BIOSCIENCE JOURNAL

APPLICATION OF LIMESTONE-MOLASSES BLENDS IN NILE TILAPIA REARING TANKS

Marcos Luiz da Silva APOLIANO[1], Francisco Roberto dos Santos LIMA[1], Davi de Holanda CAVALCANTE[2], Marcelo Vinícius DO CARMO E SÁ[3]

[1]Fisheries Engineering Postgraduate Program, Fisheries Engineering Department, Universidade Federal do Ceará, Fortaleza, Ceará, Brazil.
[2] Instituto Federal de Educação do Ceará, Acaraú Campus, Ceará, Brazil.
[3] Fisheries Engineering Department, Agrarian Sciences Center, Universidade Federal do Ceará, Fortaleza, Ceará, Brazil

Corresponding author:
Marcelo Vinícius do Carmo e Sá
marcelo.sa@ufc.br

How to cite: APOLIANO, M.L.S., et al. Application of limestone-molasses blends in Nile tilapia rearing tanks. *Bioscience Journal*. 2023, **39**, e39065. https://doi.org/10.14393/BJ-v39n0a2023-63393

Abstract
The work aimed at determining the effects of applying different blends between liquid molasses and agricultural limestone on water quality and growth performance of Nile tilapia juveniles, *Oreochromis niloticus.* The study employed 24 indoor tanks of 100 L and 24 outdoor tanks of 250 L. Four control groups were set up (no product applied, only molasses, only limestone, molasses-limestone blend with 48% limestone but no fish) and two experimental groups (molasses-limestone blends with 32% and 48% molasses), with four replicates each. Over 11 weeks, the water quality was monitored systematically. The 24-h cycle monitoring of temperature, pH, specific conductance, dissolved O_2 and total alkalinity were carried out at the 8th week. The blends between molasses and limestone accelerated the dissolution rate of the agricultural limestone in water. The blend containing 48% molasses led to greater increases in water alkalinity and pH in relation to tanks with only limestone applications. However, the molasses-limestone blend applications impaired the Nile tilapia growth performance, especially in the outdoor tanks. While the fish yield was 25.1 g m^{-3} day^{-1} in the molasses outdoor tanks, it was equal to 22.8 g m^{-3} day^{-1} in the molasses (32%)-limestone units ($P<0.05$). It has been concluded that the blending between limestone and molasses brings no clear benefits to Nile tilapia's rearing tanks when compared to the limestone-only tanks.

Keywords: Aquaculture. Calcium carbonate. Carbon dioxide. Fish culture. Liming.

1. Introduction

Liming increases the pH, alkalinity (HCO_3^-, CO_3^{-2}) and hardness (Ca^{+2}, Mg^{+2}) of water as well as it neutralizes the soil acidity. The bicarbonate and carbonate ions buffer the pH of water, moderating its changes over the 24-h cycle. Calcium and magnesium ions are essential elements for animal health and growth, being demanded in numerous physiological activities, such as nerve transmission, blood coagulation, enzymatic activity, muscle contraction and osmoregulatory regulation (Peng et al. 2019). Therefore, fish and shrimp tanks with greater alkalinity and hardness have favorable growth performance (Cavalcante et al. 2009). Frequent liming of water is also essential to the success of BFT and RAS aquaculture systems (Furtado et al. 2015).

Agricultural limestone, $CaCO_3$ is the most used product for aquaculture liming because it has proved efficacy and safety for routine applications. In aquaculture tanks, limestone is generally applied at dosages of 1000 - 5000 kg ha^{-1} (Nobre et al. 2014). However, limestone has low water solubility and, by consequence, a longer period after its application is necessary to observe significant changes in the alkalinity and hardness of water (Queiroz et al. 2004). Hydrated lime, $Ca(OH)_2$ is a better option when faster responses are needed by the farmer because it has a higher water solubility than limestone. In distilled water, the solubilities of $CaCO_3$ and $Ca(OH)_2$ are equal to 14 mg L^{-1} and 1200 mg L^{-1}, respectively (Boyd 2017; Sá et al. 2019). Therefore, the benefits of liming can be achieved more quickly by applying lime instead of limestone in water and soil.

The application of lime in aquaculture tanks, however, may be dangerous because it may cause fish and shrimp stress and mortality due to the sudden pH increase observed after its application (Whangchai et al. 2004). A safer option to lime would be sodium bicarbonate, which also has a high-water solubility. Sodium bicarbonate, however, does not increase the hardness of water and it is a more expensive product. However, the long use of sodium bicarbonate could unbalance the water's alkalinity: hardness ratio and, consequently, impair fish growth performance (Cavalcante et al. 2014; Martins et al. 2017).

The concentrations of CO_2 in water affect the dissolution rates of limestone because CO_2 reacts with $CaCO_3$ as follows: $CaCO_3 + CO_2 + H_2O \rightarrow Ca^{+2} + 2HCO_3^-$ (Boyd et al. 2016). Thus, the release of CO_2 after the decomposition of organic matter could hasten the dissolution rate of limestone in water (Han and Boyd 2018). Boyd and Tucker (2014) have proposed the application of a highly biodegradable organic matter source in aquaculture tanks, simultaneously to limestone, to achieve faster liming results. The effectiveness of that practice, however, had not been evaluated in Nile tilapia rearing tanks yet. The present work aimed at determining the effects of different blends between molasses and agricultural limestone on water quality and Nile tilapia growth performance.

2. Material and Methods

Fish and rearing system

The work was carried out at … Juveniles of Nile tilapia with 1.5 ± 0.11 g were obtained from a regional producer and transported to the laboratory facilities. Initially, fish were maintained for 7 days in one 1000-L tank for acclimatization. Potassium permanganate was applied at 4 mg L^{-1} to prevent bacterial infections. The same dosage of sodium thiosulfate was applied after 48 h to neutralize the residual permanganate. During the acclimatization, fish were fed four times a day, at 8, 11, 14 and 17 h, with a commercial powdered diet for omnivorous fish containing 45% crude protein at 10% (Aquamix, Integral Mix, Fortaleza, Ceará).

The experimental systems were comprised by twenty-four 100-L circular indoor tanks and twenty-four 250-L circular outdoor tanks. The outdoor tanks were covered with a Sombrite screen to prevent the overheating of water. The water was continuously aerated by one 2.5-hp air blower. Three air diffusers and one 15-cm aeration hose were used per tank in the outdoor and indoor systems, respectively. At the onset, eight and eighteen Nile tilapia juveniles (2.8 ± 0.14 g) were stocked in the indoor and outdoor tanks, respectively. That represented an initial fish biomass of ≈ 200 g m^{-3} at both systems. The fish were maintained inside the experimental tanks for eleven rearing weeks.

