Biofloc-based co-culture systems of Nile tilapia (Oreochromis niloticus) and redclaw crayfish (Cherax quadricarinatus) with different carbon–nitrogen ratios

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Abstract

This study aims to evaluate the production performance of biofloc-based co-culture systems of Nile tilapia and redclaw crayfish with varying carbon–nitrogen ratios (C/N). The experiment was conducted for 80 days in plastic-lined ponds with dimensions of $1 \times 1 \times 0.6$ m. Two biofloc systems with two C/N ratios (10 and 15), using molasses as the carbon source and a control system without molasses were compared. Mixed-sex Nile tilapia with an initial average body weight of 7.00 ± 0.25 g were stocked at a density of 30 fish m[−]2. After 30 days of culture, redclaw crayfish with initial average body weight of 11.50 ± 0.55 g were added to the culture system at a density of 10 crayfish m⁻². There were no significant differences in total animal biomass or total weight gain between treatments $(P > 0.05)$. The total feed and overall feed conversion ratio (FCR) differed significantly ($P < 0.05$) between the biofloc systems and the control. Total ammonia nitrogen (TAN), nitrite nitrogen $(NO₂−N)$ and nitrate nitrogen (NO3−N) concentrations in biofloc systems with a C/N ratio of 15 were generally lower than those of other treatments. Dissolved oxygen concentrations decreased with the increase in the culture period in (4.13–4.40 mg L[−]1). Based on the results of this study, it can be concluded that an increase in the C/N ratio has positive effects on the feed utilization efficiency and water quality in biofloc-based tilapia–redclaw crayfish co-culture systems.

Keywords Tilapia–redclaw crayfish co-culture \cdot Cherax quadricarinatus \cdot C/N ratio \cdot Biofloc technology. Growth performance . Water quality

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Introduction

Aquaculture as a food-producing sector is important for human livelihood (Martinez-Porchas and Martínez-Córdova [2012\)](#page-10-0). With the increase in global population, there is a compelling need to intensify aquaculture production. This can be carried out by applying new technology in fish or shrimp aquaculture activities aimed at increasing sustainable production in the context of meeting human needs for protein sources that is a highly efficient use of water resources in an environmentally friendly manner (Naylor et al. [2000,](#page-10-0) Avnimelech [2009](#page-9-0)). One of the technologies that can be applied to increase productivity in intensive aquaculture is biofloc technology.

Biofloc technology is an aquaculture system that aims to improve water quality and feed utilization efficiency by converting inorganic nitrogen waste into microbial biomass that can be consumed in situ by the cultured organisms (Crab et al. [2012](#page-9-0); Martínez-Córdova et al. [2015](#page-10-0)). In biofloc systems, the toxic waste is maintained at low concentrations, allowing the application of minimal water exchange during the culture period (Crab et al. [2010,](#page-9-0) [2012](#page-9-0)). Biofloc technology has been widely applied and studied in fish and shrimp monoculture systems such as Pacific white shrimp (Poli et al. [2019\)](#page-10-0); Nile tilapia (Azim and Little [2008](#page-9-0); Crab et al. [2009;](#page-9-0) Widanarni et al. [2012;](#page-11-0) Pérez-Fuentes et al. [2016\)](#page-10-0) and in polyculture systems such as Mirror carp, Silver carp and Bighead carp (Zhao et al. [2014](#page-11-0)).

Polyculture is an aquaculture system formed by adding one or more species to the main units of the culture system (Martínez-Porchas et al. [2010\)](#page-10-0). These additional species have different food and eating habits and ecological characteristics (Zimmermann et al. [2010](#page-11-0); Wang and Lu [2015\)](#page-11-0). In polyculture, nutrients are used by all species that are cultivated in order to increase productivity from aquaculture activity (Joyni et al. [2011;](#page-10-0) Uddin et al. [2007](#page-11-0)). The application of a polyculture system in biofloc-based aquaculture may bring about more benefits, in particular in the utilization of biofloc biomass.

