Usage of alkalizers in the nursery culture of *Piaractus brachypomus* with Biofloc technology - BFT

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Abstract. During 19 days, was assessed the application of three different alkalizers on the water quality and performance of *Piaractus brachypomus* fingerlings cultivated with Biofloc technology (BFT). Three treatments were tested, T1: Sodium bicarbonate ($NaHCO_3$), T2: Calcium hydroxide ($Ca(OH)_2$) and T3: Calcium and magnesium carbonate ($MgCa(CO_3)_2$) which were performed in triplicate. It was worked with animals of 5.65±1.59 g of weight and 6.70±0.59 cm of length, using 354 fingerlings per tank and a total density of 8 kg m^{-3}. Alkalinity and pH of T1 was significantly higher than the other treatments except with the T2 that did not differ statistically. The highest concentration of alkalinity and pH was not associated with the best treatment in terms of zootechnical parameters. T2 was the best treatment in terms of weight (15.52 g), final length (8.42 cm), growth rate (0.59 g D^{-1}), survival (99.18%), final biomass (21.99 kg m^{-3}) and feed conversion (0.68). Results indicate that application of $Ca(OH)_2$ as alkalizing for the nursery of *P. brachypomus* under BFT is quite attractive and is the most efficient in terms of water quality and performance.

Key Words: BFT, water quality, alkalinity, pH, Cachama.

Introduction. Aquaculture products account for approximately 47% of total global fish production (171 million tonnes), from 4.710.000 tonnes for 1980 to 80.030.000 for 2016 (FAO 2018). Multiple and complex effects of this activity require regulations that consider environmental costs, where fish farms contemplate the possibility of incorporate environmental friendly culture technologies, such as crop systems with water recycling or integrated crop systems (Avnimelech 2015; Martínez-Córdova et al 2010; Buschmann 2001). Between implemented technologies with most relevance, are aquaculture production models: the RAS technology (Timmons & Ebeling 2010) and the Biofloc Technology BFT (Avnimelech 2009).

Fish farming with Biofloc technology-BFT is interpreted as the cultivation of aquatic organisms, in containers with minimum water exchange, where microorganisms thrive in a high C:N ratio and saturated oxygenation, guaranteeing water quality conditions (Figure 1) for intensive cultivation, in a true concept of nutrient recycling in situ (Collazos-Lasso & Arias-Castellanos 2015). The sage of total ammonia nitrogen (TAN) for the microorganisms and especially bacteria, that use this excretion product of fish culture along with carbon available in aquatic environment, achieving to oxidize and reduce TAN concentration, allow to maintain water quality parameters in suitable crop ranges, generating in situ microbial protein with a high nutritional value (Avnimelech 2015; Nur Syuhada et al 2015; Burford et al 2003).

The teleosts fish (ammonotelic) excrete from 70 to 95% mainly non-ionized ammonium or ammonia (NH_3^-), however depending of conditions such as pH, temperature and salinity, in the aqueous environment can be found also a ionized form NH_4^+; thus both forms of nitrogen related to excretion of aquatic organisms are known as total ammonia nitrogen (Baldisserotto 2013). Ammonia is a constraint when it comes to intensifying fish culture, and that is when the understanding and knowledge of processes involved in the dynamics of nitrogen allows improving production from aquaculture and minimizes environmental impact as posed by Jiménez Ojeda et al (2018).

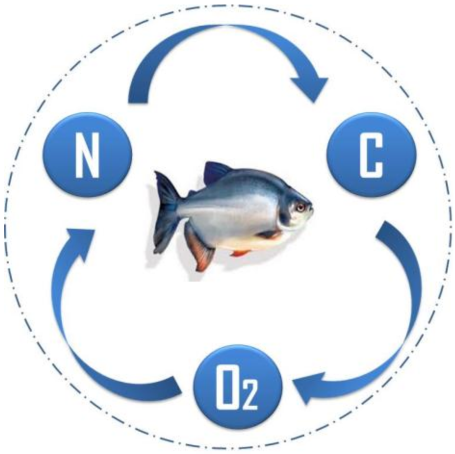

Figure 1. Carbon, nitrogen and oxygen dynamics, in fish culture in Biofloc technology.

