



Alternative liming blends for fish culture

Mikaely Karine Barbosa Nobre, Francisco Roberto dos Santos Lima, Frederico Batista Magalhães and Marcelo Vinícius do Carmo e Sá*

Laboratório de Ciência e Tecnologia Aquícola, Departamento de Engenharia de Pesca, Centro de Ciências Agrárias, Universidade Federal do Ceará, Av. Mister Hull, s/n, 60356-000, Fortaleza, Ceará, Brazil. *Author for correspondence. E-mail: marcelo.sa@ufc.br

ABSTRACT. Calcium carbonate (limestone) is a safe but slow-acting product for aquaculture liming. Calcium oxide (lime) is a fast-acting but dangerous product. It would be interesting to have simultaneously a safe and fast-acting liming product. The present work aimed to evaluate two alternative liming blends for tilapia culture. The culture water was initially acidified with HCl. Next, the same quantity of CaCO_3 (control), $\text{Na}_2\text{CO}_3 + \text{CaSO}_4$ or $\text{Na}_2\text{CO}_3 + \text{CaCl}_2$ was applied to the culture water. There were also non-limed tanks. One hundred and twenty Nile tilapia juveniles (1.6 ± 0.1 g) were stocked in twenty 250-L culture tanks for 10 weeks. All products used were similarly efficient in increasing the alkalinity of water. The probable better environment for algal growth in the limed tanks caused an increase in the DO_2 concentrations and a decrease in CO_2 , total ammonia nitrogen, nitrite and phosphorus, in relation to the non-limed tanks. The highest final body weight of fish was observed in the $\text{Na}_2\text{CO}_3 + \text{CaCl}_2$ tanks. The lowest final body weight was verified in the CaCO_3 tanks. It was concluded that the alternative blend of Na_2CO_3 plus CaCl_2 is a better liming product than the standard CaCO_3 for Nile tilapia culture.

Keywords: limestone, lime, tilapia, alkalinity, aquaculture.

Misturas alternativas para calagem em piscicultura

RESUMO. Carbonato de cálcio é um produto seguro, mas de ação lenta para calagem em aquicultura. O óxido de cálcio é um produto de ação rápida, mas perigoso. Seria de interesse ter um produto para calagem em aquicultura que fosse simultaneamente seguro e de ação rápida. O presente projeto de pesquisa teve por objetivo avaliar duas misturas alternativas para calagem no cultivo de tilápia. A água de cultivo foi inicialmente acidificada com HCl. Em seguida, a mesma quantidade de CaCO_3 (controle), $\text{Na}_2\text{CO}_3 + \text{CaSO}_4$ ou $\text{Na}_2\text{CO}_3 + \text{CaCl}_2$ foi aplicada à água. Havia ainda tanques não submetidos à calagem. Cento e vinte juvenis de tilápia do Nilo ($1,6 \pm 0,1$ g) foram estocados em 20 tanques de cultivo de 250 L por dez semanas. Todos os produtos testados foram igualmente eficientes no aumento da alcalinidade da água. Os tanques submetidos à calagem apresentaram maiores concentrações de O_2D e menores de CO_2 livre, nitrogênio amoniacal total, nitrito e fósforo. O maior e o menor peso corporal final dos peixes foram observados nos tanques $\text{Na}_2\text{CO}_3 + \text{CaCl}_2$ e CaCO_3 , respectivamente. Concluiu-se que a mistura alternativa $\text{Na}_2\text{CO}_3 + \text{CaCl}_2$ é superior ao CaCO_3 na calagem de tanques de tilápia do Nilo.

Palavras-chave: calcário, cal, tilápia, alcalinidade, aquicultura.

Introduction

Liming is an ancient agricultural practice to amend the pH of acidic soils. Higher crops can be achieved by neutralizing the soil acidity with limestone (PEIXOTO et al., 1998). In aquaculture, the same principle is valid because acidic soils and waters can produce poor growth performance results (CAVALCANTE et al., 2012).

