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Growth and Feed Efficiency of Gourami Fish Reared in Biofloc Media with Different C/N Ratios

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Abstract

The study was conducted to determine C/N ratio which gave the best growth, feed conversion ratio, feed efficiency, survivability, mortality rate, bacteria abundance and reduced nitrogen content in the production media of gourami fish. Treatments consisted of different C/N ratios including control, CN ratio 12, CN ratio 16, and CN ratio 20 in the production media. The trial was conducted in 45 days. Daily weight gain, feed conversion ratio, feed efficiency, survivability, mortality rate, abundance and types of bacteria, total ammonia-N (TAN), total nitrite, and total nitrate contents were evaluated. Results showed that different C/N ratios gave significant effects on feed conversion ratio, feed efficiency, survivability, and mortality rate, but not on daily weight gain. The best feed conversion ratio and feed efficiency was found in C/N ratio 12 while the best survivability and mortality rate were found in CN ratio 20. The bacteria spreading over the treatment groups were found to reduce from 11 types in the beginning of the trial period to only 8 types in the end of the trial period. In all treatment groups Micrococcus sp. was found in the beginning of the trial period while Bacillus sp. was found in the end of the trial period. Based on TAN, nitrate, and nitrite measurements, in production media with high C/N ratios, N content was found to be reduced and in the amount ranges that were good for fish growth even though no water changes were done.

Keywords: gourami fish; growth; feed efficiency; biofloc; mortality; bacterial abundance; C/N ratio.

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1. Introduction

Feed and environment are important factors affecting the success of fish production. Intensive aquaculture depends 100% on artificial feed. Only 20-30% of feed given to fish will be stored in the fish body [1,2] and the remaining will be wasted into the aquaculture environment. Uneaten, undigested, and metabolically wasted feed is an organic material which will accumulate in the aquaculture environment and become toxic to fish. This toxic organic material in nitrogen is in the form of ammonia. Unionized ammonia is harmful to cultured organisms as it is toxic [3;4]. One of the main sources of nitrogen in aquaculture environment is feed [5]. Overfeeding in intensive aquaculture can cause high accumulation of nitrogen in the environment of the aquaculture. Reduction of nitrogen in the medium of fish production should be done in order to maintain the aquaculture environment in a good condition. Reduction of nitrogen in an aquaculture environment can be done by increasing the population of heterotrophic bacteria which can utilize nitrogen from organic compounds, feces, and feed remains as their energy sources. These bacteria utilize inorganic and organic materials synthesize bacterial protein [2;6]. C/N ratio is an important factor affecting the growth of heterotrophic bacteria [6]. Increased C/N ratio which can increase the density of heterotrophic bacteria and maintain C/N ratios 10-30 can be done by continuously adding organic carbon sources [2;7;8]. The addition of carbon to an aquaculture environment improves C/N ratio which subsequently allows heterotroph bacteria to assimilate ammonia-nitrogen directly from water and metabolize ammonia into cell biomass [7;8]. The utilization of these bacteria is now being developed to overcome some problems in the field of aquaculture [2;9;10;11;12] and this method is commonly called biofloc technology. The use of biofloc technology can reduce the inorganic nitrogen waste through the activity of heterotrophic microbes [13] and allows the utilization of aquaculture waste as bacterial biomass which is potential as fish feed [14]. Studies on the utilization of biofloc technology and its effects on growth performance, feed efficiency, water quality have been done in nile tilapia fish and shrimp [13;14;15]. In this study, the use of biofloc technology in gourami fish with the addition of carbon to increase C/N ratios was conducted to assess its effects on nitrogen waste reduction, growth rate and feed efficiency increment, and bacterial abundance and types.

2. Materials and methods

2.1 Materials

Six-hundred gourami fish obtained from fishermen in Parung area, Bogor was used. The incoming fish was put in fiber containers at Fishery Laboratory of Djuanda University, Bogor for a one-week adaption period. The initial bodyweight of the fish was 13.42-13.94 g per head. Artificial pelleted feed containing 30% protein was used. As carbon sources, molasses containing 44.56% carbon content was added into biofloc media.

2.2 Procedures of trial

This study was a laboratory trial with treatments consisting of biofloc media with different C/N ratios. These included control, C/N ratios 12, 16, and. 20. Cylindrical containers with a diameter of 1 m and a height 1 m were cleaned and dried before they were filled with water to the height of 70 cm. In order to grow bacteria which were compatible to the environment where fish would be reared, the water used to fill the containers was taken from a pond and a well (50:50) located around the laboratory. After undergoing a week adaption period, the fish was kept in the containers with a density of 50 fish per container for 45 days. Feed was given at satiation twice a day at 08.00 and 16.00. Feed was weighed daily to determine the amount of molasses to be given per container per day. During the trial period, all fish growing media except control were provided with molasses proportional to treatment C/N ratios. Molasses were given once per day one hour after the last feeding. During the trial period no water change was done and water addition was done to compensate water loss caused by evaporation. In order to maintain water quality in control containers, water change was done daily.

