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The utilization of sodium bicarbonate, calcium carbonate or hydroxide in biofloc system: water quality, growth performance and oxidative stress of Nile tilapia (*Oreochromis niloticus*)

Gabriel Bernardes Martins [a,*], Fábio Tarouco [b], Carlos Eduardo Rosa [b], Ricardo Berteaux Robaldo [a,c]

[a] Instituto de Oceanografia, Programa de Pós Graduação em Aquicultura, Universidade Federal do Rio Grande - FURG, CP 474, 96201-900, Rio Grande - RS, Brazil
[b] Instituto de Ciências Biológicas, Programa de Pós-Graduação em Ciências Fisiológicas – Fisiologia Animal Comparada, Universidade Federal do Rio Grande - FURG, Avenida Itália, Km 8, 96201-900, Rio Grande - RS, Brazil
[c] Instituto de Biologia, Laboratório de Fisiologia Aplicada à Aquicultura, Universidade Federal de Pelotas - UFPel, Campus Universitário, 96160-000, Capão do Leão - RS, Brazil

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ABSTRACT

In *biofloc technology* (BFT) systems, nutrient cycling for microorganisms takes place. This results in minimal or null water exchange. The sum of fish biomass and microorganisms favors alkalinity consumption and, consequently, pH reduction. It is in this context that the present study evaluates alkalinity and pH maintenance using sodium bicarbonate ($NaHCO_3$), calcium carbonate ($CaCO_3$) or calcium hydroxide ($Ca(OH)_2$) on BFT during a Nile tilapia (*Oreochromis niloticus*) nursery. In this study, 25 fishes/tank was distributed in nine experimental units (useful vol. 37.5 L) and the performance was evaluated at 60 days. During the assay, the temperature, oxygen and pH were maintained within the ideal range levels for Nile tilapia growth. All alkalizing compounds were able to pH and alkalinity correction, but when using $NaHCO_3$, the alkalinity and pH were more elevated than the other treatments. Furthermore, at the beginning these assay, the total ammonia (TAN; $NH_3 + NH_4^+$) and NO_2^--N accumulate and it caused a peak, but mostly experiment remained to very low levels because of the total nitrification activity, resulting in NO_3^--N accumulation. Because the non water exchange, at the final experiment the ion Na^+ accumulate when utilized $NaHCO_3$, resulting in level similar to brackish water. While using $CaCO_3$ or $Ca(OH)_2$, the Ca^{2+} ion accumulate, resulting in extremely hard water. Despite this, the fish survival was similar between treatments (about 80%). Moreover, the final weight, daily growth rate and net yield for $NaHCO_3$ and $Ca(OH)_2$ they were higher than $CaCO_3$ treatment. This may have been because of the higher total suspended solids (TSS) and lower protein content of the bioflocs in this treatment. In order to assess the possible physiologic alterations of the fish associated with the production system, the hematocrit, glycemia and plasmatic osmolality were evaluated. Furthermore, the antioxidant capacity against peroxy radicals (ACAP), lipid peroxidation (LPO) and catalase (CAT) and superoxide dismutase (SOD) activities on the gills and liver were also evaluated. There were no differences in biochemical/physiological parameters when the different alkalizing compounds were utilized. The results demonstrate that the use of sodium bicarbonate, hydroxide or calcium carbonate is effective on the alkalinity and pH adjustments of the final proportion of 14.64 ± 0.49, 7.18 ± 0.32 e 24.09 ± 2.32% in relation to the feed consumption, respectively. Thus, the study demonstrates that the use of $NaHCO_3$ and $Ca(OH)_2$ are recommended for alkalinity and pH correction during Nile tilapia nursery on BFT systems, because of the higher growth and net yield, and this sum to less amount of these compounds may represent important economic gain.
Statement of relevance: The results of this manuscript demonstrate:

- Amount of alkalizing compound utilized for maintenance of alkalinity and pH
- Best growth to utilize $NaHCO_3$ and $Ca(OH)_2$
- The importance of evaluate the ionic concentration, specially Na^+ and Ca^{+2}, because the imbalance ionic
- The difficulty to solids control in BFT system.

* Corresponding author.
E-mail address: martins.aqua@gmail.com (G.B. Martins).

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1. Introduction

The Nile tilapia production in *biofloc technology* (BFT) has been well developed due to high productivity, reduction in water consumption and nutrient cycling for bacteria that consumes inorganic nitrogen. For the initial formation of bioflocs, the water is fertilized with organic carbon to the proportion of carbon:nitrogen 15:1, which in turns favors heterotrophic growth (Avnimelech, 1999).

Apart from heterotrophic, the BFT system permits the growth of autotrophic bacteria, or also denominated nitrifying bacteria, which are responsible for ammonia oxidation to nitrate. Although the heterotrophic bacteria also consume alkalinity, the autotrophic microorganisms are responsible for higher inorganic carbon consumption on alkalinity forms (Ebeling et al., 2006; Hargreaves, 2013), favoring the reduction of alkalinity and pH during the production cycle.

For the pH correction of systems with low water renovation, e.g., recirculation aquaculture systems, sodium bicarbonate ($NaHCO_3$) is used. When $NaHCO_3$ dissociates in water, a HCO_3^- base is produced. Besides bicarbonate, lime with limestone, which is the trade name of calcium carbonate ($CaCO_3$), has been traditionally used on aquaculture. This has a slow impact on the alkalinity and pH correction and, when diluted in water, reacts with CO_2, producing Ca^{2+} and HCO_3^- (Thunjai et al., 2004). A further compound that is utilized is the calcium hydroxide or hydrated lime ($Ca(OH)_2$). In water, this produces the neutralization reaction: $Ca(OH)_2 + 2H^+ \rightarrow 2H_2O + Ca^{2+}$, which causes faster pH elevation, mainly when the alkalinity is low.

