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EFFECT OF THE C:N RATIO ON *Daphnia magna* (Straus, 1820) PRODUCTION USING TILAPIA FARMING WASTEWATER*

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ABSTRACT

The purpose of this study was to produce *Daphnia magna* in wastewater from Tilapia farmed in a biofloc system, to evaluate three different carbohydrate:nitrogen ratios. The experiment was conducted for 30 days following a completely randomized experimental design, with the three treatments: C:N 10:1, C:N 15:1 and C:N 20:1, all fed with *Chlorella vulgaris*. The physical-chemical variables of the water analyzed were: temperature, dissolved oxygen (DO), pH, TAN, NO_2, hardness and alkalinity. The following growth variables of the *Daphnia* were also evaluated: Maximum average density (MAD), Maximum density day (MDD), Specific growth rate (SGR), Doubling time (DT) and yield (Y). The water quality variables remained in the range of ideal conditions for the species, presenting significant differences (p <0.05) among the treatments for: pH, DO, hardness and alkalinity. Regarding the growth variables, significant differences (p <0.05) were observed, with higher MAD, Y and SGR values for the 10:1 treatment (3,433 ± 267 ind L^{-1}, 245 ± 19 ind L^{-1} day^{-1} and 45.3 ± 0.6% day^{-1}, respectively) and lower for the 20:1 (1,011 ± 283 ind L^{-1}, 55 ± 15 ind L^{-1} day^{-1} and 28.3 ± 1.6% day^{-1}, respectively). The MDD occurred on day 12 for the 10:1 treatment and on day 18 for the 15:1 and 20:1 treatments. Alkalinity and hardness had stronger influence on the growth variables, which was also indicated by the simple linear regression. The principal component analysis (PCA), with 80% of explanation, identified high values of SGR, density and Y for the 10:1 and high values of alkalinity, hardness, DT and NO_2 for the 20:1. Thus, the use of effluent from Tilapia farming in a biofloc system with a C:N ratio of 10:1 provided better production results for *D. magna*, demonstrating that it is an option for the production of live feed for aquaculture.

Key words: Cladocera; biorremediation; biofloc; microalgae.

EFEITO DA RELAÇÃO C:N NA PRODUÇÃO DE *Daphnia magna* (Straus, 1820) UTILIZANDO EFLUENTE DO CULTIVO DE TILAPIA

RESUMO

O presente trabalho teve como objetivo produzir *Daphnia magna* em água residual do cultivo de Tilápia em sistema de bioflocos, avaliando relações carboidrato:nitrogênio. O experimento teve duração de 30 dias seguindo delineamento experimental inteiramente casualizado, sendo os tratamentos: C:N 10:1, C:N 15:1 e C:N 20:1, todos com oferta de *Chlorella vulgaris* na dieta. Foram analisadas as variáveis físico-químicas: temperatura, OD, pH, TAN, NO_2, dureza e alcalinidade; e as variáveis de crescimento: Densidade média máxima (DMX), Dia de máxima densidade (DMD), Taxa de crescimento específico (TCE), Tempo de duplicação (TD) e Rendimento (R). As variáveis de qualidade de água mantiveram-se na faixa ideal para a espécie, apresentando diferenças significativas (p<0,05) entre os tratamentos para: pH, OD, dureza e alcalinidade. Com relação às variáveis de crescimento, foram observadas diferenças significativas (p<0,05), com maiores valores de DMX, R e TCE para o tratamento 10:1 (3.433 ± 267 ind L^{-1}, 245 ± 19 ind L^{-1} dia^{-1} e 45,3 ± 0,6 % dia^{-1}, respectivamente) e menor para o 20:1 (1.011 ± 283 ind L^{-1}, 55 ± 15 ind L^{-1} dia^{-1} e 28,3 ± 1,6 % dia^{-1}, respectivamente). O DMD ocorreu no 12º dia para o 10:1 e no 18° dia para os 15:1 e 20:1. As variáveis alcalinidade e dureza tiveram destaque sobre as variáveis de crescimento sendo também evidenciada na regressão linear simples. Já a análise de componentes principais (PCA), com 80% de explicação, identificou altos valores de TCE, densidade e R para o 10:1 e, elevados valores de alcalinidade, dureza, TD e NO_2 para o 20:1. Assim, o uso de efluente proveniente do cultivo de Tilápia em sistema de bioflocos com relação C:N de 10:1 propiciou melhores resultados produtivos para a *D. magna* demonstrando ser uma opção para a produção de alimento vivo para a aquicultura.