Tilapia is one of the most widely cultivated fish in the world, as it is easy to culture and breed and is of high economic value (Wang and Lu [2015\)](#page-11-0). The culture of tilapia can be done in monoculture or polyculture. A tilapia polyculture system is commonly practiced with other species such as giant prawn (Macrobrachium rosenbergii) or black tiger shrimp (Penaeus monodon) (Cruz et. al. [2008;](#page-9-0) Tidwell et. al. [2010;](#page-11-0) Wang and Lu [2015\)](#page-11-0). In this study, a tilapia– redclaw co-culture in biofloc systems was evaluated. Redclaw crayfish is an aquaculture species that can be used as an ornamental fish commodity and for human consumption (Saoud et al. [2013\)](#page-10-0), with Indonesia being one of the world's largest exporters of this species, mainly as an ornamental fish product (Patoka et al. [2018](#page-10-0)). Compared to other aquaculture species, there is relatively limited information on the cultivation of redclaw crayfish in an environmentally sustainable manner. In this context, the aim of the present study was to evaluate the effects of biofloc technology with varying carbon–nitrogen ratios on the production performance of Nile tilapia–redclaw crayfish co-culture systems.

Materials and Methods

Biological Materials

The research was conducted at the Laboratory of Airlangga University in Banyuwangi, Indonesia. Nile tilapia (Oreochromis niloticus) were obtained from Pusat Pelatihan dan Penyuluhan Perikanan Banyuwangi, and redclaw crayfish (Cherax quadricarinatus) were obtained from local fish farmers in Tulungagung, East Java. The tested biota was adapted for 1 week in the pond before being used in the experimentation. During the adaptation phase, tilapia were fed three times daily using commercial floating pellets with a protein content of 31%, whereas redclaw crayfish were fed a commercial sinking diet (40 % crude protein) and sliced carrot at the same proportion of 5% of their biomass.

Experimental design

This study was conducted for 80 days using a complete randomized design with three treatments in quadruplicate, that is two biofloc systems with different C/N ratios (10 and 15) via the addition of molasses as the carbon source and a control system without the addition of carbon sources. Nile tilapia and redclaw crayfish were cultured in a semi-outdoor plastic-lined pond (1 m x 1 m x 0.6 m) with a water volume of 600 L and aerated using an air blower (Resun LP 100, 140 L/ min).

Experimental procedure

Water for experimental used was chlorinated at a dose of 25 mg/L and was strongly aerated for 3 days, neutralized using sodium thiosulfate (15 mg/L) and aerated again. Mixed-sex Nile tilapia juveniles with an initial average body weight of 7.00 ± 0.5 g were randomly distributed to each experimental pond at a density of 30 fish/ $m²$ (Table 1). On day 30 of culture, redclaw crayfish with an initial average body weight of 11.50 ± 0.5 g were added to the pond at a density of 10 individuals/ m², and four plastic ropes were placed at each corner of the pond as shelters for the crayfish.

Nile tilapia were fed three times daily using a floating commercial diet (31% protein content) at a feeding level of 5% of biomass. Redclaw crayfish were fed three times a day using a sinking commercial diet (40% protein content) and carrot slices at a level of 5% of biomass. Commercial diet for the crayfish was given three times a week while carrot was given four times a week (Karplus et al. [2001](#page-10-0)). Feeding of crayfish was done 30 min after feeding the tilapia (Hernández-Vergara et al. [2018](#page-10-0)).

The addition of molasses as the carbon source in biofloc systems was carried out according to Avnimelech (1999), which was done 2 h after the first feeding time in the morning (Ekasari et al. [2014\)](#page-10-0). Every 5 days, siphoning was carried out in all treatment ponds at a level of about 5% of the total water volume to discharge solid waste accumulated at the bottom of the pond. Sampling was performed every 10 days by taking about 25% of the population biomass in the experimental pond.