Both carbonate and calcium hydroxide are interesting alternatives to BFT cultivation. This is because they are both sources of Ca^{2+}, which is important to bioflocs for changing composition (Luo et al., 2013) and increasing the sedimentation rate (Peeters et al., 2011). Ca^{2+} is also important for Nile tilapia to balance relations between the hardness and alkalinity of the water (Cavalcante et al., 2014).

In systems, where high stocking density is applied, a strict evaluation of the biochemical and physiological characteristics would optimize the productive performance. Oxidative stress in fish has previously been evaluated as a biomarker for environmental pollution (Monserrat et al., 2007). Indeed, oxidative parameters are also considered important tools in the evaluation of challenged for the cultivation conditions that are related to the feed (Azaza et al., 2015), temperature changes (Castro et al., 2012), ammonia exposure (Hegazi et al., 2010) and other stress sources like the stocking density (Andrade et al., 2015) and pathogens (Ali et al., 2011). An oxidative stress is characterized by a situation where the antioxidant capacity is not able to compete against the reactive oxygen species (ROS) formation, affecting cellular function by protein, nucleic acids and lipids oxidation (Jones, 2006).

Therefore, in order to improve the technical production of BFT, the maintenance of alkalinity is fundamental to avoid pH oscillations, providing a more stable system for bioflocs and the physiologic status of fish. The present study investigated the alkalinity and pH correction on BFT during a nursery of Nile tilapia, utilizing $NaHCO_3$, $CaCO_3$ and $Ca(OH)_2$, it evaluated the water quality, growth, proximate composition of the bioflocs, hematology and oxidative stress of the Nile tilapia juveniles.

2. Material and methods

2.1. Design experimental

The monosex Nile tilapia juveniles (Premium genetic) were acquired from a commercial hatchery Aquabel (Rolândia, PR, BR) and transported to "Laboratório de Piscicultura do Chasqueiro" (Universidade Federal de Pelotas, RS, BR), where the experiment took place. For acclimation, the fish were maintained on a recirculation system for 15 days until the experiment began.

During acclimation and the experiment, the Nile tilapias were fed with a commercial feed (Guabi, BR) at 08:00, 11:00, 14:00 and 17:00 h, according to Table 1. For the adjustment of feeding (size, crude protein (CP) and feeding rate), the biometrics were realized weekly ($n = 10$ fish/tank).

Table 1
Feeding of Nile tilapia *Oreochromis niloticus* juveniles during acclimation and experiment.

Feed	Wet weight (g)	Feeding rates (biomass%)	Frequency (freq/day)
1 mm - 45%CP	0.5–5	15–7	4
1.7 mm - 42%CP	5–20	6	4
2-4 mm - 40%CP	20–50	4	4

CP – crude protein.

The assay occurred for 60 days between June and August of 2015. The treatments that were tested for alkalinity and pH correction were sodium bicarbonate - $NaHCO_3$ (P.A., Synth, BR), calcium carbonate - $CaCO_3$ (P.A., Synth, BR) and calcium hydroxide - $Ca(OH)_2$ (P.A., Synth, BR), which were all realized in triplicate.

For this experiment, 25 fish/tank were distributed in experimental units (useful vol. 37.5 L) with an initial weight of 3.68 ± 0.93 g and length 6.03 ± 0.50 cm. The tanks had two air stones on the bottom for continued aeration, heaters (100 W) with a thermostat (regulated to 29 °C) and a natural photoperiod. The microbial flocs that were previously produced on tanks stocked with the Nile tilapias and, were inoculated in the biofloc experimental system (10% of the total volume). During the assay, water renovation did not occur and the weekly volume was readjusted due to evaporation and clarifications losses.

When the total ammonia (TAN) reached a value superior to 1.0 mg/L, was added molasses of sugar cane (45% C) for the correction of C:N (15:1) (Avnimelech, 1999). For maintenance, the total suspended solids (TSS) between 400 and 600 mg/L was realized and, when exceed these value, 25% of the water were removed for sedimentation on a collector of solids for 1 h. After this period, the supernatant was returned to the tank and the sediment was discarded.

The alkalinity and pH correction occurred daily. After the ultimate feeding, the amount of the alkalinizing compound was calculated according to the values of the daily pH. The amount of alkalizing compound utilized was determinate according to previous laboratory practices, that for security of fishes and bioflocs determine the utilization (minimal–maximal) of 5.0–100, 10–250, 2.0–50 mg/L of $NaHCO_3$, $CaCO_3$ and $Ca(OH)_2$, respectively. On the first days of experiment, was used the lower concentration of alkalizing compound, and it was sequentially increased until reached the desired pH. The final assay was the total quantity of alkalinizing compound (total AC) utilized and the consumption of this compound (relative AC%) was determined according to the formula: AC = (total chemical compound (g) × 100)/total food (g).

2.2. Water quality and growth performance

The temperature and oxygen were measured twice a day (07:45 and 16:00) with a digital oxymeter (PRO 20, Yellow Springs, OH, USA). The results are expressed as the daily mean of treatments. The pH was measured at 16:00, utilizing digital pHmeter (HI 2212, HANNA Instrument, Woonsocket, RI, USA). Twice a week waters sample are collected and the total ammonia (TAN) (UNESCO, 1983), nitrite (NO_2^--N) and total alkalinity (APHA, 1998), the total suspended solids (TSS) (Strickland and Parsons, 1972), the floc volume (FV) (Imhoff cone – 30 min) and total hardness (Adad, 1982) were measured. For TAN and NO_2^--N dosages, were collected water samples before the first feeding, while the TSS, FV, alkalinity and hardness the samples were collected about the 13:30.