Palavras-chave: Cladocera; biorremediação; bioflocos; microalga.

INTRODUCTION

One of the primordial factors for the success of aquatic species cultivation is nutrition, especially in the early stages of development, when the supply of live food can improve levels of survival, growth and sexual maturation (Ocampo et al., 2010).

In most species, the supply of live food during the early stages is considered indispensable because the lipids present in these organisms provide energy and the essential fatty acids that act on the defense mechanisms and components of the cell membranes (Fereidouni et al., 2013).

This dependence on live food of many aquatic animal species, mainly fishes and crustaceans, can be met by providing zooplankton organisms, for example cladocerans (Lavens and Sorgeloos, 1996). The wide diversity of Cladocera is highlighted by the genus *Daphnia*, which comprises about 100 species, the best known being: *D. magna*, *D. pulex*, *D. longispina* and *D. similis* (Ebert, 2005). *Daphnia* are abundant in environments with high concentrations of organic matter, where bacteria, yeasts and microalgae propagate that the *Daphnia* use as food (Ocampo et al., 2010).

The nutritional content of these cladocerans is strictly related to their food sources (Lavens and Sorgeloos, 1996). *Daphnia* present the following relevant nutritional content, at dry weight: protein (50%), lipids (20-27%), and essential fatty acids (4.2%) (Hoff and Snell, 2004). They also provide vitamins and enzymes that act on the intestines of fish larvae. The *Daphnia* reproduce rapidly and have a short life cycle, optimizing their production (Otero et al., 2013). One of the common foods supplied for cladocerans are microalgae (Hoff and Snell, 2004).

Among the microalgae that can be offered to these cladoceran, *Chlorella vulgaris* is one of the most widely used, not only because of its ideal size, ease of cultivation and accelerated growth (Lourenço, 2006) but also because it has a significant nutritional content. Its protein content is more than 40% of its dry weight (Matosi et al., 2015) and it has a high concentration of fatty acids, which are about 10% of its dry weight (Villarruel-López et al., 2017). Of the total amount of fatty acids, 40.8% are polyunsaturated fatty acids (PUFA) (Tibbetts et al., 2017). These characteristics strengthen the immune system, improve the growth of fish and crustacea larvae and consequently offer higher productive efficiency (Chiu et al., 2015).

Meanwhile, biofloc systems have become consolidated in aquiculture because of their good productive results, for *Oreochromis niloticus* for example (Wambach, 2013; Zapata-Lovera et al., 2017; Bosisio et al., 2017; Lima et al., 2018). This is due to the equilibrated microbial community in biofloc systems and their need for low water exchange, which helps reduce the release of effluents into the environment and avoid the introduction of pathogens (Azim and Little, 2008; Luo et al., 2014). A remarkable characteristic of these systems is the formation of microbial aggregates (bacteria, yeasts, protozoa, phytoplankton and zooplankton), which is induced by manipulating the C:N ratio of the system, varying from 10 to 20:1 (Avnimelech, 2012). Different C:N ratios induce growth of different microbiota and different bacterial groups (autotrophic and heterotrophic), some more than others, depending on the C:N ratio established in the system (Ebeling et al., 2006). This microbial community allows the nitrogen, generated by the unconsumed foods and excreta of the organisms, to be converted into protein biomass, which is made available again in the culture and consumed by these organisms (Day et al., 2016).

However, after successive production cycles, residues form with a high proportion of organic matter, nitrate and phosphorus (Emerenciano et al., 2017), which makes it necessary to study the quality of the water before its disposal. Filtering and nutrient-assimilating organisms have been used, such as microalgae (Souza et al., 2015), macroalgae (Samocha, 2015), mollusks (Brito et al., 2018) and microcrustaceans (Pau et al., 2013), for bioremediation of these wastes. Thus, taking advantage of the characteristics of the wastewater from tilapia cultivation in a biofloc system for use as a culture medium for the production of *Daphnia* can be an interesting option, since water from the biofloc system has attributes in common with the natural habitat of *Daphnia*, as previously discussed. Campos (2017) used the effluent from tilapia culture in a biofloc system (C:N 12:1) to cultivate *D. similis* and found that the use of the bioflocs associated with the microalgae *C. vulgaris* obtained better growth results and that using only the biofloc as food was not able to induce high growth. However, the influence of the use of Tilapia culture effluents from biofloc systems with different C:N ratios on the production of *Daphnia* has not yet been documented.