2.3 Measurement of experimental parameters

2.3.1 Giant gourami performance

Before being put in the treatment containers, the fish was weighed to obtain the initial fish biomass . In the end of the trial period, the fish was also weighed to obtain the final fish biomass. Daily weight gain rate (%) was obtained based on data of initial fish biomass, final fish biomass, and length of trial. The amount of feed (gram) given during the trial period was determined to obtain data on feed efficiency (%) and feed conversion ratio. Feed efficiency (%) and feed conversion ratio were calculated based on fish growth (difference between fish biomass in the end and beginning of the trial period) and the amount of feed given. Survivability rate (%) of fish was calculated based on the ratio of the number of fish alive in the end of the trial period and that in the beginning of the trial period. Similarly, mortality rate (%)was calculated based on the ratio of the number of fish dead in the end of the trial period and that in the beginning of the trial period. For these purposes, the number of fish left in the end of the trial period was counted and compared to the initial number of fish (50) in the beginning of the trial period.

2.3.2 Abundance and types of bacteria

The abundance and types of bacteria were measured and determined in the beginning, middle, and end of the trial period. The abundance of bacteria was counted by using a spread plate method. Sample water was taken from each container, diluted, and spread over the Trypticase Soy Agar (TSA) in petri dishes. The petri dishes were incubated for 24 hours before the abundance of bacteria was measured by using the [18] formulation.

2.3.3 Water quality

Measurement of dissolved oxygen (DO) and temperature was conducted daily in the morning and afternoon by using a DO meter. Acidity level (pH) was measured weekly by using a pH meter. Total ammonia-N (TAN), nitrite, and nitrate levels of sample water from each container were measured every 15 days by using an indophenol/phenate method [16].

2.4 Data Analysis

Data were subjected to an analysis of variance and a least significant difference test. Data of water quality, nitrate level, nitrite level, TAN level, bacterial abundance, and bacterial types were subjected to a qualitative analysis.

3. Results

3.1 Giant gourami performance

Table 1: Daily Growth Rate (SGR), Feed Efficiency (EP), Feed Conversion Ratio (FCR), Survival Rate (SR), Mortality (M) of Gourami Fish during trial period

Parameters measured	Treatments (C/N ratio)			
	Control	12	16	20
SGR (%)	1.65	1.80	1.47	1.53
EP (%)	55.37^{a}	76.29^{b}	63.52^{ac}	68.68^{bc}
FCR	1.82^{a}	1.31^{b}	1.57^{c}	1.46^{bc}
SR (%)	73.00^{a}	97.00^{b}	97.33^{b}	100.00^{c}
M (%)	27.00^{a}	3.00^{b}	2.67^{b}	0.00^{c}

Note : different ssuperscript indicates singnificant difference ($P<0.05$)

It was found that giant gourami fish reared in C/N ratio 12 had the best daily growth rate (1.80%) (Table 1). However, no significant effect ($P>0.05$) of C/N ratio treatments was found on daily growth rate. Gourami fish reared in medium with C/N ratio 12 had the highest feed efficiency (76.29%) and fish in control medium had the lowest feed efficiency (55.37%) as shown in Table 1. C/N ratio treatments were found to give significant effect ($P<0.05$) on feed efficiency. Fish in control group had different feed efficiency from those of fish reared in C/N ratio 12 and 20 treatments but not from that of fish reared in C/N ratio 16. Feed efficiency of fish reared in C/N ratio 12 treatments was different from that of fish reared in C/N ratio 16 treatments but not from that of fish reared in C/N ratio 20 treatments. Fish reared in growth media with C/N ratio of 16 and 20 had no different feed efficiency. The best feed conversion ratio (1.31) was found in Gourami fish reared in C/N ratio 12 (Table 1). Treatments were found to give significant differences ($P<0.05$) in feed conversion ratio. Feed conversion ratio in control group was significantly different from that in other treatment groups. Fish in control group had the highest feed conversion ratio (1.82). C/N 12 treatment gave a feed conversion ratio which was significantly different from that of C/N ratio 16 treatment, but not from that of C/N ratio 16 treatment. Meanwhile, feed conversion ratio of fish in C/N ratio 16 treatments was not different from that of fish in C/N ratio 20 treatment. Survivability of Gourami fish in C/N ratio 20 treatments was found to be the highest (100%) while that of fish in control group was the lowest (73.00%) (Table 1). C/N ratio treatments were found to give different effects ($P<0.05$) on Gourami fish survivability. Fish survivability in control group was different from that in other treatment groups. Fish in C/N ratio 16 and 20 treatments had no different survivability but fish in both treatments had different survivability from fish in C/N ratio 20 treatments. Gourami fish in C/N ratio 20 treatment group had the best mortality rate (0%, with no dead fish) (Table 1). It was found that treatments gave significant effects ($P<0.05$) on fish mortality rate. Fish mortality rate in control group was different from that in C/N ratio 12, 16, and 20 treatments. Fish in C/N ratio 12 and 16 treatments had no different mortality rate but fish in both treatments had different mortality rate from fish in C/N ratio 20 treatments