Experimental design

Tap water was used to fill all tanks. An 1N HCl solution was applied at 1.0 mL L^{-1} to reduce the total alkalinity and pH of water to ≤ 20 mg L^{-1} and ≈ 5.0, respectively. The designed amounts of liquid molasses (50% C, dry matter; AgroBio Cearense, Tianguá, Ceará) and agricultural limestone (PRNT = 81%, CaO = 32%, MgO = 15%; Chaves Mineração e Indústria, Maracanaú, Ceará) were weighed and mixed to obtain homogeneous products. The limestone-molasses blends were applied to the tilapia tanks 48 h after the water acidification. Separate applications of the molasses and limestone were also made in the control tanks (Table 1).

There were six experimental treatments as follows: 1 – control tanks with no application of any product; 2 – control tanks with application of agricultural limestone only; 3 – control tanks with application of liquid molasses only; 4 – test tanks with application of the limestone-molasses blend with 32% molasses; 5 – test tanks with application of the limestone-molasses blend with 48% molasses; 6 – control tanks with application of the limestone-molasses blend with 48% molasses but no fish (Table 1). The amounts of limestone and molasses in the blends were based on previous works carried out in our laboratory. After the initial applications, the products and blends were applied every two weeks at half of the initial dosage. The Nile tilapia stockings occurred one week after the first applications of molasses, limestone, and limestone-molasses blends.

Table 1. Initial application dosages of agricultural limestone, liquid molasses, and limestone-molasses blends in 100-L indoor and 250-L outdoor Nile tilapia rearing tanks.

Product applied in water	Nile tilapia stocking	Molasses (g)		Limestone (g)		% Molasses
		100-L tank	250-L tank	100-L tank	250-L tank	
None	+	-	-	-	-	-
Agricultural limestone	+	-	-	20	50	-
Liquid molasses[1]	+	18.6	46.6	-	-	-
Limestone-molasses-32	+	9.3	23.3	20	50	≈ 32
Limestone-molasses-48	+	18.7	46.6	20	50	≈ 48
Limestone-molasses-48	-	18.7	46.6	20	50	≈ 48

[1] The liquid molasses contained 81.6% dry matter.

Over the experimental period, fish received the same commercial diet used during the acclimatization phase. Initially, the artificial diet was allowed daily at three times (8, 12 and 16h) at 5% of the stocked biomass. That feeding rate was reduced to 3% after the 2nd rearing week. Fortnight weightings of fish were performed to adjust the dietary allowances. In all tanks, there was no water exchange throughout the study but the maintenance of the initial level with acidified freshwater.

Experimental variables and statistical analysis

The water's temperature, pH (pH meter mPA210, MS Tecnopon, Piracicaba, SP), specific conductance (SC, conductivity meter CD-850, São Paulo, SP) and dissolved O_2 concentrations (Oximeter YSI-55, Xylem, São Paulo, SP) were monitored daily between 8 – 10 h. Water samples from all tanks were collected weekly to determine total alkalinity, total hardness and calcium hardness; and fortnightly to determine total ammonia nitrogen (TAN), nitrite ($N\text{-}NO_2^-$) and nitrate ($N\text{-}NO_3^-$). Total alkalinity was determined by titration with a standard solution of H_2SO_4; total hardness and calcium hardness were determined by titration with a standard EDTA solution. The concentrations of TAN, $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$ were determined by the indophenol, sulfanilamide and Cd reducing column methods, respectively. At the end of the 8th experimental week, water samples were collected to determine the concentrations of chlorophyll *a* by spectrophotometry at 665 and 750 nm and a 24-h pH monitoring was performed in the outdoor tanks. For that, the samples were collected at 8, 11, 14, 17, 20, 23, 2, 5 and 8 h in the following day. The determinations of water quality were performed according to the methodologies described by Clesceri et al. (1998).

Fortnight weightings of fish were performed to determine survival, weekly growth rate and feed conversion ratio (FCR). The water quality and growth performance variables were submitted to one-way ANOVA for completely randomized designed experiments. The Tukey's test was employed to compare the means two by two when there were significant differences between them. The 5% significance level was adopted in all statistical tests. The SigmaPlot for Windows software v.12.0 (Systat Software, San Jose, CA, USA) was used to perform the statistical analysis.

3. Results

Water quality

The pH of water increased fast in both rearing systems after the application of the products and blends, especially in the tanks receiving agricultural limestone either alone or blended with molasses (Figures 1A and 2B). From the 2nd experimental week on, the pH of the water stabilized in all tanks but at different levels: pH ≈ 8.0 for the treatments with limestone; and pH ≈ 7.5 for the other treatments. At the end, the pH of water in the indoor and outdoor tanks receiving the limestone-molasses blends was significantly higher than in the other units (Figures 1A and 1B).