Performance	Control	CN10	C/N 15
Nile tilapia			
Biomass (g tank ⁻¹)	218.3 ± 3.4	216.2 ± 2.0	215.0 ± 2.3
Density (fish m^{-2})	30	30	30
Mean body weight (g fish ⁻¹)	7.28 ± 0.11	7.21 ± 0.07	7.17 ± 0.08
Redclaw crayfish			
Biomass (g tank ⁻¹)	116.2 ± 1.4	116.5 ± 1.8	$115. \pm 1.3$
Density (crayfish m^{-2})	10	10	10
Mean body weight $(g \text{ crayfish}^{-1})$	11.62 ± 0.14	11.65 ± 0.18	11.58 ± 0.13

Table 1 Initial biomass, density and mean body weight of Nile tilapia and redclaw crayfish in biofloc-based coculture systems

Water quality

Temperature, dissolved oxygen (DO) and pH were measured daily using a thermometer, DO meters (YSI Pro20), and pH meters (Lutron YK2001PH, Taiwan), respectively. Total ammonia nitrogen (TAN), NO_2^- and NO_3^- were measured every 10 days using the procedures described in the American Public Health Association standards (APHA [1998](#page-9-0)). Floc volume was measured every 10 days using an Imhoff cone.

Zootechnical parameters

Zootechnical parameters observed in this study included weight gain (WG), daily weight gain (DWG), survival, specific growth rate (SGR), total biomass, and overall feed conversion ratio (FCR) (Zhao et al. [2014](#page-11-0); Pérez-Fuentes et al. [2016](#page-10-0); Poli et al. [2019\)](#page-10-0).

Data Analysis

Data variance homogeneity and normality were assessed using the Kolmogorov–Smirnov test. The data for Nile tilapia and crayfish growth performance were analyzed using one-way analysis of variance (ANOVA) at a 95% confidence interval (α = 0.05) followed by a post hoc Duncan test. Repeated-measures ANOVA using a linear model with two factors (water quality and time) was performed to analyze water quality data. Statistical analyses were conducted using SPSS version 18 statistical software for Windows (SPSS Inc.)

Results

Growth Performance

The growth performance of Nile tilapia and redclaw crayfish in term of biomass, WG, DWG, SGR, and survival did not differ significantly $(P> 0.05)$ between the treatments (Table [2\)](#page-4-0). The total feed used in biofloc systems was significantly lower than that for the control, and the biofloc system with a C/N ratio of 15 was lower than that with a C/N ratio of 10. The food conversion ratios in the biofloc systems were significantly lower than that of the control $(P<0.05)$, but there was no effect of C/N ratio on this parameter.

Water quality

Nitrite, nitrate, TAN and floc volume monitored were significantly different $(P<0.05)$ between the control and biofloc treatments. However, no significant differences were observed in DO, pH or temperature concentrations (P>0.05) (Table [3](#page-4-0)).

Temperature, pH and DO parameters tended to decrease at the end of the maintenance period but were still in the optimal range for the organism cultivated (Fig. [1\)](#page-5-0).

The levels of pH in all treatments decreased at the end of the culture period, but levels did not differ significantly $(P > 0.05)$ among treatments (Fig. [1](#page-5-0)a). Similarly, DO levels in all treatments tended to decrease over time. The higher DO levels were found in the treatment with a C/N ratio of 10, which was significantly different ($P < 0.05$) from that of a C/N ratio of 15 (Fig. [1b](#page-5-0)), but not significantly different $(P> 0.05)$ from the control. The

Table 2 The growth performance of Nile tilapia and redclaw crayfish in biofloc-based co-culture systems

Mean values $(± standard deviation)$ followed by different superscript letters in the same row are significantly different ($P<0.05$). ABW = average body weight, SGR = specific growth rate, FCR = feed conversion ratio

temperature of the culture media was relatively stable throughout the culture period, with no significantly difference $(P> 0.05)$ among all treatments (Fig. [1c](#page-5-0)).