Therefore, the objective of this study was to evaluate the different carbohydrate:nitrogen ratio of the wastewater from a Tilapia (*Oreochromis niloticus*) culture in a biofloc system for the production of *Daphnia magna* with the supply of *Chlorella vulgaris* in the diet.

MATERIAL AND METHODS

The experiment was conducted at the Livestock Food Production Laboratory (LAPAVI), located at the Department of Fisheries and Aquaculture (DEPAq) of the Federal Rural University at Pernambuco (UFRPE).

Experimental Design

The experiment had a duration of 30 days following a completely randomized experimental design, with the different treatments having different carbohydrate:nitrogen ratios (C:N 10:1, C:N 15:1 and C:N 20:1) with three replicates each, totaling nine experimental units.

Characteristics of residual water and distribution in the experimental units

Water was used from Tilapia cultivated in biofloc systems with different carbohydrate:nitrogen ratios. The carbon source used was molasses. The C:N ratios tested were 10:1, 15:1 and 20:1 according to the methodology of Samocha et al. (2007) and Avnimelech (2009). The Nile Tilapia were cultivated for 62 days, at a stocking density of 40 ind m^{-3} and a temperature of approximately 27 °C throughout the culture. The Nile Tilapia were fed a quantity equivalent to 5% of their biomass per day and the pH of the water was adjusted to about 7.5 using sodium bicarbonate ($NaHCO_3$). The sedimentable solids of the 10:1, 15:1 and 20:1 treatments were 20, 60 and 80 ml L^{-1}, respectively, with alkalinities of 100, 120 and 135 mg $CaCO_3$ L^{-1}, respectively.

The residual water was diluted and its alkalinity was adjusted to the ideal conditions for *D. magna* growth, which was approximately 60 mg $CaCO_3$ L^{-1}, as adapted from Jin et al. (1991). For this reason, the volume of biofloc water used in the three treatments was mixed with clear freshwater, in amounts that varied according to the three C:N ratios, using 3.0 to 4.0 L $tank^{-1}$ (approximately 30-40% of the volume) of biofloc water and 6.0 to 7.0 L $tank^{-1}$ (approximately 70-60% of the volume) of clear freshwater (alkalinity 20 mg $CaCO_3$ L^{-1}), totaling 10 L $tank^{-1}$. The clear freshwater was previously treated with chlorine (2 mL L^{-1}), dechlorinated, and aerated for three days before use.

Water quality

The physical-chemical variables of temperature (ºC), dissolved oxygen (mg L^{-1}) and pH were measured daily using a multi-parameter MultiProbe System 5565 YSI. Total ammoniacal nitrogen (TAN) (Koroleff, 1976), nitrite (NO_2) (Golterman et al., 1978), alkalinity (mg $CaCO_3$ L^{-1}) and hardness (mg $CaCO_3$ L^{-1}) were monitored twice a week (APHA, 2005).

Daphnia magna cultivation

The cultivation was carried out in 10 L tanks, where 6 ind L^{-1} (Buratini and Aragão, 2012) were initially stored, totaling 60 individuals per tank with constant aeration and with light intensity kept at 40 mol photons m^{-2} s^{-1} using a metal halide lamp with a 12-h light/dark photoperiod. Water was replaced and removed to compensate for evaporation or the increase of water volume caused when adding microalgae.

The microalgae *Chlorella vulgaris* was cultivated in a Provasoli culture medium (Provasoli, 1968), containing in µmol L^{-1}: 1,235,000 $NaNO_3$, 69,431.5 $C_3H_7Na_2O_6P.H_2O$, 66,892.3 $C_{10}H_{14}N_2O_8Na_2.2H_2O$, 24,760 $Fe(NH_4)_2(SO_4)_2.6H_2O$, 48,520 H_3BO_3, 550 $FeCl_3.6H_2O$, 3,030 $MnCl_2.4H_2O$, 550 $ZnCl_2$, 6.3 $CoCl_2.6H_2O$, 2.21 cyanocobalamin and 6.14 biotin; using 1.0 mL L^{-1} of the culture medium in each experimental unit. The cultivation was conducted in a semi-continuous system, with an integral photoperiod and light intensity of approximately 40 mol photons m^{-2} s^{-1}. *D. magna* was supplied daily in the exponential phase of microalgae growth, at the same time of day throughout the growing period, at a concentration of 3.2 x 10^6 cél mL^{-1} ind^{-1} (Buratini and Aragão, 2012).