Currently, the standard liming product for aquaculture is agricultural limestone that is the commercial name of calcium carbonate (CaCO_3). In the water, calcium carbonate reacts with carbon dioxide forming soluble calcium and bicarbonate ions (THUNJAI et al., 2004). Beside limestone, it is

also used burned (CaO) or hydrated ($\text{Ca}(\text{OH})_2$) lime to raise the pH of soil and water. However, lime has a better use as a disinfectant due to its explosive reaction with water (very fast pH raising; YUVANATEMIYA et al., 2011).

Although calcium carbonate has a proven efficacy and safety for routinely use its effects in water are slow, requiring considerable time to be achieved (QUEIROZ et al., 2004). Lime is the first option when a rapid response is needed by the fish farmer. Lime, however, as stated above, is a dangerous product to be applied directly in the culture water because it can cause severe mortalities of fish (BOYD; MASSAUT, 1999). Therefore, it would be interesting to have a liming product simultaneously safe to the aquatic biota,

as the calcium carbonate, and short-term acting, as the calcium oxide. The present work aimed to compare the growth performance of juvenile Nile tilapia reared in acidified polyethylene tanks subjected to two alternative liming blends with the fish performance observed in calcium carbonate limed tanks.

Material and methods

Fish, acclimatization and experimental system

One thousand juvenile Nile tilapia (BW = 1.0 ± 0.05 g) were obtained from a regional fish farmer and transported by road to the laboratory facilities (03°44'39.9"S; 38°34'55.9"W). In the lab, fish were transferred to one 1,000-L polyethylene tank filled with green water and supplied with constant aeration. Fish remained in the reception tank for four days, when they were fed on a powdered fish 50% CP diet (FriAcqua Inicial, Nutreco Fri-Ribe Animal Nutrition Inc., Maracanaú, Ceará State, Brazil) every three hours, from 8:00 am up to 5:00 pm, delivered at a rate of 10% total biomass per day. After acclimation, fish were transferred to the experimental system which contained twenty 250-L polyethylene outdoor tanks filled with clear water (pH = 7.2; total alkalinity = $50.7 \text{ mg L}^{-1} \text{ CaCO}_3$; total hardness = $70.7 \text{ mg L}^{-1} \text{ CaCO}_3$) previously exposed to the air to loss its residual chlorine. No mechanical aeration was provided to the experimental tanks. Six fishes (BW = 1.57 ± 0.1 g) were assigned to each tank for a rearing period of 10 weeks.

Experimental design

The artificial acidification of the culture water was carried out in two distinct phases. Initially, all tanks were acidified with a same volume of concentrated HCl p.a to reduce total alkalinity to close to $20 \text{ mg L}^{-1} \text{ CaCO}_3$. For this, the following mathematical expression was used $y = (0.002 \cdot x) - 0.006$ where x is the desired reduction in the water's total alkalinity (in $\text{mg L}^{-1} \text{ CaCO}_3$) and y is the required volume of the concentrated HCl to apply in water (in mL L^{-1}). After 48 hours, five randomly designed CaCO_3 control tanks received one application of 50 g of calcium carbonate p.a. per tank and ten experimental tanks the following alternative liming blends: $\text{Na}_2\text{CO}_3 + \text{CaSO}_4$ and $\text{Na}_2\text{CO}_3 + \text{CaCl}_2$, analytical grade quality, five tanks each one. The liming blends were also applied at 50 g per tank in a 1:1 proportion of the different salts used. Besides, there were also five non-limed control tanks. Afterwards, a new application of concentrated HCl was carried out at the onset of the 3rd experimental week to reduce again the water's total alkalinity to $20 \text{ mg L}^{-1} \text{ CaCO}_3$. No HCl application was performed on tanks with total alkalinity lower than $20 \text{ mg L}^{-1} \text{ CaCO}_3$.

As the fish's growth performance in the non-limed tanks was not affected by the initial acidification, it was decided to strengthen the water acidification process. Accordingly, daily applications of 2.0 mL of concentrated HCl were carried out in all tanks in the second phase of the study which started in the 5th experimental week. The 2nd phase of the study aimed to produce a more pH challenging environment to tilapia. New applications of the liming products were performed in the middle of the rearing period at a rate of 18 g of the product or blend (1:1) per tank.