While the nitrate (NO_3^--N) and orthophosphate (PO_4^-) (Aminot and Chaussepied, 1983), Na^+ (photometer flame, B462, Micronal, BR) e Ca^{2+} (Doles, BR) were only measured at the final assay. The floc volume index (FVI) was determined using the following formula: FVI = FV (mL)/TSS (g) (Yousuf, 2013).

At the end of the assay, all of the fish were quantified and their weight was measured for the evaluation of survival, daily growth rate

(DGR), net yield and feed conversion rate (FCR), according to the following equations:

a) Survival rate = 100 × (Nf-Ni)/Ni
b) DGR = fish biomass increase (g)/time (days)
c) Net yield = fish biomass increase (g) × 1000/Vol (L)
d) FCR = feed supply (g)/fish biomass increase (g)
Where Ni e Nf are the initial and final number of fishes.

2.3. Tissue collection and hematological analyses

At the final experiment, the tissue samples ($n = 6$ fishes/tank) were collected for analyses. The fish were anesthetized in a benzocaine bath (500 mg/L) for blood, gill and liver collection. The blood was withdrawn via the arterial caudal fin (anticoagulant EDTA 10%, 1 mL sterile syringe, 25 gauge needle) and centrifuged (10 min, 1500 ×g) for plasma achievement. After the blood collection, the fish were euthanized for spinal medulla rupture and then the other tissue samples were collected. The samples of the blood plasma, gill and liver were initially maintained at −180 °C and after to −90 °C until the analyses.

Immediately after the blood collection, glucose (Accu Chek Performa, UK) and hematocrit (15 min, 12,000 RPM) were measured, while the plasma was subsequently utilized for the osmolality measurement (Vapro®Vapor Pressure Osmometer, Wescor 5600, Logan, UT).

2.4. Proximate analyses

The proximate analyses of the bioflocs were realized on duplicate. The methodologies utilized are described for AOAC (1999). For the ash content (method access number: #942.05), the samples were burned in muffle for 4 h at 600 °C. The Kjeldhal method was used for the total protein determination (#984.13) after the acid digestion samples and nitrogen distillation. To calculate the protein content, the 6.5 coefficient was used. While for the ethereal extraction (#920.39), Soxhlet extraction was used for 6 h, with petroleum ether as the solvent.

2.5. Biochemical measurements

For biochemical dosages, two pools of two fish/tanks, totaling six pools, were utilized for each treatment. The samples were homogenized (1:4 *w*/*v*) in buffer (specific for each methodology), centrifuged (20.000 ×*g*, 20 min, 4 °C) and the supernatant was utilized. The total protein content was determined in triplicate, utilizing the Biuret assay (Doles, BR) and was realized in a microplate reader (550 nm).

The samples for catalase (CAT) and superoxide dismutase (SOD) activities were homogenized (1:4 *w*/*v*) in buffer adjusted to pH 7.6, containing Tris base (20 mM), EDTA (1 mM), dithiothreitol (DTT, 1 mM), sucrose (500 mM), KCl (150 mM) and phenylmethylsulfonyl fluoride (PMSF, 100 mM).

The CAT was measured for an initial decomposition rate of 50 mM H_2O_2 at 240 nm (Beutler, 1975). The results are expressed as CAT units, where one unit is the enzyme amount that hydrolyzes 1 μmol of H_2O_2 for 1 m and for the protein mg at 30 °C and pH 8.0.

The SOD was determined based on the inhibition and auto-oxidation of epinephrine on the alkali medium of the SOD enzyme, which was monitored at 480 nm. The activity is expressed as U SOD/mg of protein at 30 °C and pH 8.0, where one unit is defined as the enzyme amount that inhibits 50% of epinephrine auto-oxidation (Misra and Fridovich, 1972). For SOD activity, only the liver activity was possible accomplish.

For the antioxidant capacity against peroxy radicals (ACAP) determination, the tissue were homogenized in buffer adjusted to pH 7.75, containing Tris-HCl (100 mM), disodium EDTA (2 mM) e $MgCl_2 \cdot 6H_2O$ (5 mM). This was determined employing the fluorescence dye 2,7-dichlorofluorescein diacetate (H_2DCF-DA – Molecular Probes) in the presence or absence of a peroxyl radical generator 2, 2′-azobis 2 methylpropionamidine dihydrochloride (ABAP; 4 mM; Aldrich), which decomposes at 37 °C producing peroxyl radical (Winston et al., 1998). The difference in the fluorescence area (at excitation and emission wave lengths of 488 nm and 525 nm, respectively) with or without ABAP was considered as the antioxidant capacity measure (Amado et al., 2009).

For the lipid peroxidation (LPO) determination, the samples were homogenized (1:4 *w*/*v*) in methanol (100%, 4 °C). The homogenized samples were centrifuged (1000 ×g for 10 min, 4 °C) and the supernatant was employed for determination. The LPO was measured using the FOX method (Hermes-Lima et al., 1995), which is based on Fe^{2+} oxidation by lipid hidroperoxides (FOX reactive substances) at an acid pH with Fe^{+} complexing the xylenol orange dye. The cumene hydroperoxide was used as the standard. The results are expressed as ηmol cumene hydroperoxide/g tissue.