The organisms were counted every two days using the volumetric method. Homogeneous 2 L samples were taken from each experimental unit, and densities of less than 250 individuals were counted one by one and those greater than 250 individuals were counted with the help of a 10 mL graduated pipette (Manso, 2007).

Growth variables of *Daphnia magna*

Maximum average density (MAD) was verified, with the maximum density of organisms obtained in the cultivation indicated as (ind L^{-1}), and the Maximum density day (MDD), was the day with the highest number of organisms in the cultivation. The specific growth rate (SGR), doubling time (DT) and yield (Y) were calculated using the following equations (Otero et al., 2013):

$$SGR = [(LnNt1-LnNt0)/t1]*100$$

were:

Nt1: Final number of individuals

Nt0: Initial number of individuals

t1: Day of maximum density

$$DT = Ln2/k$$

where:

Ln2: Natural logarithm of 2

k: Specific growth rate (SGR)

$$Y = Nt1-Nt0/t1$$

where:

Nt1: Final number of individuals

Nt0: Initial number of individuals

t1: Day of maximum density

Statistical analysis

The data were evaluated for normality and homogeneity by the Shapiro-Wilk and Bartlett tests, respectively. The normal data were submitted to one-way ANOVA, followed by a Tukey's test, at the significance level of 0.05. For the non-normal data, the Kruskal-Wallis non-parametric test was applied followed by the Dunn posthoc ($p < 0.05$). The Pearson correlation test and a simple linear regression (95% confidence) were applied to the water quality and growth data of *D. magna*, as well as a Principal Component Analysis (PCA) with data previously logarithmized (log x + 1). Statistical analysis was conducted with the software R 3.4 (R Core Team, 2015; Gross and Ligges, 2015; Wickham, 2016).

RESULTS

Water quality

The water quality variables remained within the ideal range for the species. The average pH varied around 7.5, dissolved oxygen between 3.65 and 4.18 mg L^{-1}, and temperature around 26 ± 1 ºC. For pH, there was a significant difference between the treatments ($p < 0.05$), with higher mean values for treatments 10:1 and 15:1, whereas the temperature was similar among the treatments ($p > 0.05$). In relation to dissolved oxygen, there was a significant difference ($p < 0.05$), being higher in treatment 10:1 (4.18 ± 0.55 mg L^{-1}) and lower in treatments 15:1 (3.65 ± 0.68 mg L^{-1}) and 20:1 (3.78 ± 0.67 mg L^{-1}) (Table 1).

For the chemical variables TAN and NO_2 there were no significant differences ($p > 0.05$). TAN averaged 0.146 to 0.33 mg L^{-1}, while NO_2 ranged from 0.142 to 0.378 mg L^{-1}. There were significant differences in hardness and alkalinity ($p < 0.05$), with a mean variation from 75 to 133.33 mg $CaCO_3$ L^{-1} and 51.67 to 60.18 mg $CaCO_3$ L^{-1}, respectively (Table 1).

Growth of *Daphnia magna*

When evaluating growth variables, a significant difference ($p < 0.05$) was observed among all treatments (Table 2), with higher MAD values for the 10:1 treatment (3,433 ± 267 ind L^{-1}) and lower for the 20:1 (1,011 ± 283 ind L^{-1}). The highest SGR and Y values were obtained in the 10:1 treatment, 45.3 ± 0.6% day^{-1} and 245 ± 19 ind L^{-1} day^{-1}, respectively. While the 20:1 treatment obtained the lowest values, 28.3 ± 1.6% day^{-1} and 55 ± 15 ind L^{-1} day^{-1}. Doubling time (DT) followed the same trend as the other variables, especially the 10:1 treatment (Table 2).

The maximum density day (MDD) was reached on day 12 for the 10:1 treatment and on day 18 for the treatments 15:1 and 20:1 (Table 2) with a tendency to decline shortly after MAD (Figure 1).

Table 1. Mean, minimum and maximum values of water quality variables in *D. magna* cultivation in wastewater from Nile Tilapia cultures in biofloc systems with different C:N ratios.