Fish husbandry

Over the experiment, fish was fed on two artificial diets (powdered and a 2-3 mm pellet diet). Initially, the 50% CP powdered diet was delivered to all fish, the same diet used in the acclimation period. That diet was given to fish in four equal meals at 8:00 am, 11:00 am, 2:00 pm and 5:00 pm. The feeding rates adopted in that phase declined from 10% up to 7% of the stocked biomass. The initial diet was then changed by the 40% CP 2-3 mm pellet diet (FriAcqua Alevinos, Nutreco Fri-Ribe Animal Nutrition, Maracanaú, Ceará State, Brazil) when the average body weight of fish in the tank was ≥ 5.5 g. Thereafter, the 2-3 pellet diet was supplied to all fish also in four equal meals at the same previous feeding hours. The feeding rates adopted along the final phase of the experiment declined from 7% up to 3.5% when fish reached 25 g BW or over. Fortnightly weighing of fish were carried out to adjust the amounts of diet delivered to each tank. The photoperiod was the natural (nearly 12h light: 12h dark).

Experimental variables

Twice a week, at 8:00 am and 4:00 pm, water temperature, electrical conductivity (EC) and water pH were monitored using portable equipment (Instrutherm digital thermometer, Lutron CD-4301 water conductivity meter and Marconi MA522 pH-meter, respectively). Weekly, always by the morning (8 a.m.), the concentrations of dissolved oxygen (DO_2 ; Winkler method with azide modification), free CO_2 (titration with standard sodium carbonate solution), total alkalinity (titration with standard sulfuric acid solution) and total hardness (titration with standard EDTA solution) were determined in all tanks.

Fortnightly, water samples were taken from the experimental tanks to determine concentrations of total ammonia nitrogen (TAN; phenate method), nitrite (diazotization and coupling method) and reactive phosphorus (ammonium molybdate method). These water quality variables were determined following standard methods (APHA, 1999).

The growth performance variables examined in the present work were fish survival, final body weight, specific growth rate ($SGR = [(\ln \text{ final body weight} - \ln \text{ initial body weight}) / \text{rearing days}] \times 100$), yield and food conversion ratio ($FCR = \text{weight of feed offered (g)} / \text{fish weight gain (g)}$).

Statistical analysis

The results of water quality and growth performance 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 variance 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, USA).

Results and discussion

Water quality

Over the experiment, the average monthly rainfall was 259 mm. Therefore, the fish culture carried out took place in the rainy season. The water temperature of the tanks remained within the appropriate range for normal growth of juvenile Nile tilapia (AZAZA et al., 2008), ranging from 24.6°C at 8:00 am up to 32.2°C at 4:00 pm. On average, the water temperature was $26.7 \pm 0.7^\circ\text{C}$ and $29.0 \pm 1.6^\circ\text{C}$ at 8:00 am and 4:00 pm, respectively. No significant differences were detected between the treatments for water temperature (Table 1).

The applications of the different liming products (CaCO₃, Na₂CO₃, CaSO₄ and CaCl₂) on the artificially acidified fish culture water have significantly affected most water quality indicators monitored in the present work (Table 1). The initial application of the concentrated HCl in the culture water has decreased pH to 5.8-5.9 in all tanks (initial pH = 7.2 ± 0.1). Afterwards, the applications of the different salts in the culture water have significantly increased the pH to 7.7-8.7. However, the different liming salts have not differed between themselves for their effects on the water pH increase. Therefore, the alternative liming blends used in the present work (Na₂CO₃+CaSO₄ and Na₂CO₃+CaCl₂) were as effective as the standard CaCO₃ in the purpose of increasing water pH.