2.6. Statistic analysis

Initially, the data normality and homoscedasticity were analyzed. The results were compared by ANOVA (One Way) and posterior Tukey test ($p < 0.05$). The analyses were realized on SigmaPlot 12.0 software (Systat Software, Inc., Chicago, IL). Data were presented as average ± standard deviation.

3. Results

3.1. Water quality

The physical and chemical parameters of the water are demonstrated in Table 2. The temperature and dissolved oxygen did not differ among the treatments. The TAN (Fig. 1a) and NO_2^--N (Fig. 1b) values during the experiment did not demonstrate any differences among the treatments and, during most of the experimental period remained at very low levels. Furthermore, at the final assay, NO_3^--N did not demonstrate any differences among the treatments, while the orthophosphate was more elevated for the $NaHCO_3$ treatment than the other treatments.

The alkalinity and pH averages (Table 2) were superior when $NaHCO_3$ was utilized and, in Fig. 2 are shown the daily averages of pHs along the assay. The hardness and Ca^{2+} concentration were higher for the treatments $CaCO_3$ or $Ca(OH)_2$, while the Na^+ was superior for $NaHCO_3$.

Table 2
Water quality in biofloc technology for Nile tilapia nursery utilizing $NaHCO_3$, $CaCO_3$ and $Ca(OH)_2$ for alkalinity and pH correction.

	$NaHCO_3$	$CaCO_3$	$Ca(OH)_2$
Temperature (°C)	27.70 ± 1.17 (24.45–30.6)	27.55 ± 1.22 (24.95–31.1)	27.60 ± 1.16 (23.9–30.3)
O_2 (mg/L)	5.91 ± 0.61 (4.34–7.41)	5.93 ± 0.51 (4.55–7.29)	5.97 ± 0.55 (4.74–7.46)
pH	7.53 ± 0.02 a	7.33 ± 0.03 b	7.41 ± 0.03 ab
Total alkalinity (mg/L $CaCO_3$)	75.76 ± 3.78 a	48.95 ± 0.75 b	54.58 ± 6.87 b
Total hardness (mg/L $CaCO_3$)	101.33 ± 4.23 a	322.44 ± 34.02 b	340.44 ± 17.45 b
TSS (mg/L)	501.47 ± 37.59 a	707.49 ± 49.34 b	577.77 ± 43.43 a
FV (ml/L)	49.75 ± 10.56	44.37 ± 10.67	47.50 ± 4.75
FVI (mL/g)	71.47 ± 21.68 a	41.34 ± 16.75 b	62.54 ± 16.75 ab
TAN (mg/L)	1.17 ± 3.50	0.73 ± 2.69	1.04 ± 3.38
NO_2^--N (mg/L)	7.03 ± 10.61	5.94 ± 10.59	8.14 ± 13.65
NO_3^--N (mg/L)	140.00 ± 34.64	117.50 ± 10.60	143.33 ± 14.43
PO_4^--P (mg/L)	8.30 ± 0.51 a	1.75 ± 0.49 b	3.60 ± 1.9 b
Na^+ (mg/L)	597.05 ± 279.29 a	66.46 ± 14.30 b	72.43 ± 38.58 b
Ca^{2+} (mg/L)	58.54 ± 5.17 a	239.73 ± 25.10 b	299.15 ± 63.99 b
Total AC (g)	120.46 ± 6.05 a	186.56 ± 3.51 b	55.60 ± 3.83 c
Relative AC (%)	14.64 ± 0.49 ab	24.09 ± 2.32 b	7.18 ± 0.32 a

Data represents mean ± S.D. (Tukey, $p < 0.05$). Between parentheses are maximum and minimum values. TSS – total solid suspension; FV – floc volume; FVI – floc volume index; TAN – total ammonia; AC – alkalizing consumption.

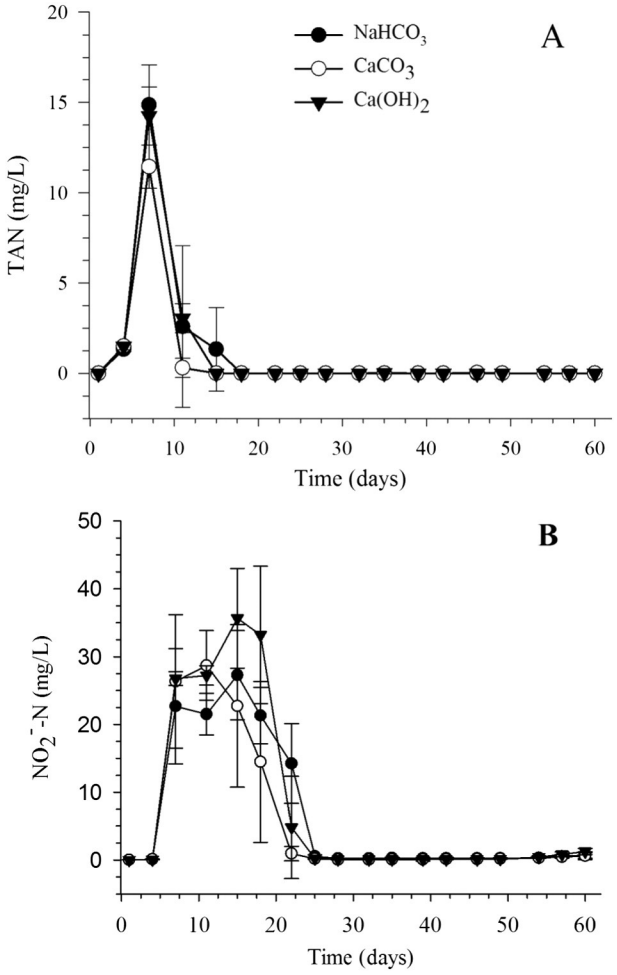

Fig. 1. Mean of total ammonia (TAN; $NH_3 + NH_4^+$) (A) and NO_2^--N (B) on the culture of Nile tilapia *Oreochromis niloticus* in biofloc technology utilizing different chemical compounds for alkalinity correction. Data are mean ± SD of three replicate tanks per sampling time.