BFT considers three main ways of removing TAN, by autotrophic communities (photoautotrophic and chemoautotrophic) and heterotrophic communities, each with different requirements and products of their metabolism (Avnimelech 2015).

Photoautotrophic or phytoplankton communities comprise algae and photosynthetic bacteria are able of produce their own food by converting solar energy into chemical energy by photosynthesis and producing their biomass from CO_2 and water (Avnimelech 2009). In order to reduce forms of nitrogen, phytoplankton presents the following consumptions: 3.13 g alkalinity per 1g NH_4^+; 4.02 g alkalinity per 1g NO_3^-; 18.07 g CO_2 per 1 g NH_4^+ and 24.40 g CO_2 per 1 g NO_3^-, and produce: 15.40 g O_2 per 1 g NH_4^+; 19.71 g O_2 per 1 g NO_3^-; 15.85 g volatile suspended solids (VSS) per 1 g NH_4^+ and NO_3^-.

On the other hand, heterotrophic bacterial communities require mainly sources of organic carbon like sugar or molasses for their growth and to be able to counteract the increments of ammonium. These bacteria require 15.17 g of organic carbon, 4.71 g of dissolved oxygen and 3.57 g of alkalinity to remove one gram of ammonia nitrogen and produce 9.65 g CO_2, 8.07 g of VSS.

Finally, chemoautotrophic bacterial communities use carbonates and bicarbonate as a source of energy during the process of nitrification, they consume 7.05 g alkalinity and 4.18 g of O_2 for each gram of ammonia nitrogen and produce 5.85 g CO_2; 0.976 g NO_3^- and 0.20 g VSS (Ebeling et al 2006).

As stated in the preceding paragraph, the three ways of reducing nitrogen compounds require carbonates, in this sense the alkalinity is diminished as a product of metabolic reactions of removal and oxidation of TAN, which indicates that the Biofloc system lose buffer capacity and therefore frequent additions of carbonates and bicarbonate are required (Chen et al 2006; Azim & Little 2008). Accordingly, the present study assessed the addition of three alkalizing corrections: sodium bicarbonate ($NaHCO_3$), Calcium hydroxide ($Ca(OH)_2$) and calcium and magnesium carbonate (CaO 37.5% and MgO 13.5%) in a nursery culture of *Piaractus brachypomus* under biofloc technology.

Material and Method. The present study was carried out in the experimental unit of bioassays of the Institute of Aquaculture of the Llanos (IALL) of the University of the Llanos, Villavicencio-Meta-Colombia.

Biological material and experimental design. Fingerlings of *P. brachypomus* cultivated in Biofloc system were distributed in 9 plastic tanks (250 L each) with an initial weight of 5.65±1.59 g and length of 6.70±0.59 cm at a density of 354 fingerlings per tank (8 kg m^{-3}). The experimental design consisted of three treatments, where different alkalizers were used to established and stabilized biofloc microorganisms according to the indications mentioned by Ebeling et al (2006) and Avnimelech (1999). The treatments were: T1: sodium bicarbonate ($NaHCO_3$), T2: calcium hydroxide ($Ca(OH)_2$) and T3: calcium and magnesium carbonate ($MgCa(CO_3)_2$), each of the treatments were evaluated in triplicate for 19 days and experimental units were randomly distributed. The assay was carried out in a C:N ratio of 15:1 using molasses as a source of organic carbon (40% C), with daily addition according to De Schryver et al (2008) and Avnimelech (2009). Aeration was supplied by diffusion and consisted of a blower of 2 hp (HP), and aero-tubeTM diffuser hose that provided a suitable air bubble size for diffusion in the water ensuring proper oxygenation and water movement. The animals were fed with commercial concentrate of 32% protein (PB) to 6% of their live weight with a frequency of three times per day (8:00; 12:00 and 16:00 hrs).