The salts applications have significantly increased the electrical conductivity (EC) of water. However, that response has varied according to the salts used. The highest EC increases were observed for the alternative liming blends (Na₂CO₃+CaSO₄ and Na₂CO₃+CaCl₂), especially for the Na₂CO₃+CaCl₂ whose EC differed significantly from that for the

standard CaCO₃. These results of water EC are in accordance with the salts' degrees of solubility in water. While the chloride salts are water soluble, including the CaCl₂, the carbonate salts are insoluble in water. Although the sulfate salts are water soluble in general, the CaSO₄ is an exception, being also insoluble. The degrees of water solubility at 20°C for those salts, in g per 100 g of water, are as follows: CaCl₂ (74.5), Na₂CO₃ (21.5), CaSO₄ (0.25) and CaCO₃ (0.0006) (REGER et al., 2009). Hence, a stronger effect on water EC would be expected for CaCl₂ compared with CaSO₄ and, especially, with CaCO₃. Based solely on the degree of solubility in water, better liming results should be obtained by the use of CaCl₂ and Na₂CO₃ instead of CaSO₄ (agricultural gypsum) and CaCO₃ (limestone). Therefore, it would be advisable to use the alternative liming blend Na₂CO₃+CaCl₂ if faster results are required. On the other hand, CaCO₃ still would be the best choice if the liming effects are not urgent.

The applications of the different liming blends to the culture water have significantly increased its total alkalinity compared with the acidified non-limed tanks. However, no significant differences were recorded between the products for the total alkalinity. These results strengthen the belief that alternative liming blends used herein (Na₂CO₃+CaSO₄ and Na₂CO₃ + CaCl₂) are liming materials as suitable as the standard CaCO₃ for fish culture.

The liming effects on the total hardness of water varied depending on the salts used. The hardness of CaCO₃ tanks was significantly higher than found in the Na₂CO₃+CaSO₄ tanks. It seems that CaCO₃ is a better product than CaSO₄ for hardness increase. That suggestion, however, disagrees with the degrees of water solubility of those salts and it is a difficult point to explain. Although not statistically different, CaCl₂ seems to be a better product for hardness increase than CaSO₄.

Regardless the salts used, significantly lower DO₂ concentrations were observed in the acidified non-limed tanks compared to the limed ones. The acidity of water in the non-limed tanks has probably decreased algal density. Consequently, the input of DO₂ through photosynthesis was impaired in the non-limed tanks. The applications of carbonate salts (CaCO₃ and Na₂CO₃) held in the present work adjusted upwards the water pH allowing a normal phytoplankton growth. Therefore, the acidification of water, besides its direct and negative effect on fish physiology (VAN DER SALM et al., 2005), has also indirect effects, such as the reduction of the DO₂ concentrations of water. Moreover, liming can indirectly increase the DO₂ concentration by allowing a greater bloom of phytoplankton.

Table 1. Water quality of 250-L acidified tanks subjected or not to different liming products and stocked with juvenile Nile tilapia for ten weeks (mean \pm S.D.; n = 5).

Variable ¹	Liming				ANOVA P
	None	CaCO ₃	Na ₂ CO ₃ +CaSO ₄	Na ₂ CO ₃ +CaCl ₂	
8 a.m. temp	26.71 \pm 0.71	26.66 \pm 0.70	26.64 \pm 0.70	26.74 \pm 0.72	ns ³
4 p.m. temp	29.06 \pm 1.64	29.01 \pm 1.72	28.96 \pm 1.68	29.07 \pm 1.63	ns
8 a.m. pH	5.79 \pm 1.62 a ²	7.72 \pm 0.65 b	7.71 \pm 0.75 b	7.68 \pm 0.76 b	<0.001
4 p.m. pH	5.89 \pm 2.05 a	8.43 \pm 0.74 b	8.72 \pm 0.93 b	8.73 \pm 0.90 b	<0.001
8 a.m. EC	603 \pm 57 c	674 \pm 79 bc	761 \pm 113 ab	857 \pm 107 a	<0.001
4 p.m. EC	602 \pm 47 c	668 \pm 84 bc	757 \pm 123 ab	859 \pm 105 a	<0.001
Alkalinity	7.44 \pm 5.8 a	60.99 \pm 23.3 b	58.74 \pm 21.4 b	58.24 \pm 20.7 b	<0.001
Hardness	116.1 \pm 12.6 c	180.6 \pm 25.0 a	141.0 \pm 16.6 b	162.9 \pm 14.1 ab	<0.001
DO ₂	4.0 \pm 1.1 a	6.8 \pm 1.9 b	6.4 \pm 2.1 b	6.4 \pm 2.0 b	0.031
Free CO ₂	33.1 \pm 7.6 a	11.9 \pm 3.0 b	11.1 \pm 2.2 b	11.5 \pm 2.2 b	<0.001
TAN	2.00 \pm 0.27 a	0.56 \pm 0.16 b	0.48 \pm 0.15 b	0.38 \pm 0.04 b	< 0.001
Nitrite	0.063 \pm 0.01 a	0.054 \pm 0.01 ab	0.048 \pm 0.01 b	0.045 \pm 0.00 b	0.009
Reactive-P	515 \pm 135 a	70 \pm 24 b	96 \pm 17 b	53 \pm 10 b	< 0.001