C:N	pH*	T (°C)	DO (mgL^{-1})	TAN (mgL^{-1})	NO_2 (mgL^{-1})	Alkalinity (mg $CaCO_3$ L^{-1})	Hardness* (mg $CaCO_3$ L^{-1})
10:1	7.58 (6.80- 7.98)^{a}	26.99 (26.2- 27.57)	4.18 (2.72- 5.25)^{a}	0.333 (0-2.0)	0.142 (0-0.5)	51.67 (35- 85)^{a}	75 (50- 150)^{a}
15:1	7.55 (6.41- 9.45)^{ab}	26.98 (26.5- 27.52)	3.65 (2.15- 5.10)^{b}	0.208 (0-0.5)	0.378 (0-1.8)	53.15 (40- 80)^{ab}	87.5 (50 -100)^{ab}
20:1	7.53 (6.71- 9.11)^{b}	26.97 (26.8- 27.45)	3.78 (2.25- 5.18)^{b}	0.146 (0- 0.25)	0.253 (0- 1.75)	60.18 (50- 90)^{b}	133.3 (50 - 200)^{b}
p	< 0.001	0.643	< 0.001	0.623	0.343	0.003	0.015

*The variables pH and Hardness were submitted to a Kruskal-Wallis test followed by a posthoc Dunn's test ($p<0.05$), while Temperature, DO, Alkalinity, TAN and NO_2 were submitted to one-way ANOVA followed by a Tukey test ($p<0.05$). Different letters between the lines show significant differences ($p<0.05$) among the treatments.

Table 2. Mean ± standard deviation of the growth variables in *D. magna* cultivation using wastewater from Nile Tilapia cultures in biofloc systems with different C:N ratios.

C:N	MAD (ind L^{-1})	SGR (% day^{-1})	DT (day)	Y (ind L^{-1} day^{-1})	MDD
10:1	3,433 ± 267a	45.3 ± 0.6^{a}	0.791 ± 0.012^{a}	245 ± 19^{a}	12
15:1	2,100 ± 200^{b}	32.5 ± 0.5^{b}	1.123 ± 0.016^{b}	116 ± 11^{b}	18
20:1	1,011 ± 283^{c}	28.3 ± 1.6^{c}	1.262 ± 0.056^{c}	55 ± 15^{c}	18
p	< 0.001	< 0.001	< 0.001	< 0.001	

The growth variables were submitted to a one-way ANOVA followed by a Tukey's test ($p<0.05$). Different letters between the lines show significant differences ($p<0.05$) among the treatments. MAD: Maximum average density, SGR: Specific growth rate, DT: Doubling time, Y: Yield; MDD: Maximum density day.

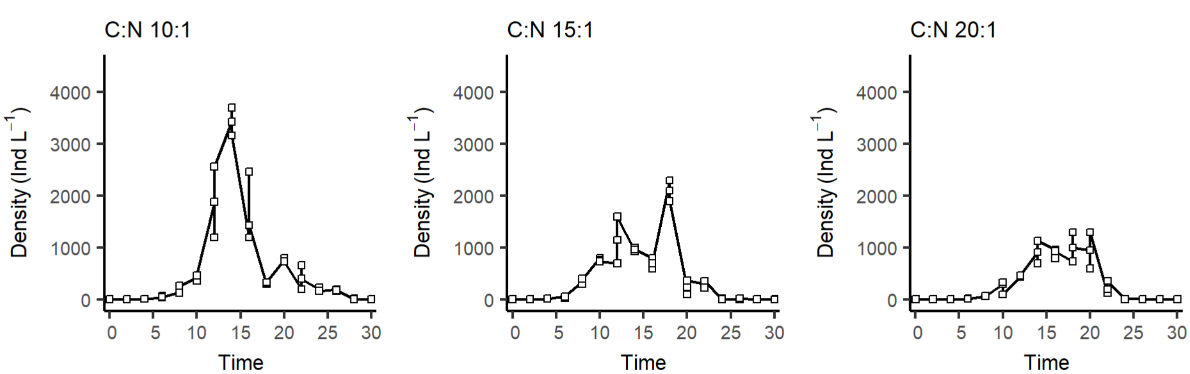

Figure 1. Growth of *D. magna* using wastewater from the cultivation of Nile Tilapia in biofloc systems with different C:N ratios.