Along the experiment, TSS elevation (Fig. 3) occurred and $CaCO_3$ had superior values, while the FV (Table 2) was equal for all of the treatments. However, when the carbonate was utilized, the FVI was lower than the $NaHCO_3$ and equal to $Ca(OH)_2$.

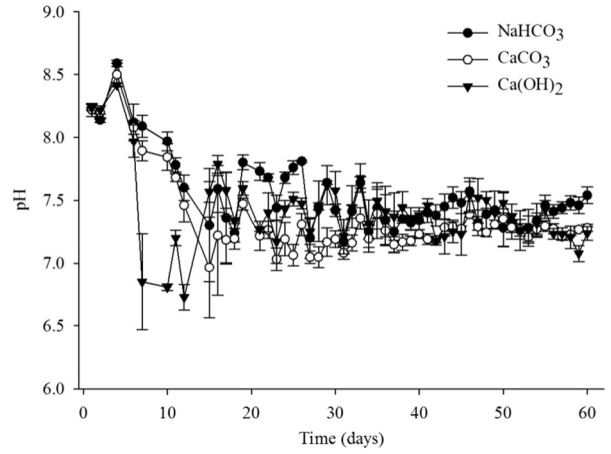

Fig. 2. pH values on the culture of Nile tilapia *Oreochromis niloticus* in biofloc technology utilizing different chemical compounds for pH correction. Data are mean ± SD of three replicate tanks per sampling time.

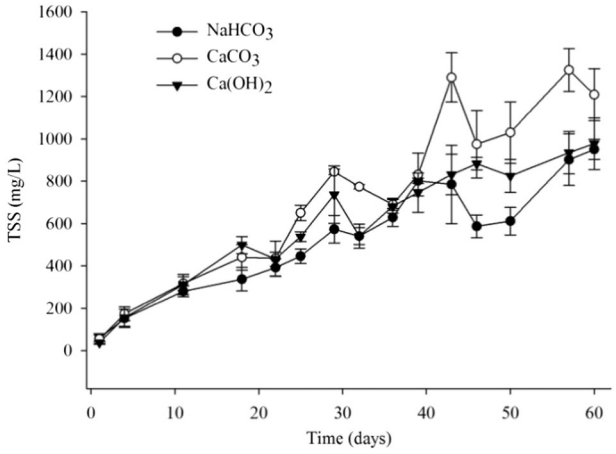

Fig. 3. Total suspended solids (TSS) on the culture of Nile tilapia *Oreochromis niloticus* in biofloc technology utilizing different chemical compounds for alkalinity correction. Data are mean ± SD of three replicate tanks per sampling time.

The total amount of alkalinizing compound (total AC) utilized and the relative amount (relative AC) to feed (Table 2) were superior for CaCO3, followed by the $NaHCO_3$ and $Ca(OH)_2$ treatments.

3.2. Growth and proximate analyses

The growth results are shown in Table 3. The final weight, DGR and net yield were higher for $NaHCO_3$, but statistically equal to $Ca(OH)_2$. This treatment also has final weight, DGR and net yield statistically equal to $CaCO_3$. Meanwhile, the final length, survival and FCR did not differ among the treatments.

The proximate composition of bioflocs is represented in Table 4. The protein and ash content was more elevated for the bicarbonate and calcium hydroxide treatments, while the lipid content did not differ among the treatments.

3.3. Hematological analyses and stress oxidative

The hematological parameters of glucose, hematocrit and osmolality (Table 5) did not demonstrate alterations and were equal among the treatments. Similarly, the CAT (Fig. 4 A) and SOD (Fig. 4 B) activities, as well the ACAP (Fig. 5) and LPO (Fig. 6), did not differ among the treatments.

4. Discussion

During the assay, the water temperature (28–29 °C) and dissolved oxygen (>3 mg/L) levels remained at a rate that is considered ideal for Nile tilapia *O. niloticus* growth (Santos et al., 2013; Tran-Duy et al., 2012). Furthermore, all the treatments were within the pH range that is considered ideal for the species, between 7 and 8 (El-Sayed, 2006;

Table 3
Growth performance and feed utilization of Nile tilapia raised on biofloc technology utilizing $NaHCO_3$, $CaCO_3$ and $Ca(OH)_2$ for pH and alkalinity correction. Fishes (3.68 ± 0.93 g and 6.03 ± 0.50 cm) are stocked in 37.5 L tanks at 25 fishes/tank, during 60 days.

	$NaHCO_3$	$CaCO_3$	$Ca(OH)_2$
Final weight (g)	44.09 ± 0.93 a	38.29 ± 1.29 b	40.57 ± 1.67 ab
Final length (cm)	13.16 ± 0.08	13.07 ± 0.32	13.04 ± 0.12
DGR (g/day)	0.67 ± 0.01 a	0.57 ± 0.02 b	0.61 ± 0.02 ab
Survival rate (%)	80.00 ± 17.43	81.33 ± 12.85	80.00 ± 10.58
Net yield (Kg/m^3)	23.52 ± 0.49 a	20.76 ± 0.70 b	21.64 ± 0.89 ab
FCR	1.18 ± 0.07	1.10 ± 0,16	1.13 ± 0.10

Each value represents mean ± S.D. (Tukey, $p < 0.05$). DGR – daily growth rate and FCR – food conversion rate.

