

Although the variation of water pH over the 24-h cycle has been slightly greater in the CaSO_4 -aquaria, when compared to the CaCO_3 -aquaria, this fact was not capable to significantly inhibit fish growth ($p > 0.05$). On the other hand, the TH of water treated with CaSO_4 was the highest one among all treatments ($149.1 \pm 4.67 \text{ mg CaCO}_3 \text{ L}^{-1}$ and TH/TA ratio = 9.62). Thus, no advantage for fish growth was produced by the greater TH of water in the CaSO_4 -aquaria, in comparison to the CaCO_3 -aquaria. Therefore, the amount of calcium in the CaSO_4 -aquaria, although not detrimental to phytoplankton and fish growth, was probably excessive. Liming the acidified water with CaCO_3 has resulted in a more balanced relationship between TA and TH (TH/TA ratio = 1.6) than the carried out by the CaSO_4 application (TH/TA ratio = 9.6).

In the present study, except for the results of fish body length, no significant effect on tilapia's growth performance was observed by the increase in total alkalinity of water without expressive change in the water's total hardness (Table 3; $p > 0.05$). The same was observed by Soderberg et al. (2000) in a study on walleye, *Stizostedion vitreum*. On the other hand, the increase of total hardness without raise of total alkalinity has produced significantly better indices of growth performance ($p < 0.05$). These results suggest that the increase in total hardness, as elicited by CaCO_3 and CaSO_4 application, is more important for tilapia growth than only the increase of total alkalinity of the water, as elicited by Na_2CO_3 . Therefore, the total hardness of water seems to be a better index to evaluate the water quality of tilapia ponds than the total alkalinity.

Conclusion

In summary, it can be concluded that beyond a minimum level of total alkalinity ($\text{TA} \geq 20 \text{ mg L}^{-1} \text{ CaCO}_3$) and total hardness ($\text{TH} \geq 20 \text{ mg L}^{-1} \text{ CaCO}_3$), it is also important that fish culture waters have a TH/TA ratio higher than 1 to prevent harmful pH variations over the 24-h cycle and the entire culture period. Hence, just the increase of total alkalinity with no adjustment of hardness of the water, as accomplished by Na_2CO_3 liming, may be harmful for the aquatic life. On the other hand, the single increase of the total hardness of water with no correction of its alkalinity, as performed by CaSO_4 application, is harmless and may even bring positive effects to primary productivity and fish growth. Finally, the paired increase of water total alkalinity

and total hardness, as obtained by CaCO_3 liming, combines the benefits of these two water quality indicators, and, as a result, is a safer management to be regularly performed in fish culture units.

Acknowledgements

The authors would like to express their gratitude to Mr. Pedro Eymard from Departamento Nacional de Obras Contrastes Secas – DNOCS (Pentecoste, Ceará), for the Nile tilapia fingerlings kindly donated.

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Received on December 16, 2010.

Accepted on August 2, 2011.

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