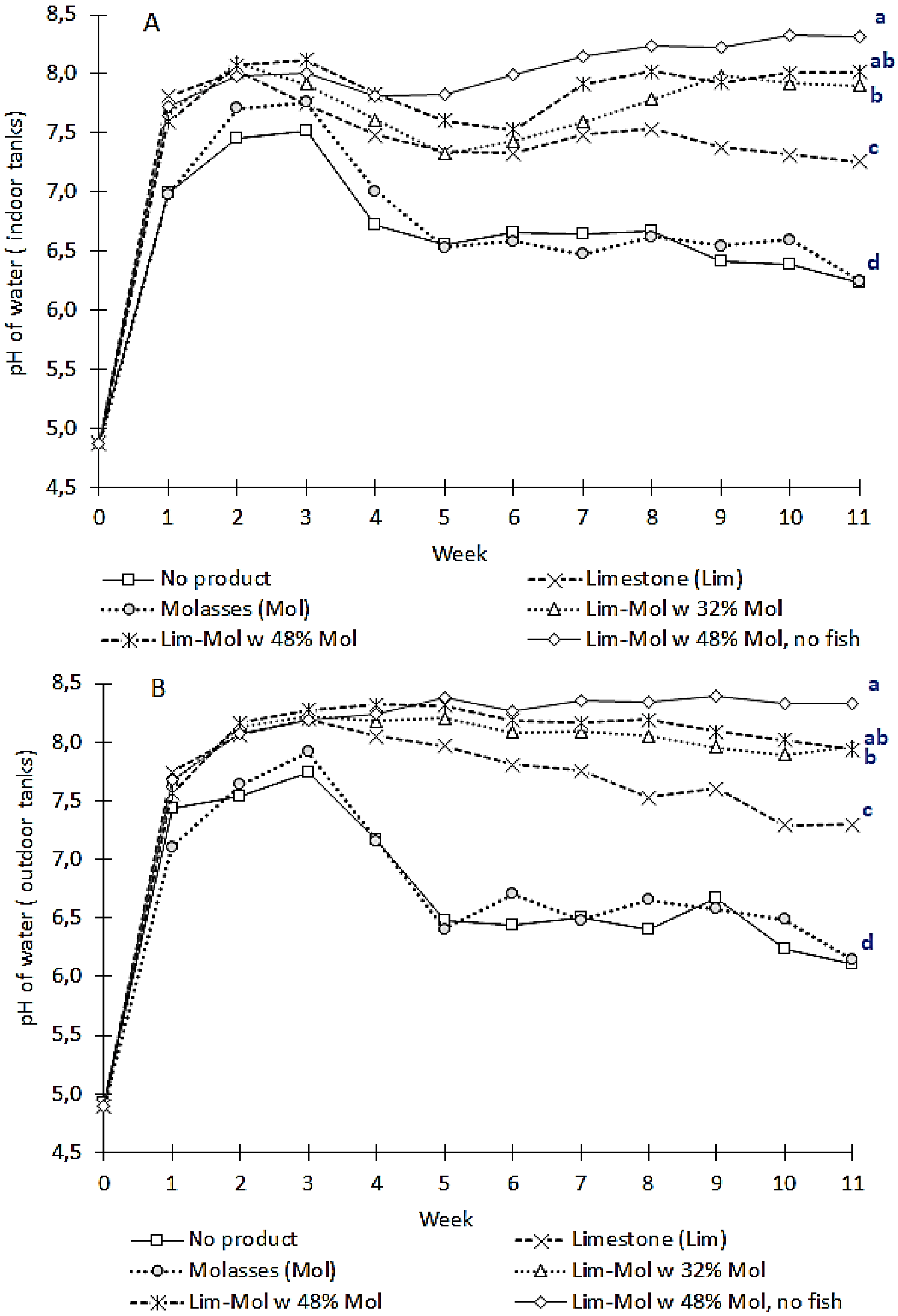

Figure 1. pH of water in Nile tilapia's rearing A - *indoor* and B - *outdoor* tanks (n = 4). In the last week, means with distinct letters are significantly different between themselves by the Tukey's test (ANOVA $P<0.05$).

The total alkalinity (TA) of water increased rapidly in all tanks after the applications of the products and blends, mainly in the treatments containing limestone (Figures 2A and 2B). The TA of water continued to rise fast in the indoor tanks that received the limestone-molasses blends, reaching values over 80 mg L^{-1} at the 2nd week. Contrarily, a maximum TA = 60 mg L^{-1} was reached in the tanks that received only limestone at 3rd experimental week. After eleven weeks, the TA of water were just 54.5 mg L^{-1} and 38.2 mg L^{-1} in the indoor and outdoor tanks that received limestone only, respectively. On the other hand, the final TA was close to 100 mg L^{-1} in the tanks that received the limestone-molasses blend with 48% molasses and stocked with tilapia. The TA of water in those last tanks remained above the values observed in the respective tanks without fish over almost the entire experimental period.

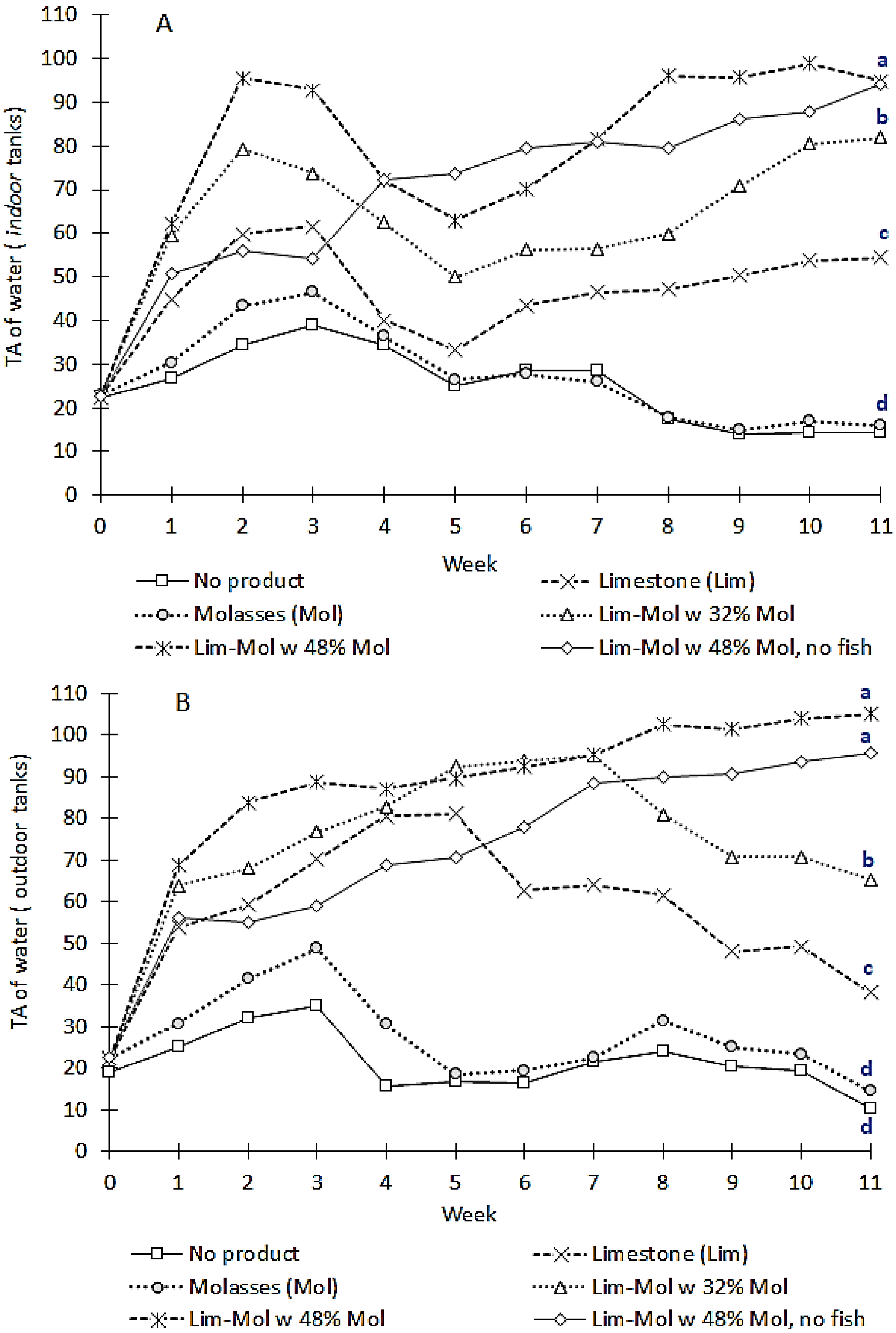

Figure 2. Total alkalinity (TA) of water (mg L^{-1} $CaCO_3$ eq.) in Nile tilapia's rearing A - *indoor* and B - *outdoor* tanks (n = 4). In the last week, means with distinct letters are significantly different between themselves by the Tukey's test (ANOVA P<0.05).