Evaluating the possible existing correlations of water quality variables with the growth variables of *D. magna*, it was observed that the variables hardness and alkalinity had an inverse influence on population density, obtaining correlations of -0.82 ($p = 0.0065$) and -0.71 ($p = 0.031$), respectively. This was also observed by correlating the hardness with SGR and Y, which revealed correlations of -0.76 ($p = 0.018$) and -0.77 ($p = 0.015$), respectively. Alkalinity directly influenced DT with a correlation of 0.69 ($p = 0.04$). The other physical-chemical variables did not show significant correlations ($p > 0.05$).

Considering the important influence found for the correlation of the variables alkalinity and hardness with the density of *D. magna*, it was possible to apply a simple linear regression model, which can be observed in Figure 2. For the variable hardness, the following equation was obtained for the line: $y = 5189 + -30.5x$ ($r^2 = 0.68$) and for the variable alkalinity: $y = 10748 + -156x$ ($r^2 = 0.51$).

From the analysis of the main components (PCA) a degree of explanation of 80% of the variation in the data was observed. This cumulative proportion was divided between two components, where PC1 represented about 57.8% of the data variation and component 2 about 22.23%. The variables alkalinity (-0.328), hardness (-0.346), TAN (0.253), DT (-0.376), SGR (0.372), yield (0.368) and density (0.367) were better explained by PC1; while the variables NO_2 (-0.517), DO (-0.323), pH (-0.478) and temperature (-0.481) were better explained by PC2 (Figure 3).

High values of SGR, density and yield were reported for the treatment with the 10:1 C:N ratio. In contrast, the high values of alkalinity, hardness, DT and NO_2 were found in the 20:1 C:N group. The 15:1 ratio did not show a trend in relation to the other variables, indicating a balance between them (Figure 3).

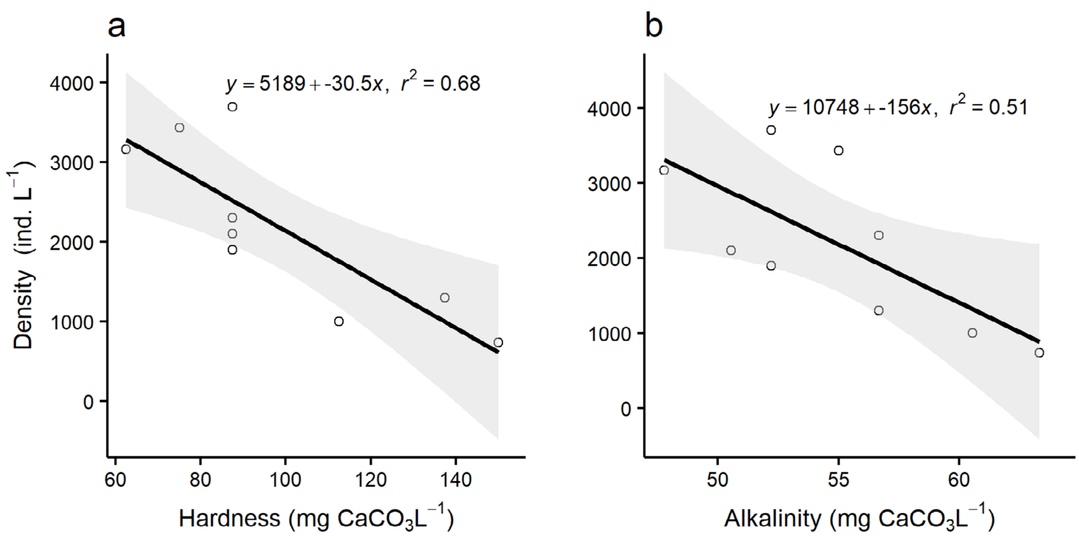

Figure 2. Correlation of the variables hardness (a) and alkalinity (b) with the density of *D. magna* throughout the cultivation. The gray area represents the 95% confidence interval.

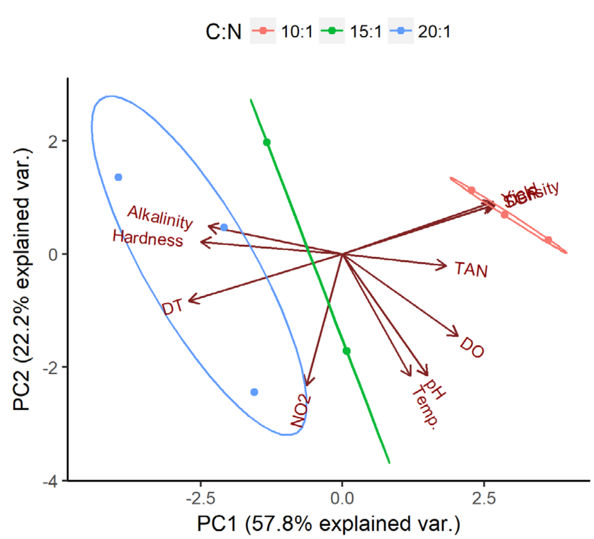

Figure 3. Principal component analysis for the response variables in *D. magna* cultivation at different C:N ratios.