Table 4
Crude protein (CP), crude lipid (CL) and ash (dry basis %) of biofloc maintained at different source correction of pH and alkalinity.

	$NaHCO_3$	$CaCO_3$	$Ca(OH)_2$
CP	16.76 ± 0.38 a	13.20 ± 0.95 b	17.85 ± 1.02 a
CL	2.11 ± 0.29	2.65 ± 0.45	2.63 ± 0.33
Ash	42.82 ± 3.25 a	35.02 ± 0.99 b	44.03 ± 3.63 a

Each value represents mean ± S.D. (Tukey, $p < 0.05$).

El-Sherif and El-Feky, 2009). This suggests that the tested alkalinizing compounds are efficient for alkalinity and pH correction.

At the beginning of the experiment, the sudden elevation of TAN and NO_2^--N occurred in all of the treatments. According to Emerson et al. (1975), that demonstrated the relation between pH and temperature, the maximum values of non-ionized ammonia (NH_3) in present study for the $NaHCO_3$, $Ca(OH)_2$ or $CaCO_3$ were 0.98, 0.96 e 0.74 mg/L, respectively. These averages are nearby to lethal concentration (CL50-96h), estimated in 0.96 mg/L of NH_3 for Nile tilapia juveniles – 12.6 g (Evans and Pasnik, 2006). However, in this study, TAN reduction quickly occurred and, remained very low during most of the experiment.

Still, the NO_2^--N values reached the maximum value of 35.66 mg/L when calcium hydroxide was used. However, this is inferior to CL50-96 h, which Atwood et al. (2001) demonstrated is 81 mg/L for Nile tilapia juveniles – 4.4 g. The NO_2^--N toxicity has mainly been associated with hematocrit and hemoglobin reduction, besides of methemoglobin formation (Yildiz et al., 2006), which impair oxygen transport. This, in addition to the TAN increase, was probably the reason for mortality in all of the treatments, verified at the beginning of the assay. Nevertheless, the survival in this experiment is very satisfactory, about the 80% for all treatments.

The fertilizations with molasses were realized when the TAN reached values higher than 1.0 mg/L, favoring the heterotrophic bacteria's growth. The fertilization were realized on the fourth, fifth and sixth days, utilized 5.0, 5.0 and 4.0 g of molasses, respectively. After the TAN reduction, the fertilization was finalized, initiating the nitrification process. During this process the partial oxidation of ammonia occurred, producing NO_2^--N, as verified between 11 and 15 days of the assay. For the initial period of the assay, the higher TAN and NO_2^--N could be attributed to the low concentration of microorganisms responsible for nitrogen cycling, therefore, indicating that 10% of inoculum not is enough to prevent the increase of TAN and NO_2^--N.

The total hardness at the final assay was very elevated for the $CaCO_3$ and $Ca(OH)_2$ treatments. According to Boyd (1990), the medium values for these treatments are considered extremely hard, while, for $NaHCO_3$, they are moderately hard. However, in this study, the total hardness does not seem to have influenced the growth, as the calcium hydroxide treatment demonstrated the same performance as the sodium bicarbonate. Cavalcante et al. (2012) demonstrates significant improvement on growth of Nile tilapia juveniles to elevation of hardness from about 55 to 150 mg/L $CaCO_3$. But further studies are needed to relate the high values of hardness and Ca^{2+} to the growth of *O. niloticus.*

The formation, structure, stability and size of the bioflocs are dependents of water ionic composition (De Schryver et al., 2008). Previous studies have demonstrated that a higher Ca^{2+} concentration increases the biofloc density, favoring sedimentation (Luo et al., 2013; Peeters et al., 2011). This is directly related to the size and FVI of the bioflocs, which have ideal values above 200 mL/g (De Schryver et al., 2008). In this study, the $CaCO_3$ treatment demonstrated lower FVI values than the other treatments, which represents a higher density and sedimentation velocity.

In addition to Ca^{2+}, the Na^+ also has an important influence on the composition and structure of the flocs, neutralizing the negative charges of the particles, favoring the adhesion (Peeters et al., 2011). The Na^+ concentration for the $NaHCO_3$ treatment was higher than the other

Table 5
Hematologic analyses of tilapia juveniles cultured at different source correction of pH and alkalinity on biofloc system.

	$NaHCO_3$	$CaCO_3$	$Ca(OH)_2$
Glicose (mg/dL)	39.50 ± 3.62	35.00 ± 3.18	37.50 ± 3.50
Hematócrito (%)	32.33 ± 1.13	27.00 ± 3.18	29.00 ± 1.27
Osmolality (mOsm)	316.66 ± 9.81	315.21 ± 9.81	312.00 ± 10.07

Each value represents mean ± S.D. ($p < 0.05$; $n = 6$).