The final concentrations of total ammonia nitrogen (TAN) in the tanks that received no product or molasses only were higher than the values observed in the unit's receiving limestone either alone or in mixture with molasses (P<0.05; Figures 3A and 3B). Final TAN was equal to 39.7 ± 8.6 mg L^{-1} and 26.2 ± 8.8 mg L^{-1} in indoor tanks receiving no product or limestone only, respectively. In the outdoor tanks, those concentrations were equal to 24.7 ± 3.6 mg L^{-1} and 18.8 ± 2.3 mg L^{-1}, respectively. In tanks that received limestone, either alone or in mixture with molasses, the final concentrations of TAN were close to zero.

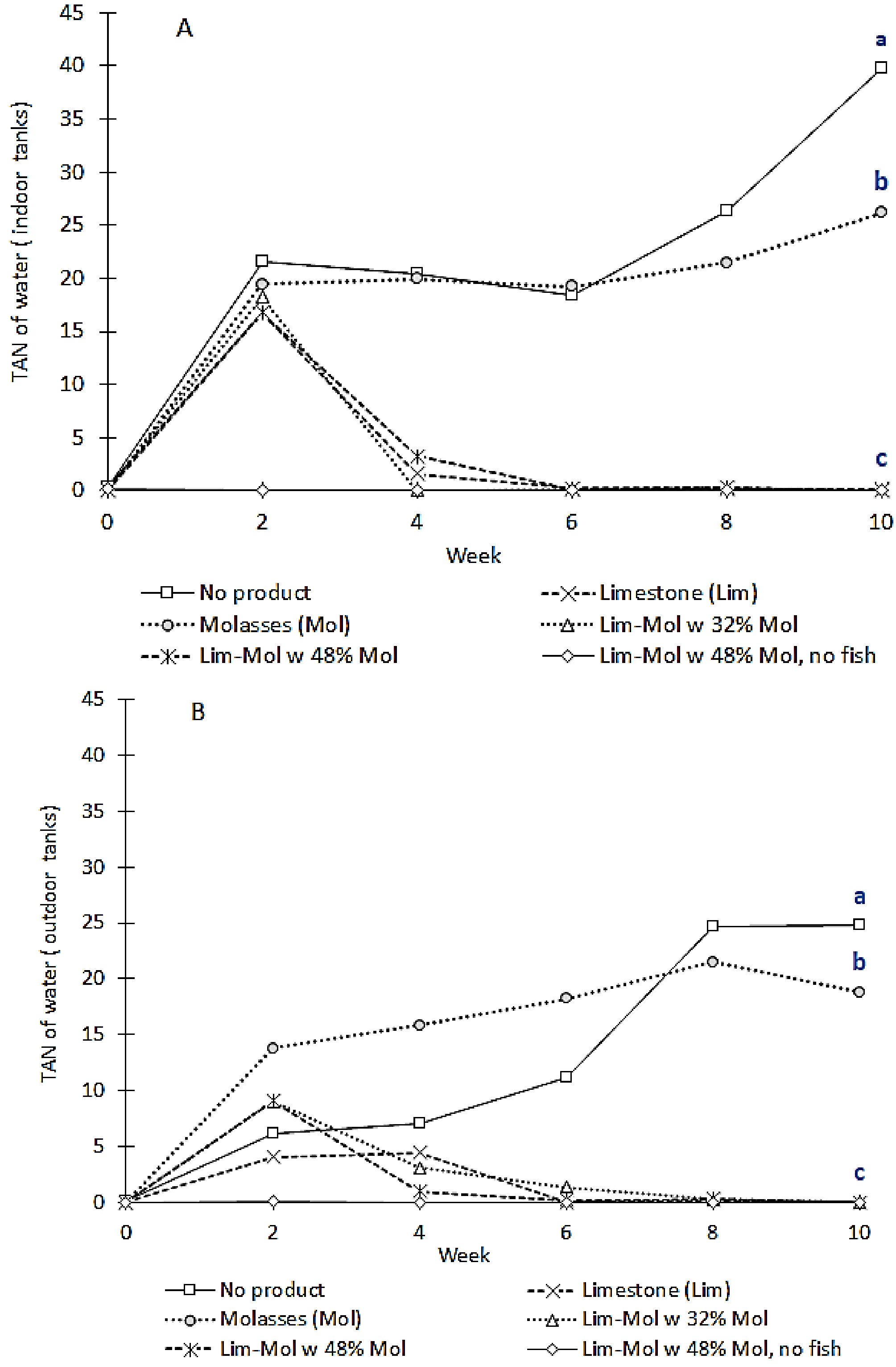

Figure 3. Concentrations of total ammonia nitrogen (mg L^{-1}) in Nile tilapia's rearing A - *indoor* and B - *outdoor* tanks (n = 4). In the last week, means with distinct letters are significantly different between themselves by the Tukey's test (ANOVA P<0.05).

The final concentration of chlorophyll *a (*Cl-*a)* in water was higher in the tanks receiving molasses only (176 ± 69 µg L^{-1}; Figure 4). The applications of agricultural limestone, either alone or in mixture with molasses, did not affect Cl-*a.* Therefore, the applications of the limestone- molasses blends have not stimulated the microalgae growth in the tilapia's rearing tanks. The lowest concentrations of chlorophyll *a* in water were observed in tanks receiving limestone only or the limestone-molasses blend with 48% molasses but no fish, with values equal to 46.6 ± 8.3 µg L^{-1} and 14.5 ± 3.2 µg L^{-1}, respectively.