DISCUSSION

The C:N ratio influenced the water quality (mainly the pH, DO, hardness and alkalinity) (Table 1), which in turn was reflected in the performance of *D. magna*. The mean pH (Table 1) remained in the ideal range for *Daphnia* cultivation, which is from 7.2 to 7.6 (Beatrici, 2004). Dissolved oxygen also varied within the ideal range for the specie (Hoff and Snell, 2004). The decrease of DO with the increase of the C:N ratio can be explained by the greater development of heterotrophic bacteria, which compete for space, nutrients and oxygen (Luo et al., 2013), and by the increase in organic matter, mainly sedimentable solids, due to the amount of the carbon source for the system (Silva et al., 2017).

Similarly, the alkalinity and hardness remained within the acceptable standards for the specie (Jin et al., 1991). The highest values of alkalinity in the 20:1 C:N ratio may be due to the low number of nitrifying bacteria, which consume much higher amounts of alkalinity (7.07 g) in nitrification processes (Chen et al., 2006; Silva et al., 2017), than heterotrophic bacteria (3.57 g) (Ebeling et al., 2006), in the production of microbial biomass for one gram of TAN, since high concentrations of carbon decrease the rate of nitrification (Ebeling et al., 2006).

The mean values of water hardness in this study were classified as moderately hard (Boyd, 2015), however the 20:1 treatment achieved high levels (Table 1), characterizing hard water, which may have influenced the reproduction of *D. magna*, since this variable strongly influences the fertility of the females and the neonatal production rate. Values closer to 80 mg $CaCO_3$ L^{-1} presented better results than concentrations of 37 (lower) and 169.5 (higher) mg $CaCO_3$ L^{-1} (Maranho and Nieweglowski, 1995).

Although the other water quality variables (temperature, TAN and NO_2) did not present significant differences among the treatments in this study, they can also influence the growth of *Daphnia*. Temperatures closer to 25 °C allow a higher average number of ovulations, starting on the 6th day of culture, whereas at 20 °C they reproduce from the 8th day and have a lower growth rate (Martínez-Jerónimo, 2012). In addition, the variations in the values of TAN and NO_2 were within the acceptable standards for the cultivation of *Daphnia*, since it can tolerate levels of 15 to 20 mg L^{-1} of total ammonia (Hoff and Snell, 2004) and up to 2 mg L^{-1} of nitrite (Xiang et al., 2012). Therefore, the temperature, TAN and NO_2 in this study did not affect the growth of *D. magna*.

In addition to the water quality variables, the diet offered the *Daphnia* is an important factor in its growth. This cladoceran is a nonselective filtering organism that can feed on phytoplankton, bacteria, ciliates and debris (Darmawan, 2014) present in the biofloc system and that are stimulated by the C:N ratio that affects the growth and diversity of the microbial community (Panigrahi et al., 2018). It can also modify the nutritional composition of the bioflocs in terms of the levels of carbohydrates, proteins and fatty acids (Crab et al., 2010). These microbial aggregates can reach protein and lipid levels of 38.43% and 3.23%, respectively in a C:N ratio of 11:1 (Azim and Little, 2008), and thus may be a nutritional source for the organisms cultivated (Khatoon et al., 2016).

A diet consisting solely of bacteria is not nutritionally adequate for *Daphnia* because it is deficient in PUFAs and sterols (Taipale et al., 2014). Therefore, the supply of microalgae is also an essential factor in the cultivation of *Daphnia*, as observed by Campos (2017) who cultivated *D. similis* in clear water and in BFT effluent, obtaining best growth results in the treatment with inoculation of *C. vulgaris* microalgae and biofloc reutilization. Like *Chlorella*, other chlorophytes are also important for feeding cladocerans, such as those used by Alcántara-Azuara et al. (2014) in the cultivation of *D. pulex*, obtaining densities of 1,309 ± 24 ind L^{-1}, 1,395 ± 24 ind L^{-1} e 1,933 ± 60 ind L^{-1} using *Sphaerocystis* sp., *C. vulgaris* and *Haematococcus pluvialis*, as feed respectively, with these density values lower than those found in the treatments 10:1 and 15:1 in this study (Table 2). Moreover, the addition of *C. vulgaris* to the system was reflected in good growth of *D. magna*.