Inorganic nitrogen concentrations

Inorganic nitrogen concentrations in the culture media were significantly different (P) <0.05) between biofloc treatments and the control. The concentrations of TAN in biofloc treatments tended to decrease with the increase in the culture period. On the contrary, the control demonstrated a consistent increase in TAN concentration throughout the culture

Table 3 Repeated measure ANOVA on water quality parameters observed in biofloc-based Nile tilapia–redclaw crayfish co-culture systems

Parameter	Control	CN ₁₀	CN ₁₅	P value
TAN	0.682 ± 0.601 ^a	0.155 ± 0.627^b	0.110 ± 0.016^b	0.001
NO ₂	0.623 ± 0.603^a	0.271 ± 0.762^b	$0.182 \pm 0.140^{\circ}$	0.001
NO ₃	$78.017 \pm 2.499^{\text{a}}$	73.807 ± 1.618^a	67.796 ± 2.607 ^b	0.005
DO.	$4.41 \pm 0.265^{\text{a}}$	4.37 ± 0.208 ^a	4.13 ± 0.577 ^a	0.769
PH	6.64 ± 0.155 ^a	6.83 ± 0.755 ^a	7.12 ± 0.154 ^b	0.118
Temperature	$25.17 \pm 0.115^{\text{a}}$	25.45 ± 0.313^a	$25.44 \pm 0.25^{\circ}$	0.360
Floc volume	$3.10 \pm 0.01^{\text{a}}$	34.67 ± 16.621^b	42.8 ± 0.544^b	0.004

Fig. 1 The level of pH (a), dissolved oxygen concentrations (b) and temperature (c). The concentrations of DO in the culture media were significantly different between C/N 10 and 15. No significant differences were found in pH or temperature values between any treatments. The value is an average value $(\pm$ SE). The concentrations of DO in the culture media were significantly different between C/N 10 and 15. The pH and temperature values showed no significant differences between any treatments. The value is an average value $(\pm$ SE)

period. TAN concentrations in the biofloc system with a C/N ratio of 15 were the lowest and were significantly different ($P \le 0.05$) from those of other treatments (Fig. [2](#page-6-0)a). Nitrite levels in all treatments fluctuated during the culture period, but the level in the control tended to increase with the increase in the culture period. In contrast, nitrite levels in biofloc treatments tended to decrease in time. The lowest nitrite concentrations were found in the treatment with a C/N ratio of 15 and were significantly different $(P < 0.05)$ from those of other treatments (Fig. [2b](#page-6-0)). Nitrate tended to accumulate in all treatments. The highest levels of nitrate were found in the control and were significantly different (P) \leq 0.05) from those of the biofloc treatments (Fig. [2c](#page-6-0)).

Biofloc volume

Up to day 60 of biofloc culture, the volume in biofloc treatments tended to increase, whereas after that it decreased gradually (Fig. [3](#page-6-0)). However, biofloc volumes were not significantly different $(P> 0.05)$ between the C/N ratios.

Fig. 2 Inorganic nitrogen concentration in biofloc-based Nile tilapia–redclaw crayfish co-culture systems throughout the culture period. a TAN. b NO₂−N. c NO₃^{−N} showed significantly different results (P <0.05). Value is average $(\pm$ SE)

Discussion

averages $(\pm \text{ SE})$

Growth Performance

The present study shows that the application of biofloc-based co-culture of Nile tilapia and redclaw crayfish demonstrated positive results on the production performance, in particular the

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total feed used and the FCR of both individual animal FCR and total FCR. Improved production performance did not show significantly different results among treatments on total biomass. But it can increase the efficiency of feeding. This can be seen from the combination of total feed and overall FCR, which is better in maintenance with C / N ratio treatment compared to control without treatment. This might be because the bioflocs generated from the addition of organic C source increased the availability of additional food in the culture pond. This confirms a previous study showing that the addition of carbon sources in biofloc systems likely increases fish production through the increased microbial protein produced in the system that can be used as an additional source of nutrition for the cultured animals (Zhao et al. [2014](#page-11-0)). The higher efficiency of feed utilization in biofloc treatments may be seen in the lower total feed in biofloc treatments.