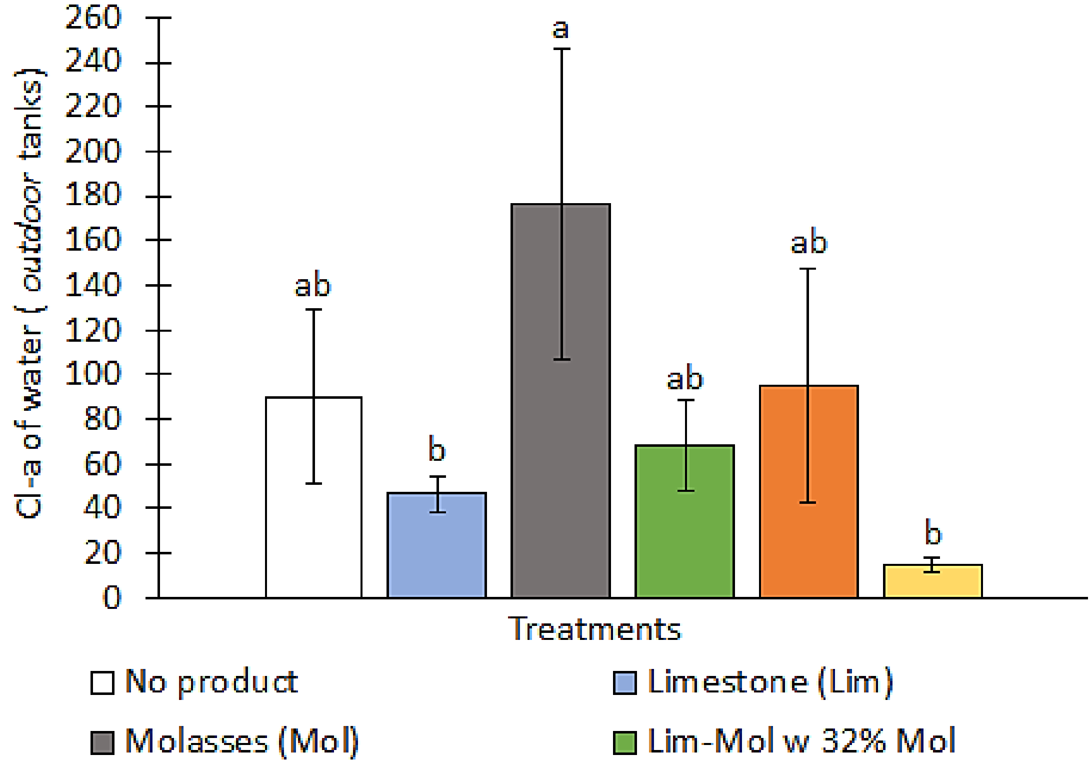

Figure 4. Final concentrations of chlorophyll *a* (Cl-*a*; µg L^{-1}*)* in Nile tilapia's outdoor rearing tanks (n = 3). Columns with distinct letters are significantly different between themselves by the Tukey's test (ANOVA P<0.05).

The pH of the water increased slowly in all treatments over the 24-h cycle (Figure 5). The 11-h pH at the tanks that received the limestone-molasses blends was higher than in the units with no product or that received molasses only (P<0.05). The pH of water began to fall after 14 h of afternoon, mainly in the tanks with no product, molasses, or limestone only. Since Cl-*a* in the tanks receiving limestone only was not higher than in the units that received the limestone-molasses blends (Figure 4), the greatest pH variations in the former tanks were probably due to their lower total alkalinity (Figure 2).

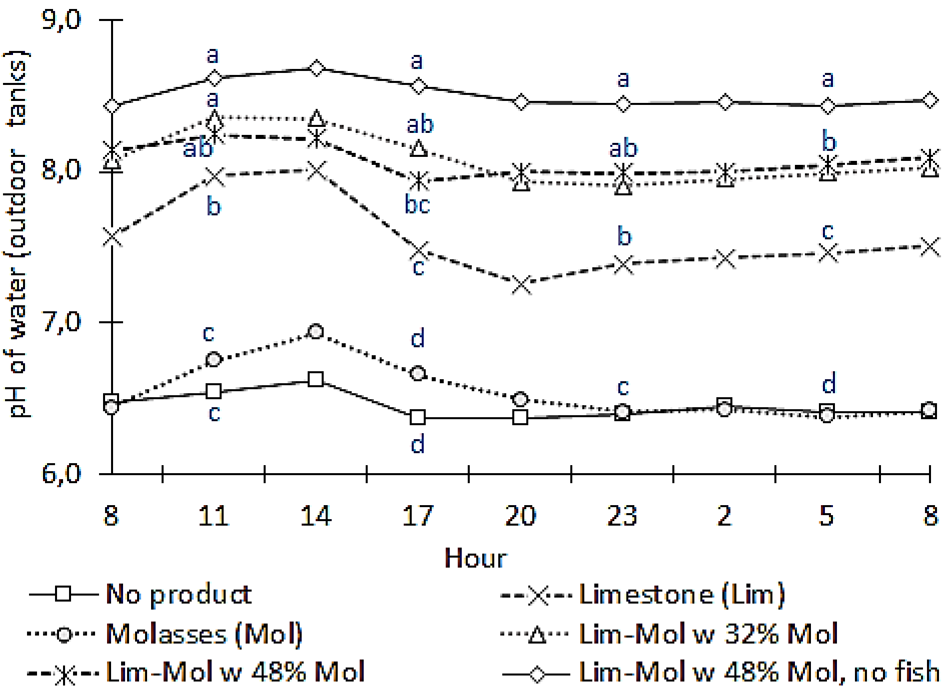

Figure 5. 24-h pH of water in Nile tilapia's outdoor tanks (n = 4). Means with different letters at 11, 17, 23 and 5 h are significantly different between themselves by the Tukey's test (ANOVA P<0.05).

Growth performance

After eleven weeks, no significant differences were observed between the treatments for fish survival with values of 82.5 ± 5.2% and 93.9 ± 1.6% for the indoor and outdoor tanks, respectively (Table 2). In the indoor tanks, no differences were observed between the treatments for fish final body weight (P<0.05; 25.3 ± 1.7 g). On the other hand, the fish final body weight in outdoor tanks was higher than in the units with no product or molasses only, when compared to the tanks that received limestone only or the limestone-molasses blend with 32% molasses (P<0.05; Table 2). Fish yield was also significantly higher in the former tanks (Table 2).

In both systems, the FCR was higher in the tanks that received applications of the limestone-molasses blend with 32% molasses than in the units with molasses only (P<0.05; Table 2). The PER results in the limestone-only indoor tanks were higher than in the units with no product or that received the limestone-molasses blend with 32% molasses. Those results were higher in the outdoor tanks with no product or molasses only applications in comparison with the units receiving limestone only or the limestone-molasses blend with 32% molasses (P<0.05; Table 2).