Besides the addition of the food (microalgae), the culture medium (mixotrophic or autotrophic) also influences the performance of *D. magna*. Studies evaluating the use of chicken manure to produce a mixotrophic culture medium obtained maximum mean densities of 4,660 ± 523 ind L^{-1} (Paray and Al-Sadoon 2016) and 2,111,788.9 ind L^{-1} (Herawati et al., 2017), the latter using manure fermented with probiotic bacteria (*Lactobacillus casei* and *Saccharomyces cerevisiae*). Darmawan (2014), used wastewater from an African catfish (*Clarias gariepinus*) farm for *D.magna* cultivation and obtained a MAD of 2,653 ind L^{-1}, which is lower than that in the 10:1 C:N treatment in this study (Table 2). Other studies using an autotrophic medium to grow cladocerans attained mean maximum densities of 550 ± 500 ind L^{-1} (*Diaphanosoma* sp.) (Otero et al., 2013) and 820 ± 9.57 ind L^{-1} for *D. magna* (Ocampo et al., 2010). Therefore, it is possible to observe that a mixotrophic environment for the cultivation of *Daphnia magna* leads to better growth results than autotrophic culture environments, a fact that was also confirmed in this study.

According to Lavens and Sorgellos (1996), mixotrophic systems have a characteristic of being self-sustaining due to the formation of algae and also because they are less subject to deficiencies, such as food shortages. But water quality variables may be less subject to control due to system dynamics. In this study, some variables stood out in relation to others, such as hardness and alkalinity, which were revealed in the simple linear regression model, highlighting their important influence on population density of *D. magna* (Figure 2). This indicates that hardness ranging from 60 to 80 mg $CaCO_3$ L^{-1} and alkalinities closer to 50 mg $CaCO_3$ L^{-1} allow better reproductive performance and consequently an increase in *D. magna* density. In addition, the simple linear regression model showed the need to dilute the biofloc water from Nile tilapia cultivation, since it has alkalinities higher than 100 mg $CaCO_3$ L^{-1} (Emerenciano et al., 2017) and hardness ranging from 100 to 340 mg $CaCO_3$ L^{-1} depending on the type of reagent used for pH and alkalinity correction ($NaHCO_3$, $CaCO_3$ or $Ca(OH)_2$) (Martins et al., 2017).

Regarding PCA, there was a clear separation of treatments and of the responses of the variables evaluated (Figure 3) as well as a correlation between these variables. In addition, the 20:1 C:N and 10:1 C:N treatments confirmed their performances in relation to *D. magna* growth and the variables that caused these results. This indicated that the C:N 10:1 ratio obtained the best values for SGR, MAD and Yield, revealing that this treatment is the best for *D. magna* growth. However, the low growth in 20:1 C:N was possibly due to the high values of hardness and alkalinity, given their importance for reproduction (Jin et al., 1991; Maranho and Nieweglowski, 1995), reflecting in the longer time needed to double the population, thus achieving high DT values.

The reuse of aquaculture residues for the production of live food has been well researched in recent decades, whether in the production of microalgae (Abreu et al., 2016), rotifers (Stevenson et al., 1998), cladocerans (Campos, 2017) or copepods (Kumar et al., 2017). The same is true for biofloc systems, where research focusing on the use of their waste, whether the flocs (dry matter) or effluent, is increasing due to the problem of high concentrations of phosphorus and nitrate in the system. The studies have led to potential alternatives for animal feed supplementation (Khatoon et al., 2016), biomass production (Abreu et al., 2016) and aquaponics systems (Pinho et al., 2017). Hence, studies of these issues are important for promoting the viability of aquaculture activity, since wastes are frequently not reused by this production sector.

CONCLUSION

It was concluded that the 10:1 C:N ratio in the effluent from Nile Tilapia farming in a biofloc system provided better results for density, growth rate, doubling time, yield and day of maximum density for *D. Magna*. The variables alkalinity and hardness were the main factors related to good production results. Future research should be encouraged about the biochemical composition of *Daphnia* produced in biofloc effluent and its use in the diet of farmed organisms.

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