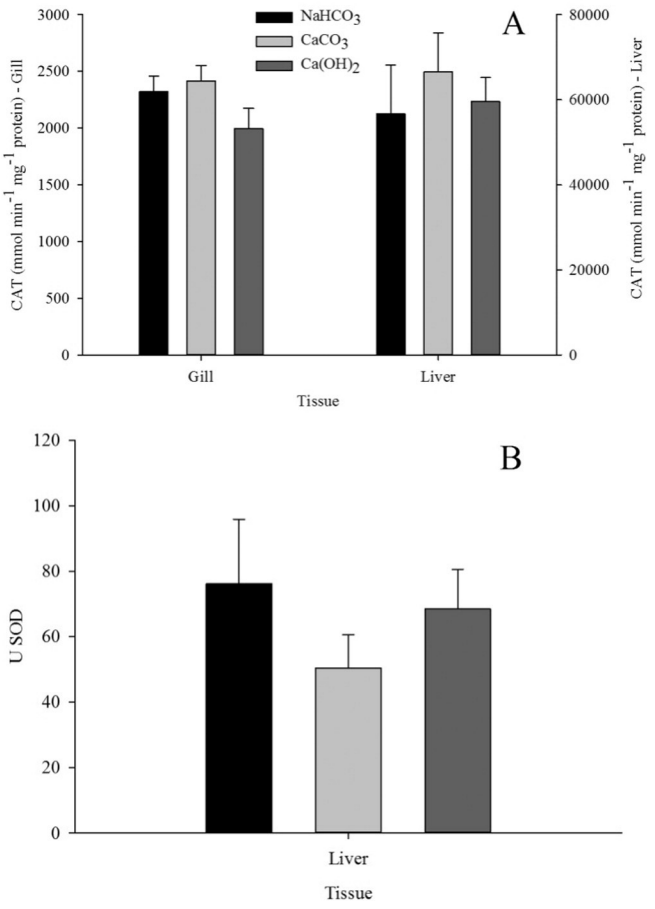

Fig. 4. Catalase (CAT) (A) and superoxide dismutase (SOD) (B) activity in gills and liver of Nile tilapia *Oreochromis niloticus* raised at different alkalizing compound for pH and alkalinity correction.(mean ± SD; $p < 0.05$; $n = 6$).

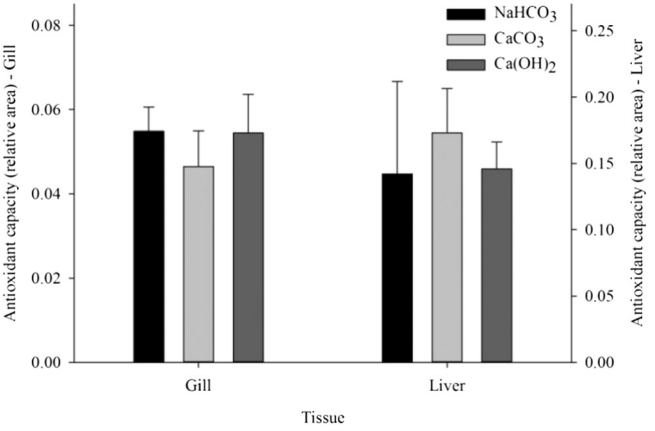

Fig. 5. Antioxidant capacity against peroxyl radicals (relative area) in gills and liver of Nile tilapia *Oreochromis niloticus* raised at different compound chemical for correction alkalinity (mean ± SD; $p > 0.05$; n = 6).

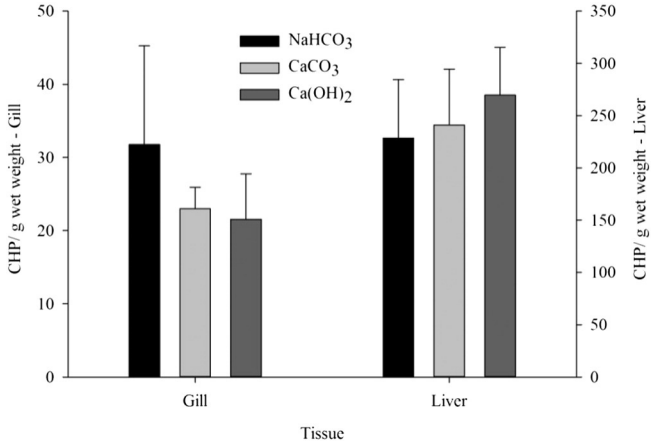

Fig. 6. Lipid Peroxidation in gills and liver of Nile tilapia *Oreochromis niloticus* raised at different compound chemical for correction alkalinity (mean ± SD; $p > 0.05$; n = 6).

treatments, reaching 597.05 ± 279.29 mg/L. Although some studies have demonstrated the brackishwater (6–8‰) benefit on growth of Nile tilapia (Jun et al., 2012; Qiang et al., 2013), this Na^+ concentration was not enough to improve the growth, because the growth performance was similar to $Ca(OH)_2$, reaching Na^+ concentration of 72.43 ± 38.58 mg/L.

The TSS control is considered limiting for the high density system and, previous studies with BFT have demonstrated TSS values superior of 500 mg/L (Avnimelech, 2007; Azim and Little, 2008; Long et al., 2015). Our results showed that, in the last 15 days, the TSS was superior for $CaCO_3$, reaching values that were superior of 1000 mg/L. This may be associated with the chemical characteristics of the alkalizing compound, because a greater total amount of $CaCO_3$ (186.56 ± 3.51 g) was used compared to $Ca(OH)_2$ (55.6 ± 3.83 g).

When $NaHCO_3$ was used, the growth and final density was equal to the $Ca(OH)_2$ and, it was higher compared to the $CaCO_3$. The lesser growth for $CaCO_3$ can be attributed to the sharp rise in the TSS, because high concentration of TSS is associate to gill clogging, causing difficulty on gas and ions exchanges through the gills (Hargreaves, 2013). Moreover, when using $NaHCO_3$, the hardness:alkalinity relation was more proximate to those of Cavalcante et al. (2012), who demonstrated an improvement in the growth of Nile tilapia juveniles, while keeping the ratio at 1:1.