4. Discussion

Water quality

Since highest pH values were observed in the tanks that received the limestone-molasses blends, the mixture of molasses with limestone boosted the dissolution of $CaCO_3$ in water. Therefore, it would be advisable to apply the limestone-molasses blends in fish tanks, instead of limestone only to control the pH of water more effectively. The limestone-molasses blend with 32% molasses had a better cost: benefit ratio than that for the limestone-molasses blend with 48% molasses, since it used less molasses and affected similarly the pH of water. Lack of statistical significancy for the pH results between the indoor and outdoor tanks suggest that the products applied in the present work had a stronger effect on the pH of water than that from the phytoplankton activity.

Table 2. Growth performance of Nile tilapia juveniles in indoor and outdoor rearing tanks after eleven rearing weeks (mean ± d.p.; n = 4).

Variable	Sys.	Treatment: No product	Limestone (Lim)	Molasses (Mol)	Lim-Mol 32% Mol	Lim-Mol 48% Mol	P-value
Survival (%)	In	84.4 ± 12	78.1 ± 12	81.2 ± 12	78.1 ± 21	90.6 ± 12	ns[1]
	Out	94.4 ± 4	94.4 ± 6	93.1 ± 5	95.8 ± 5	91.7 ± 5	ns
	P-val	ns	ns	ns	ns	ns	
Final weight (g fish^{-1})	In	23.9 ± 2	26.7 ± 4	26.7 ± 3	26.1 ± 7	23.0 ± 4	ns
	Out	28.2 ± 1 a[2]	25.6 ± 2 b	28.9 ± 2 a	25.5 ± 1 b	27.9 ± 2 ab	< 0.05
	P-val	< 0.05	ns	ns	ns	ns	
Final fish biomass (kg m^{-3})	In	1.61 ± 0.13	1.67 ± 0.25	1.73 ± 0.19	1.63 ± 0.44	1.67 ± 0.29	ns
	Out	1.92 ± 0.1 a	1.74 ± 0.1 b	1.94 ± 0.1 a	1.76 ± 0.1 b	1.84 ± 0.1 ab	< 0.05
	P-val	< 0.05	ns	ns	ns	ns	
SGR[3] (% day^{-1})	In	2.76 ± 0.1	2.94 ± 0.2	2.99 ± 0.1	2.88 ± 0.3	2.75 ± 0.5	ns
	Out	3.01 ± 0.1	2.89 ± 0.1	2.99 ± 0.1	2.84 ± 0.1	2.97 ± 0.1	ns
	P-val	< 0.01	ns	ns	ns	ns	
Fish yield (g m^{-3} day^{-1})	In	20.8 ± 1 ab	21.3 ± 1 ab	22.2 ±1 a	19.9 ±1 b	21.3 ±1 ab	< 0.01
	Out	24.9 ±0.5 a	22.5 ± 1 b	25.1 ±1 a	22.8 ± 1 b	23.9 ±2 ab	< 0.01
	P-val	< 0.01	ns	< 0.01	< 0.01	< 0.05	
FCR[4]	In	1.09 ±0.1 ab	1.05±0.1 ab	0.99±0.1 b	1.13±0.1 a	1.05±0.1 ab	< 0.01
	Out	0.96±0.1 b	1.07±0.1 a	0.95±0.1 b	1.06±0.1 a	1.01±0.1 ab	< 0.01
	P-val	< 0.01	ns	ns	ns	ns	
PER[5]	Ind	2.3 ±0.1 b	2.4 ± 0.1 ab	2.5 ± 0.1 a	2.2 ± 0.1 b	2.4 ± 0.1 ab	< 0.01
	Out	2.6 ± 0.1 a	2.3 ± 0.1 b	2.6 ± 0.1 a	2.3 ±0.1 b	2.5 ± 0.2 ab	< 0.01
	P-val	< 0.01	ns	ns	ns	ns	

[1] Not significant (P>0.05). [2] In a same line, means with distinct letters are significantly different between themselves by the Tukey's test (P<0.05). In a same column, means of a same variable with a P-value lower than 0.05 are significantly different between themselves. [3] Specific growth rate (SGR) = [(ln final weight - ln initial weight)/rearing days] x 100. [5] Feed conversion ratio (FCR) = artificial diet allowed (g)/fish body weight gain (g). [5] Protein efficiency ratio (PER) = fish weight gain (g)/dietary protein allowance(g).

The application of the limestone-molasses blends in water increased total alkalinity faster than in the tanks with limestone only, suggesting a higher dissolution rate of agricultural limestone in the former units. Similar results were observed by Han and Boyd (2018) who obtained a final total alkalinity of 45 mg L^{-1} in the aquaria with limestone, but a total alkalinity of 124 mg L^{-1} in the units where limestone was applied onto an organic substrate. The presence of fish in the tanks promoted a greater alkalinity increase due to the release of CO_2 from the respiration and decomposition of organic matter. The tanks without fish required more time to reach a same concentration of alkalinity in comparison with their respective populated tanks. As the alkalinity increase was higher in the tanks receiving the limestone-molasses blend with 48% molasses, the higher amount of molasses in the mixture promoted a greater dissolution of limestone in water. Waters with higher concentrations of alkalinity afford more osmotic comfort to the farmed animals and reduce the toxicity of gases such as NH_3 and H_2S (Sipaúba-Tavares et al. 2006).

The applications of agricultural limestone to the tanks, either alone or blended with molasses, contributed significantly to the control of TAN in water. Limestone removes ammonia by raising the pH of water and promoting a greater formation of its non-ionized form (NH_3), which is volatile. NH_3 could be transferred to the atmosphere by the vigorous mechanical aeration of water. The nitrification process also reduces ammonia by converting it to nitrite and nitrate (Avnimelech 2006; Ebeling et al. 2006). Therefore, the applications of agricultural limestone in the present work may have favored nitrification in the tilapia tanks. Ebeling et al. (2006) stated that approximately 7.1 g of alkalinity are consumed for every 1.0 g of TAN that is oxidized to nitrate. The reduction of total alkalinity in the tanks with no product and with molasses only might have negatively affected nitrification, leading to TAN accumulation. Those results confirm Furtado et al. (2015) who observed higher TAN concentrations in the BFT *L. vannamei* tanks with lower total alkalinity.