The pH and alkalinity correction were performed at the end of water quality monitoring, when alkalinity ≤60 mg $CaCO_3$ L^{-1} and according to pH values. To calculate the amount of alkalizer to be added, the reactivity or fineness rate of each limestone was determined by sieves of 2 mm, 0.75 mm and 0.35 mm in size, establishing proportion of particles ≤0.75 mm and the reactive part of the calcareous (Table 1). The dosage was calculated with the following equation:

$$CC = (DA - CA) * (100/RE) * V$$

Where: CC: Ammount of limestone to add (g)
DA: Desired alkalinity (mg L^{-1})
CA: Current alkalinity (mg L^{-1})
RE: Reactivity or fineness (%)
V: Volume

Table 1

Reactivity or finesse of the alkalizer used: $NaHCO_3$, $Ca(OH)_2$ and $CaMg(CO_3)_2$

Alkalizer	*Particle size (mm)*			
	>2.00	*2.00–0.75*	*0.75–0.355*	*<0.355*
$NaHCO_3$	0.05%	1.16%	0.20%	98.59%
$Ca(OH)_2$	0.05%	27.94%	7.26%	64.75%
$CaMg(CO_3)_2$	4.55%	36.94%	1.47%	57.05%

Water quality. Parameters like pH, concentration of dissolved oxygen (DO), percentage of oxygen saturation (% DO) and temperature were measured daily with the HANNA multiparametric probe (model HI 98196, Hanna instruments). Total ammonia nitrogen (TAN), nitrites (NO_2), nitrates (NO_3), and alkalinity was monitored with the HANNA photometer (model HI 83200, Hanna Instruments) twice a week. Settleable solids (SS) were quantified with the Imhoff cone (VITLAB, Germany), collecting samples twice a week. Volatile solids (VS), fixed solids (FS) and total suspended solids (TSS), were measured weekly according to the protocols established in APHA (1998).

Zootechnical parameters. During 19 days of trial, was assessed; final weight (fw) g, final length (fl) cm, growth rate (gr) g day^{-1} = gain of weight (gw) g x cultivation $days^{-1}$ where gw = final weight (fw) g – initial weight (iw) g; survival (%) = (No. final fish/No. initial fish) x 100; final biomass (kg m^{-3}) and feed conversion = Supplied food x biomass $gain^{-1}$.

Statistical analysis. All data were evaluated for normal assumptions and by the Shapiro-Wilk and Levene tests, respectively. The results were compared with analysis of variance of one via, subsequently of a Tukey test (P<0.05). The analyses were performed in the Infostat 2018 software.

Results

Water quality. Water quality parameters are shown in Table 2. Significant differences were evident in the pH of T1 and T2 with respect to T3 which showed the lowest value of all treatments. Nitrates for T1 were significantly higher ($p<0.05$) than T2 but without significant differences with T3. Alkalinity values sampled during the assay showed significant differences ($p<0.05$) between the three treatments and this is due to the neutralizing power and dissociation of the calcareous material, presenting greater Alkalinity in T1. Other parameters evaluated as temperature, dissolved oxygen, percentage of oxygen saturation, TAN, NO_2, SS, TSS, VS and FS, showed no statistical differences.