There can be advantages to utilizing the BFT system, favoring the mixed microbiologic system (hetero and autotrophic bacteria). For example, the lower C organic consumption and the TSS produced, as well as the reduction in O_2 and alkalinity consumptions (Ebeling et al., 2006; Browdy et al., 2012). The economy caused by reducing those elements may represent a significant reduction in the production cost, mainly related to less cost with water pumping (for clarification) and aeration power, and also less use of molasses. In this context, Ray and Lotz (2014) compared the performance of white shrimp *Litopenaeus vannamei* on BFT utilizing a hetero and chemoautotrofic system. They confirmed that the second system leads to a reduction in oxygen consumption and lower TSS production, without a loss of growth.

In the present study, there was high consumption of alkalizing compound. Due to the chemical characteristics of each compound, there were differences in consumption, which influenced the proportion that was utilized in relation to the feed. The calcium hydroxide had a capacity to increase the pH quickly, mainly when there was low alkalinity. Therefore, it can be said that it must be used with caution. Ganguly et al. (1999) reported these characteristics when they utilized 0.1–0.2 g/L. They assign the bactericidal capacity to the quick increase in pH, causing damage to the bacterial cells. Our results did not demonstrate any damage to the bioflocs or fish due to the calcium hydroxide, using the maximum daily concentration of 0.048 g/L. This was a less value than that reported by Furtado et al. (2011), when 0.15 g/L lime was employed without causing damage to bioflocs and white shrimp L. *vannamei*.

The PO_4-P levels on the final assay remained low when the $CaCO_3$ or $Ca(OH)_2$ was utilized. The reaction between Ca^{2+} and PO_4-P, producing calcium phosphate ($Ca_3(PO_4)_2$ and mineral phosphate (Li et al., 2012), was probably responsible for the lower levels of treatment with high Ca^{2+} concentration. Luo et al. (2014) also obtained low PO_4-P on yours results, however they attributed the low values to the assimilation of microorganisms present on the bioflocs, suggesting the cycling of phosphorus in addition to nitrogen. This immobilization/cycling of PO_4-P on medium is an important result, because 80–90% of the phosphorus utilized in the feed is released in the production system (Barak et al., 2003).

This is the best utilization of nutrients on BFT occur due to the absorption for the organisms that compose the bioflocs and, the harvesting of Nile tilapia. Ekasari et al. (2014) demonstrated that juveniles of Nile tilapia (9.6 g) consume around 90 g TSS/Kg wet weight. From this perspective, the biofloc represents an extra source of protein, lipid, carbohydrate and energy.

The protein content of bioflocs in all treatments presented values below those previously reported by Azim and Little (2008) and López-Elías and Moreno-Arias (2015). However, the ash content was more elevated and previous researches with Nile tilapia have demonstrated ash values between 7 and 40% (Azim et al., 2007; Ekasari et al., 2010; López-Elías and Moreno-Arias, 2015). These results probably occurred because of the intense harvesting of Nile tilapia and intense biofloc removal (clarification). Ray et al. (2010) demonstrated that increased on clarification reduced the nutrient content of biofloc on *Litopenaeus vannamei* production. These two factors result in a lower C:N relation in the system, favoring the development of autotrophic bacteria, which are responsible for the high consumption of alkalinizing compound and, consequently results in a higher ash content because the utilization of alkalizing compound and incorporation of ions in the bioflocs.

The hematological index and biochemical evaluations gave insights into the health status of the fish. The glucose and hematocrit did not demonstrate any differences among the treatments and were within the range that is considered normal for Nile tilapia (Bittencourt et al., 2003). These hematological parameters associated with none alteration in antioxidant capacity evaluation can be reflective of the favorable conditions.

The SOD and CAT enzymes act in a highly coordinated system. The alteration in the activity of both enzymes is related to the conditions of the environment that causes oxidative stress, such as management (Braun et al., 2010), hypoxia (Welker et al., 2013) and ammonia (Sinha et al., 2014). The SOD is part of the antioxidant system and, is responsible for the conversion of superoxide anions O_2^- on less toxic products H_2O_2 and O_2 (Gaté et al., 1999). The CAT catalyzes the removal of H_2O_2, transforming in water and molecular oxygen. In the present study, there were no differences in the CAT and SOD activities. Furthermore, there were no alterations on the antioxidant capacity and lipidic peroxidation. Thus, our results demonstrated similar and favorable conditions for growth when using different alkalinizing compounds.

5. Conclusions

The sodium bicarbonate utilization, calcium hydroxide or carbonate are effectives on the alkalinity and pH correction on BFT systems. Due to the lower capacity of calcium carbonate of correct the pH and alkalinity, there was utilized higher amount from these compound. This significantly contributed to the increase in the TSS, which reached superior values of 1000 mg/L and, high TSS associated with a low protein content of bioflocs, which could be responsible for the diminished growth in these treatments. However, when evaluating the hematological index and oxidative stress, the alterations were not verified, indicating similar conditions among the treatments. Thus, the best alternatives for alkalinity and pH correction are sodium bicarbonate or calcium hydroxide.

More research is necessary in order to evaluate the water quality, mainly related to the interaction and efficiency of bacteria, both autotrophics and heterotrophics, as also ionic composition of reuse water for seeking the maximum efficiency of growth in BFT system.

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