As for primary productivity, the applications of molasses in water have contributed to the phytoplankton growth. Silva et al. (2018) concluded that molasses, more than sugar or cassava starch, was the organic carbon source that most benefited the phytoplankton in tilapia BFT tanks. In our study, as the pH of water was lower in the tanks receiving molasses only, their concentrations of free CO_2 were higher in relation to the tanks with the limestone applications. Consequently, there was a greater stimulus to the primary productivity in the molasses-only tanks. Since the final concentration of Cl-*a* was lower in the tanks receiving limestone than in the molasses-only units, the phytoplankton growth might have been lessened by the applications of limestone in water. Limestone reduces the concentrations of available CO_2 to microalgae by raising the water pH. Moreover, the pH increase transformed a higher proportion of NH_4^+ into NH_3, which is the total ammonia volatile form. Part of the NH_3 was probably lost to the atmosphere by the intense aeration carried out, making it unavailable to phytoplankton uptake. Different results were obtained by Queiroz et al. (2016) who observed that the large applications of agricultural limestone did not affect the phytoplankton blooms in fish tanks.

The lower concentration of chlorophyll *a* in the tanks without fish indicates that the lack of artificial feed input has restricted the phytoplankton growth. After the decomposition of feces and other organic debris, the concentrations of nutrients in water are increased, favoring the phytoplankton development (Kim et al. 2020). The 24-h cycle changes of water pH depend mainly on the photosynthetic activity and the water's alkalinity. In waters of higher algal biomass and lower alkalinity, the pH of water tends to present greater variations over the 24-h cycle. According to Boyd et al. (2016), the daily pH amplitude in water will depend on its buffering capacity, that is, its total alkalinity.

Because of the low solubility of limestone in water (Cavalcante and Sá 2010), the release of carbonates in the limestone-only tanks was probably lower than in the units receiving the limestone-molasses blends. Therefore, it is suggested that the limestone-molasses blends afforded a greater physical-chemical stability to the tilapia rearing tanks than the other treatments. The greatest variations in the water pH were observed during the day, and the nocturnal pH remained practically constant. With no photosynthesis at night, the pH of water usually decreases due to the accumulation of free CO_2 in water (Macedo et al. 2010). As that event has not happened in the present study, the intense mechanical aeration carried out probably eliminated significant amounts of CO_2 to the atmosphere, thus avoiding the pH reduction.

Growth performance

In the present work, the Nile tilapia's growth performance results suggest that *O. niloticus* growth is higher when the fish are stocked in phytoplankton-rich tanks supplied with moderately acidic waters ($6.0 < pH < 6.5$). That condition was observed in the tanks with no limestone applications, i.e., no product applied or the molasses-only units. Therefore, the applications of limestone in water, either alone or blended with molasses, negatively affected the fish yield, suggesting that Nile tilapia juveniles have a better growth performance in acidic waters. Those results confirm Rebouças et al. (2015) who observed favorable growth of Nile tilapia reared in green waters with a pH as low as 5.5. On the other hand, they diverge from Martins et al. (2019) who obtained the best tilapia growth in waters with pH between 6.5 and 7.5. That is a subject deserving further studies.

In the tilapia indoor tanks, the highest final fish biomass was 1.73 ± 0.2 kg m^{-3} for the molasses-only treatment, followed by the limestone-only (1.67 ± 0.2 kg m^{-3}), the limestone-molasses 48% (1.67 ± 0.3 kg m^{-3}), the limestone-molasses 32% (1.63 ± 0.4 kg m^{-3}) and the no-product tanks (1.61 ± 0.1 kg m^{-3}; Table 2). Those means, however, were not significantly different between themselves ($P>0.05$). Nevertheless, the indoor results for final fish biomass suggest that fortnight applications of liquid molasses could be beneficial to Nile tilapia's growth performance. Molasses applications in fish tanks are known method to reduce ammonia levels in water by bioflocs absorption (Khanjani et al. 2022). It is expected a better tilapia growth in low-ammonia rearing tanks. On the other hand, significant differences for final fish biomass were observed in the tilapia outdoor tanks, as follows: the highest results were for the molasses-only (1.94 ± 0.1 kg m^{-3}) and the no-product treatments (1.92 ± 0.1 kg m^{-3}) which were equivalent between themselves but greater than for the limestone-only (1.74 ± 0.1 kg m^{-3}) and limestone-molasses 32% tanks (1.76 ± 0.1 kg m^{-3}; $P<0.05$; Table 2). The limestone-molasses 48% tanks assumed an intermediate position (1.84 ± 0.1 kg m^{-3}). The suggestion that regular molasses applications would be favorable to Nile tilapia tanks has been confirmed by the outdoor tanks results. However, the final fish biomass for the no-product tanks were equally good than the former ones. Therefore, there would be no economic rationale to systematically apply molasses in Nile tilapia tanks. More importantly, regular applications of limestone in tilapia tanks might impair fish growth performance. Thus, Nile tilapia juveniles' rearing tanks probably do not require frequent liming as it happens to *Litopenaeus vannamei*' tanks.

5. Conclusions

The blend between liquid molasses with agricultural limestone accelerated the dissolution rates of limestone in water. That was proved by the greater total alkalinity of the tanks receiving the limestone-molasses blend with 48% molasses. Nevertheless, the growth performance of the Nile tilapia juveniles reared in those tanks was negatively affected. Therefore, the liming practices leading to faster increases of water alkalinity are not suitable for Nile tilapia's rearing tanks. It has been concluded that the blending between limestone and molasses brings no clear benefits to Nile tilapia's rearing tanks.

Authors' Contributions: APOLIANO, M.L.S.: conception and design, acquisition of data, analysis and interpretation of data, and drafting the article,; LIMA, F.R.S.: acquisition of data and analysis and interpretation of data; CAVALCANTE, D.H.: conception and design and analysis and interpretation of data; DO CARMO E SÁ, M.V.: conception and design, analysis and interpretation of data, and critical review of important intellectual content All authors have read and approved the final version of the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

Ethics Approval: The research project (protocol number CEUA 3801290120) was approved by the Ethic Committee on Animal Use of the Federal University of Ceará (CEUA-UFC) in the meeting of 02/21/2020.

Acknowledgments: The authors would like to thank the funding for the realization of this study provided by the Brazilian agencies FAPITEC/SE (Fundação de Apoio à Pesquisa e à Inovação Tecnológica do Estado de Sergipe - Brasil), through the PROMOB (Academic Mobility Program), Finance Code 1780/2014, CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil), Finance Code 001, and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico - Brasil), Finance Code 307808/2018.