¹temp: temperature (°C); EC: electrical conductivity (μ S cm⁻¹); total alkalinity and total hardness (mg L⁻¹ CaCO₃); DO₂: dissolved oxygen in water (mg L⁻¹); free CO₂ (mg L⁻¹); TAN: total ammonia nitrogen (mg L⁻¹); nitrite (mg L⁻¹); reactive-P: reactive phosphorus (μ g L⁻¹); ²In the same row, means not sharing the same letter are significantly different by the Tukey's test (ANOVA p < 0.05); ³Non-significant (ANOVA p > 0.05).

Concentrations of free CO₂ in water were inversely related to DO₂ concentrations. Higher concentrations of CO₂ were observed in acidified non-limed tanks and no expressive differences existed between the limed tanks for CO₂. Bicarbonate ions are partially or totally transformed to free CO₂ over the acidification process. The inverse route occurs when there is an increase in water pH (GENDEL; LAHAV, 2013). Therefore, liming is an effective management to decrease the concentrations of free CO₂ in fish culture water. When there are more than 15 mg free CO₂ L⁻¹ fish can experience some respiratory stress (DANLEY et al., 2005). In the present work, this was the case in the acidified non-limed tanks in which the concentrations of free CO₂ in water exceeded 30 mg L⁻¹.

Liming has lowered the concentrations of TAN in all experimental tanks when compared to the acidified non-limed tanks. However, the differences between the limed tanks for TAN were not significant. Microalgae absorb actively ammonia from the water to use it in their growth. Accordingly, the alga community aids in lowering the TAN concentrations of the culture medium (CHEN et al., 2012). As the alga growth was probably inhibited by the acidification process carried out in the present work, the ammonia sink effect of phytoplankton was insignificant in the acidified non-limed tanks.

The concentrations of nitrite in the tanks limed with the standard CaCO₃ or with any of the alternative liming blends used were significantly lower than in the acidified non-limed tanks. As nitrite can also be absorbed by phytoplankton (GOBLER et al., 2012), nitrite concentrations in the acidified non-limed water were higher than in the limed tanks because phytoplankton community was probably smaller compared to the latter tanks.

The concentrations of reactive phosphorus in water were significantly lowered by liming, regardless the products used. However, differences for reactive

phosphorus between the limed tanks were not significant. As previously supposed, liming has enhanced the phytoplankton growth in the culture tanks. Consequently, the microalgae in limed tanks have probably absorbed intensely phosphorus from the water for their development, explaining that way the lower phosphorus concentrations in those tanks. It is notable that the removal of reactive phosphorus from water by phytoplankton cells is usually a very fast process (PENGSENG; BOYD, 2011).