The low total feed in biofloc treatments could indicate that both species consume various types of feed available in the culture media, including bioflocs suspension. Tilapia and redclaw crayfish have been known as omnivorous species that consume various types of feed including algae and detritus (Wang and Lu [2015;](#page-11-0) Ghanawi and Saoud [2012](#page-10-0)). In addition, biofloc may contain major nutrients such as protein and lipids that could contribute to the growth of the cultured animal (Crab et al. [2010\)](#page-9-0). A previous study by Ekasari et al. ([2010\)](#page-10-0) noted that the biofloc system produced proteins that could easily be absorbed, which might contribute to the lower FCR.

The food conversion ratios differed between biofloc and control treatments both for each species and in the total combination. The results of other studies showed that the culture of tilapia with biofloc systems could decrease FCR and reduce feed consumption by 20% compared to tilapia culture with a water exchange system (Azim and Little [2008;](#page-9-0) Emerenciano et al. [2013\)](#page-10-0). The authors attributed the low FCR to the availability of biofloc microorganisms that could reduce the requirement for external feeding, thereby decreasing the FCR (Ekasari et al. [2010](#page-10-0); Pérez-Fuentes et al. [2016](#page-10-0)).

Research on tilapia–crayfish co-culture has been reported by Karplus et al. ([2001](#page-10-0)), using monosex male tilapia and male crayfish. This study showed that at a combination of tilapia at a density of 33 ind m⁻³ and 10 redclaw crayfish (low density) resulted in the highest DWG. This study demonstrated that the use of monosex tilapia and redclaw crayfish has an impact on the productivity of the cultures. Our study showed that the DWG and SGR values were not significantly different $(P> 0.05)$ in all treatments. These may be explained by the use of mixedsex biota that could have an influence on the growth in both tilapia and crayfish.

The present study showed that several Nile tilapia fish and redclaw crayfish in the treatments were reproductively mature. Reproductive maturation would affect the growth of Nile tilapia and crayfish. Soowannayan et al. [\(2015](#page-10-0)) reported that female redclaw crayfish stop growing after they reach gonadal maturity. A study by Curtis and Jones ([1995](#page-9-0)) noted that the growth of female redclaw crayfish was faster without the presence of male crayfish. Likewise, monosex male Nile tilapia demonstrated a higher rate of growth than females. Male and female tilapia will grow quickly at the beginning of the maintenance period, but the rate will decline as they reach maturity (Azim and Little [2008;](#page-9-0) Chakraborty et al. [2011\)](#page-9-0).

Another study that reported a polyculture system of tilapia and crayfish in earthen ponds showed that survival of tilapia (stocking density of 1 ind m^{-3}) was in the range of 76–95%, and that of redclaw crayfish (2.5 crayfish m[−]2) was 35.8–44.55% (Brummet and Alon [1994](#page-9-0)). Karplus et al. [\(2001](#page-10-0)) showed that intensive polyculture of tilapia–crayfish for a period of 133 days resulted in no significant difference in fish and crayfish survival between the treatments $(P> 0.05)$, with a range of 88–95% and 44.5–59.5%, respectively. In the present study, bioflocbased polyculture resulted in tilapia and crayfish survivals of 94.4–95.6 and 60–70%,

respectively, which is higher than those reported previously (Karplus et al. [2001](#page-10-0)). The higher survival was likely because of the use of shelter in the culture media. Mortality mainly occurred in the beginning of the culture regardless of the treatment, and thus may have been caused by the environmental influence and stress during the adaptation process of tilapia and the territorial behavior of redclaw crayfish.