Table 2

Water quality parameters in a nursery culture system with biofloc technology, adding different alkalizers T1: sodium bicarbonate ($NaHCO_3$), T2: calcium hydroxide ($Ca(OH)_2$) and T3: calcium and magnesium carbonate ($CaMg(CO_3)_2$)

Parameters	*Treatments*		
	T1 ($NaHCO_3$)	*T2 ($Ca(OH)_2$)*	*T3 $CaMg(CO_3)_2$*
Temperature (°C)	28.16±0.74	28.31±0.60	28.16±0.70
DO (mg L^{-1})	6.73±1.04	6.53±1.17	6.89±0.96
% DO (%)	90.69±13.69	88.20±15.52	92.85±12.83
pH	8.46±0.47^{a}	8.17±0.40^{a}	7.46±0.62^{b}
TAN (mg L^{-1})	3.79±2.60	4.18±2.78	5.24±4.17
NO_2 (mg L^{-1})	0.71±0.68	0.77±0.62	0.50±0.41
NO_3 (mg L^{-1})	19.38±8.96^{a}	10.35±6.95^{b}	15.63±4.07^{ab}
Alkalinity (mg $CaCO_3$ L^{-1})	170.00±53.2^{a}	82.62±29.77^{b}	16.67±20.51^{c}
SS (ml L^{-1})	19.80±13.37	34.67±27.51	46.83±41.64
TSS (mg L^{-1})	474.33±90.67	489.11±46.43	509.44±136.69
VS (mg L^{-1})	92.76±45.44	112.00±43.52	102.00±37.51
FS (mg L^{-1})	381.57±67.49	377.11±24.00	407.44±111.92

Values presented as mean ± standard deviation. Different superscripts in the same row indicate significant differences ($P<0.05$). Dissolved oxygen (DO), percentage of oxygen saturation (% DO), total ammonia nitrogen (TAN), nitrites (NO_2), nitrates (NO_3), settleable solids (SS), total suspended solids (TSS), volatile solids (VS) and fixed solids (FS).

Zootechnical parameters. Table 3 shows the zootechnical parameters obtained during the assay. T2 showed better results in terms of final weight, final length, growth rate, survival, final biomass and feed conversion compared to T1 and T3.

Table 3

Zootechnical parameters of *Piaractus brachypomus* fingerlings cultivated 19 days with biofloc technology, adding different alkalizers T1: sodium bicarbonate ($NaHCO_3$), T2: calcium hydroxide ($Ca(OH)_2$) and T3: calcium and magnesium carbonate ($CaMg(CO_3)_2$)

Parameters	*Treatments*		
	T1 ($NaHCO_3$)	*T2 ($Ca(OH)_2$)*	*T3 $CaMg(CO_3)_2$*
Initial weight (g)	5.65±1.59	5.65±1.59	5.65±1.59
Final weight (g)	10.17±3.56^{c}	15.52±5.24^{a}	12.77±3.94^{b}
Final length (cm)	7.82±0.90^{b}	8.42±1.02^{a}	8.39±0.88^{a}
Growth rate (g*day^{-1})	0.27±0.2^{c}	0.59±0.31^{a}	0.42±0.24^{b}
Survival (%)	83.76±12.64^{b}	99.18±0.62^{a}	97.61±0.70^{a}
Final biomass (kg*m^{-3})	12.05±1.07^{c}	21.99±1.27^{a}	17.79±1.22^{b}
Feed conversion	1.77±0.03^{b}	0.68±0.06^{a}	0.96±0.11^{b}

Values presented as mean ± standard deviation. Different superscripts in the same row indicate that the means differ significantly ($P<0.05$).

Discussion. During the assay, temperature, dissolved oxygen and oxygen saturation were maintained in parameters suitable for the BFT system (Avnimelech 2009; Emerenciano et al 2017) and for the species under study (Jiménez-Ojeda & Collazos-Lasso 2018).