Growth performance

The final survival of fish was very good for all treatments, leveling or exceeding 90%. However, no significant differences were seen between the treatments for fish survival (Table 2). Overall, fish growth was fast along the experimental period independent of the treatment. The best fish growth rate was observed in the Na₂CO₃+CaCl₂ tanks. The final fish body weight in the Na₂CO₃+CaCl₂ tanks was significantly higher than found in the CaCO₃ tanks (Table 2). Surprisingly, the fish growth in the acidified non-limed tanks was acceptable, being not statistically different from the limed tanks. These results suggest that juvenile Nile tilapia between 1.5 to 40 g BW are capable to thrive well in moderately acidified waters (pH 5-6). Therefore, it seems unnecessary to proceed liming in such acidic culture water. This finding, however, disagrees with Wudtisin and Boyd (2006) that recommended liming, whenever the pH of water or sediments is lower than 6. However, Wudtisin and Boyd (2006)'s liming recommendation is generic and not focus on any fish genera or species in particular. Perhaps juvenile Nile tilapia is an acid-loving fish such as the Amazonian tambaqui, *Colossoma macropomum* (ARIDE et al., 2007). That supposition, however, disagrees with El-Sherif and El-Feky (2009) who concluded that the best water pH for Nile tilapia growth is between 7 and 8. This issue deserves a further examination.

Table 2. Growth performance of juvenile Nile tilapia stocked for ten weeks in 250-L acidified tanks, subjected or not to different liming products (mean ± S.D.; n = 5).

Variable ¹	Liming				ANOVA P
	None	CaCO ₃	Na ₂ CO ₃ +CaSO ₄	Na ₂ CO ₃ +CaCl ₂	
Survival	90.0 ± 14.9 ²	93.3 ± 14.9	90.0 ± 9.1	93.3 ± 9.1	ns ³
Initial BW	1.56 ± 0.13	1.51 ± 0.12	1.58 ± 0.06	1.62 ± 0.08	ns
Final BW	35.3 ± 3.7 ab	31.1 ± 1.9 b	34.9 ± 2.8 ab	37.0 ± 2.3 a	0.025
SGR	4.46 ± 0.24	4.32 ± 0.16	4.42 ± 0.10	4.47 ± 0.13	ns
Yield	10.4 ± 3.1	10.9 ± 0.9	11.2 ± 1.1	11.9 ± 1.7	ns
FCR	1.86 ± 0.66	1.42 ± 0.09	1.53 ± 0.14	1.35 ± 0.11	ns

¹Survival (%); initial and final body weight (BW; g); SGR (% body weight day⁻¹): specific growth rate = [(ln final BW - ln initial BW)/n^o of days] x 100; yield (g m⁻³ day⁻¹); FCR = feed conversion ratio (feed allowed/fish weight gain); ²Means in the same row not sharing the same letter are statistically different by the Tukey's test (ANOVA p < 0.05); absence of letters indicates no statistical significance; ³Non-significant (ANOVA p > 0.05).

The superior performance of fish stocked in the Na₂CO₃+CaCl₂ tanks, compared with the CaCO₃ tanks, suggests that the alternative liming blend of Na₂CO₃ plus CaCl₂ is a better liming product than the standard CaCO₃. The higher water solubility of Na₂CO₃ and CaCl₂ in contrast with that of CaCO₃ (REGER et al., 2009) may explain partially this result. Firstly, the alternative liming blend of Na₂CO₃ plus CaCl₂ is capable to simultaneously increase the alkalinity and hardness of water as well as the CaCO₃. Secondly, unlike CaCO₃, the Na₂CO₃+CaCl₂ blend also provides Na⁺ and Cl⁻ ions to the culture water, which are very important to fish osmoregulation (HIROSE et al., 2003). Therefore, the Na₂CO₃+CaCl₂ blend has extra benefits for fish growth not provided by the CaCO₃ liming. The SGR, yield and FCR results were not significantly different between the treatments (Table 2).

Conclusion

The results obtained in the present study allow us to conclude that:

The alternative liming blends Na₂CO₃+CaSO₄ and Na₂CO₃+CaCl₂ are capable to increase the pH and the total alkalinity of water as efficiently as the standard CaCO₃;

The alternative blend Na₂CO₃+CaCl₂ is a better liming product than the standard CaCO₃ for Nile tilapia culture.

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