Water quality

The levels of TAN and nitrite in the culture media were still within a normal range for both the tilapia culture (Cavalcante et al. [2014](#page-9-0); Caldini et al. [2015](#page-9-0); Dagne et al. [2013](#page-10-0)) and crayfish (Masser and Rouse [1997](#page-10-0); Naranjo-Páramo et al. [2018\)](#page-10-0) (Table 4). The lowest average TAN-N, nitrite-N, and nitrate-N concentrations during the culture period were observed in the biofloc treatment with a C/N ratio of 15. The low concentration of TAN in biofloc ponds compared with that of the control may be attributed to the presence of heterotrophic bacteria in BFT systems which accelerated the rate of ammonia reduction in the media (Widanarni et al. [2012](#page-11-0)).

The availability of nitrite and nitrate in all ponds could indicate the presence of a nitrification process by the autotrophic nitrifying bacteria which oxidize ammonia to nitrite and nitrite to nitrate (Ebeling et al. [2006\)](#page-10-0). However, the nitrification of nitrifying bacteria can be limited by the growth of heterotrophic bacteria (Brune et al. [2003](#page-9-0)). Autotrophic nitrifying bacteria was also likely to be present in and dominate the control treatments (Verstraete and Alexander [1973;](#page-11-0) Watson et al. [1981](#page-11-0)). The lowest TAN and nitrite levels were both observed in the treatment with a C/N ratio of 15, indicating efficient conversion of these compounds in heterotrophic systems such as biofloc systems.

The values of pH and temperature did not differ significantly among treatments, and were within optimal conditions for normal growth of tilapia. The pH value (Fig. [2](#page-6-0)a) tended to decrease as the culture progressed, which might be due to the increase in microbial biomass as a result of the accumulating organic matter coming from the uneaten feed, biofloc biomass and the metabolic waste of the organisms. In addition, one of the metabolic wastes from both the cultured organisms and the microbes that directly affects water pH is $CO₂$ that dissolves in water, forming carbonic acid which subsequently increases water acidity. Furthermore, microbial activity such as nitrification is likely to contribute to the decreased pH. High microbial activity also resulted in a gradual reduction in the dissolved oxygen concentration in all

Parameter	Acceptable range		Notes
	Tilapia	Crayfish	
TAN $(mg L^{-1})$	$0.17 - 3.87$ ^b	< 1.00 d,f	
Nitrite N (mg L^{-1})	$0.02 - 0.12$ ^a	$< 1.00^{\circ}$	
Nitrate(mg L^{-1})	$0.2 - 219b$	$< 10^e$	
pH	$6.00 - 9.00$ ^a	$6.50 - 9.00$ ^e	
DO $(mg L^{-1})$	$3.00 - 9.00$ c	$> 5.00^{\circ}$	Adult > 1.0 mg L^{-1} , juveniles are more sensitive to low dissolved oxygen
Temperature $({}^{\circ}C)$	$25.00 - 32.00^{\circ}$	$23.00 - 31.00^e$	

Table 4 Acceptable range of water quality parameters for Nile tilapia and redclaw crayfish

^a Cavalcante et al. ([2014](#page-9-0)); ^b Caldini et al. ([2015](#page-9-0)); ^c Dagne et al. [\(2013\)](#page-10-0); ^d Masser and Rouse [\(1997](#page-10-0)); ^e Naranjo-Páramo et al. (2018) (2018) (2018) . TAN = total ammonia nitrogen, DO = dissolved oxygen

treatments (Fig. [2](#page-6-0)b) (Fleckenstein et al. [2018;](#page-10-0) Avnimelech 2009). As expected, floc volume in both biofloc treatments was significantly higher than that in the control. This may relate to the addition of carbon sources in these treatments, which increased the growth of biofloc (bacterial biomass) (Pérez-Fuentes et al. [2016](#page-10-0)).

Conclusion

Biofloc-based polyculture in Nile tilapia–redclaw crayfish significantly reduced the FCR and the total feed given and improved the water quality. Biofloc treatment with a C/N ratio of 10 gave the lowest FCR and the highest total biomass values among treatments. However, the best water quality was observed in the biofloc treatment with a C/N ratio of 15.

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Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interest.

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