The addition of an inorganic carbon source such as sodium bicarbonate ($NaHCO_3$), calcium hydroxide $Ca(OH)_2$ and calcium and magnesium carbonate $CaMg(CO_3)_2$, has a differential effect on water quality parameters in terms of alkalinity and pH principally. The alkalizers react forming bicarbonate HCO_3^- and carbonate CO_3^- ions, generating a buffer effect on water. The limes used in the assay provided differential amounts of these molecules, causing the buffer effect to be particular in each treatment, a phenomenon that affects the pH. Moreover nitrite (NO_2^-), nitrate (NO_3), solids (SS, SST, SSV, SF) in terms of its conformation and quantity, and the percentage of ammonia (NH_3^-) of TAN would be affected (Jiménez-Ojeda et al 2018; Avnimelech 2015; Hargreaves 2013; Emerson et al 1975).

Alkalinity and pH remained dissimilar in the three treatments, being the greatest value for sodium bicarbonate followed by calcium hydroxide. Calcium and magnesium carbonate presented the lowest alkalinity and pH values, this can be explained by the lower rate of finesse compared to bicarbonate and calcium hydroxide as shown in Table 1, where 57.05% of the particles of $CaMg(CO_3)_2$ were <0.0355 mm.

Ammonia concentration did not show differences between treatments, however they were with values above 3.5 mg L^{-1}, being these concentrations similar to the lethal concentration (LC) 50 of *Piaractus mesopotamicus* as reported by Barbieri & Vigliar (2015). Emerson et al (1975) determined the amount of ammonia (NH_3) present in total ammonia nitrogen (TAN) in relation to pH and temperature; according to the mentioned paper NH_3 concentration of T1, T2 and T3 is: 0.68, 0.27, 0.11 mg/L respectively, values that show the great tolerance and rusticity of *P. brachypomus* taking in account the survivals. Values of nitrite (NO_2) were below 1 for all treatments, being permissible ranges for the species as reported by Torres-Tabares et al (2007) and Ochoa et al (2002). Presence of nitrates (NO_3) indicates processes of nitrification as proposed by Hargreaves (2013).

Total suspended solids generated in the system represent a relevant parameter that requires control, since the culture species present differential concentrations of tolerance of this parameter. Studies in *Oreochromis niloticus* report the use of $Ca(OH)_2$ and $NaHCO_3$ limes with values in TSS of 577.77±43.43 mg L^{-1} and 501.47±37.59 mg L^{-1} respectively in BFT system (Martins et al 2017). Larvae of South American catfish *Rhamdia quelen* in BFT culture, showed tolerances of up to 1000 mg/L of TSS, however the best performance was evidenced at 200 mg L^{-1}. In the present study, the SST did not presented differences between treatments being in range of 384-645 mg L^{-1}, being in recommended ranges for cultivation in BFT (Avnimelech 2015).

In the present study, the best performance of the culture was given in T2 with addition of $Ca(OH)_2$ achieving biomasses of 21.9 Kg m^{-3}, final weight of 15.52±5.24 g, food conversion of 0.68±0.06 and a survival of 99.18±0.62%. Bru-Cordero et al (2017) reported biomasses of 11.4±1.3 kg m^{-3}, feed conversion of 0.9±0.3 and 98% survival in a BFT biculture of *P. brachypomus* and *O. niloticus* which were fed with a protein content of 32%.

Finally Martins et al (2017) reported sodium bicarbonate and calcium hydroxide like good choices for pH and alkalinity correction in the cultivation of *O. niloticus* with Biofloc system technology, which is consistent with the present study.

Conclusions. In the present study has reported the importance of the alkalizers implemented to supplement and correct pH and alkalinity in fish cultures under biofloc technology. This study show that the addition of calcium hydroxide $Ca(OH)_2$ is the most recommended to correct the alkalinity and pH in the cultivation of *P. brachypomus* with Biofloc system technology, obtaining a better productive biomass of 21.9 Kg m^{-3}, a final weight of 15.52±5.24 g and survival of 99.18±0.62%. Alkalizers used to supplement this system can vary in availability, purity and price depending on the place where they are, however, it is relevant to evaluate the efficiency in the correction of the parameters pH and alkalinity, which are necessary to maintain the comfort conditions of the cultivation species and microorganisms growth.

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