References

AVNIMELECH, Y. Bio-filters: The need for a new comprehensive approach. *Aquacultural Engineering*. 2006, **34** (3), 172-178. https://doi.org/10.1016/j.aquaeng.2005.04.001

BOYD, C.E. Use of agricultural limestone and lime in aquaculture. *CAB Reviews*. 2017, **12** (15), 1-10. https://doi.org/10.1079/PAVSNNR201712015

BOYD, C.E. and TUCKER, C.S. Handbook for aquaculture water quality. 1th ed. Auburn: Craftmaster Printers, 2014.

BOYD, C.E., TUCKER, C.S. and SOMRIDHIVEJ, B. Alkalinity and Hardness: Critical but Elusive Concepts in Aquaculture. *Journal of the World Aquaculture Society*. 2016, **47** (1), 1-36. https://doi.org/10.1111/jwas.12241

CAVALCANTE, D.H., et al. Effects of $CaCO_3$ liming on water quality and growth performance of fingerlings of Nile tilapia, *Oreochromis niloticus*. *Acta Scientiarum Animal Sciences*. 2009, **31** (3), 327-333. https://doi.org/10.4025/actascianimsci.v31i3.6263

CAVALCANTE, D.H., et al. Imbalances in the hardness/alkalinity ratio of water and Nile tilapia's growth performance. *Acta Scientiarum Technologyy*. 2014, **36** (1), 49-54. https://doi.org/10.4025/actascitechnol.v36i1.18995

CAVALCANTE, D.H. and SÁ, M.V.C. Efeito da fotossíntese na alcalinidade da água de cultivo da tilápia do Nilo. *Revista Ciência Agronômica*. 2010, **41** (1), 67-72. https://doi.org/10.5935/1806-6690.20100009

CLESCERI, L.S., GREENBERG, A.E. and EATON, A.D. Standard methods for the examination of water and wastewater. 20th ed. Washington: American Public Health Association, 1998.

EBELING, J.M., TIMMONS, M.B. and BISOGNI, J.J. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia–nitrogen in aquaculture systems. *Aquaculture*. 2006, **257** (1-4), 346-358. https://doi.org/10.1016/j.aquaculture.2006.03.019

FURTADO, P. S., POERSCH, L. H., WASIELESKY, W. The effect of different alkalinity levels on *Litopenaeus vannamei* reared with biofloc technology (BFT). *Aquaculture International.* 2015, **23** (1), 345-358. https://doi.org/10.1016/j.aquaculture.2006.03.019

HAN, Y. and BOYD, C. E. Effect of organic matter concentration on agricultural limestone dissolution in laboratory soil–water systems. *Aquaculture Research*. 2018, **49** (10), 3451-3455. https://doi.org/10.1111/are.13810

KHANJANI, M.H., SHARIFINIA, M. and HAJIREZAEE, S. Recent progress towards the application of biofloc technology for tilapia farming. Aquaculture. 2022, 552, 738021. https://doi.org/10.1016/j.aquaculture.2022.738021

KIM, K.B., et al. Stochastic modeling of chlorophyll-a for probabilistic assessment and monitoring of algae blooms in the Lower Nakdong River, South Korea. *Journal of Hazardous Materials*. 2020, **400** (1), 123066. https://doi.org/10.1016/j.jhazmat.2020.123066

MACEDO, C.F. and SIPAÚBA-TAVARES, L.H. Eutrophication and water quality in pisciculture: consequences and recommendations. *Boletim do Instituto de Pesca*. 2010, **36** (2), 149-163.

MARTINS, G.B., et al. Growth, water quality and oxidative stress of Nile tilapia *Oreochromis niloticus* (L.) in biofloc technology system at different pH. *Aquaculture Research*. 2019, **50** (4), 1030–1039. https://doi.org/10.1111/are.13975

MARTINS, G.B., et al. The utilization of sodium bicarbonate, calcium carbonate or hydroxide in biofloc system: water quality, growth performance and oxidative stress of Nile tilapia (*Oreochromis niloticus*). *Aquaculture*. 2017, **468** (1), 10-17. https://doi.org/10.1016/j.aquaculture.2016.09.046

NOBRE, M.K.B., et al. Alternative liming blends for fish culture. *Acta Scientiarum Animal Sciences*. 2014, **36** (1), 11-16. http://dx.doi.org/10.4025/actascianimsci.v36i1.21282

PENG, M., et al. Tolerance, Growth, and Physiological Responses of the Juvenile Razor Clam (*Sinonovacula constricta*) to Environmental Ca^{2+} and Mg^{2+} Concentrations. *Frontiers in Physiology*. 2019, **10** (1), 1-14. https://doi.org/10.3389/fphys.2019.00911

QUEIROZ, J.F., BOEIRA, R.C., NICOLELLA, G. *Efeitos da aplicação de grandes quantidades de calcário agrícola em viveiros de piscicultura*. 1th ed. São Paulo: Embrapa Meio Ambiente, 2016.

QUEIROZ, J.F., et al. Lime application methods, water and bottom soil acidity in freshwater fish ponds. *Scientia Agricola*. 2004, **61** (1), 469-475. https://doi.org/10.1590/S0103-90162004000500001

REBOUÇAS, V.T., et al. Tolerance of Nile tilapia juveniles to highly acidic rearing water. *Acta Scientiarum. Animal Sciences*. 2015, **37** (1), 227-233. http://doi.org/10.4025/actascianimsci.v37i3.27031

SÁ, M.V.C., CAVALCANTE, D.H., LIMA, F.R.S. Dissolution rates of hydrated lime, $Ca(OH)_2$ in fresh, oligohaline, mesohaline and euhaline waters and significance it's for liming of shrimp culture ponds. *Aquaculture Research*. 2019, **50** (6), 1618-1625. https://doi.org/10.1111/are.14039

SILVA, U.L., et al. Response of Phytoplankton to Different Carbon Sources and C:N Ratios in Tilapia Fingerling Culture With Bioflocs. *Boletim do Instituto de Pesca*. 2018, **44** (2), 1-8. https://doi.org/10.20950/1678-2305.2018.255

SIPAÚBA-TAVARES, L.H., CELESTE, C.C. and BRAGA, F.M.S. Efeito do óxido de cálcio sobre variáveis limnológicas em viveiros de criação de *Piaractus mesopotamicus* (pacu) e *Colossoma macropomum* (tambaqui). *Boletim do Instituto de Pesca*. 2006, **32** (2), 191-198.

WHANGCHAI, N., et al. Strategies for alkalinity and pH control for ozonated shrimp pond water. *Aquacultural Engineering*. 2004, **30** (1-2), 1-13. https://doi.org/10.1016/j.aquaeng.2002.11.001

Received: 27 September 2021 | **Accepted:** 20 December 2022 | **Published:** 31 March 2023

This is an Open Access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.