3.2 Abundance and types of bacteria

In the beginning of trial period, the bacterial abundance was $3.00x10^4$-$2.30x10^5$. The lowest bacterial abundance was found in C/N ratio 20 medium and the highest was in control group. Bacteria count in control group was decreasing until the end of trial period while that in C/N ratio 12 medium was found to increase in the middle of the trial period (day 30) and slightly decrease on day 45. In C/N ratio 16 medium, bacterial counts decreased in the middle of the trial period but it rebound in the end of the trial period. Bacterial count in C/N ratio 20 medium was found to steadily increase until the end of the trial period. Types of bacteria found in each C/N ratio medium varied since the beginning to the end of the trial period (Table 2). *Micrococcus* sp. was found to be dominant in the beginning but it was replaced by *Bacillus* sp. in the end of the trial period. Types of bacteria in control, C/N ratio 16, and C/N ratio 20 groups were found to increase in the end of the trial period. In contrast, in C/N ratio 16 groups, types of bacteria were found to decrease.

Table 2: Abundance (CFU/ml) and types of bacteria in growing media of Gourami fish

Treatment	Beginning		Middle		End	
	Bacterial count	Type of bacteria	Bacterial count	Type of bacteria	Bacterial count	Type of bacteria
Control	**$2.30x10^5$**	Bacillus sp. Micrococcus sp. Clostridium sp.	**$5.61x10^4$**	Pseudomonas sp. Cromobacterium lixidium, Micrococcus sp. Bacillus sp. Streptococcus sp. Enterobacteria sp. Campylobacter sp.	**$7.50x10^4$**	Cromobacterium lixidium, Pseudomonas sp. Acinetobacter sp. Bacillus sp. Enterobacteria sp. Campylobacter sp.
C/N ratio 12	**$1.39x10^5$**	Acinetobacter sp. Enterobacteria sp. Kurthia sp. Cromobacterium lixidium Campylobacter sp Haemophylus sp. Micrococcus sp.	**$3.37x10^5$**	Enterobacteria sp. Pseudomonas sp. Cromobacterium lixidium. Acinetobacter sp. Flavobacterium. Aeromonas sp.	**$1.41x10^5$**	Bacillus sp., Enterobacteria sp. Cromobacterium sp. Pseudomonas sp.
16	**$1.12x10^5$**	Micrococcus sp. Campylobacter sp. Eubacterium sp. Cromobacterium lixidium Bacillus sp.	**$3.17x10^4$**	Bacillus sp. Campylobacter sp. Pseudomonas sp. Cromobacterium sp. Enterobacteria sp. Streptobacillus sp. Acinetobacter sp.	**$1.22x10^5$**	Bacillus sp. Cromobacterium lixidium, Campylobacter sp. Enterobacteria sp. Campylobacter sp. Acinetobacter sp.
20	**$3.00x10^4$**	Streptobacillus sp., Campylobacter sp. Micrococcus sp. Cromobacterium lixidium	**$2.02x10^5$**	Campylobacter sp. Acinetobacter sp. Streptobacillus sp. Bacillus sp. Micrococcus sp. Clostridium sp.	**$1.79x10^5$**	Streptobacillus sp. Acinetobacter sp. Pseudomonas sp. Bacillus sp. Campylobacter sp. Clostridium sp.

3.3 Water quality

The quality of water in all Gourami fish growing media during the trial period was found to be within the acceptable range (Table 3). Dissolved oxygen (DO) values were fairly high (5.1-7.2 ppm), temperature was 25.0-28.4^{o}C, and pH was 6.3-7.2.

Table 3: Quality of water in all Gourami fish growing media during the trial period

Water quality parameter	Treatment (C/N ratio)			
	Control	12	16	20
Dissolved oxygen (DO) (mg/L)	5.2-7.1	5.1-7.2	5.1-7.1	5.1-7.1
Temperature ($^{\circ}$C)	25.0-27.8	25.0-28.4	25.0-27.7	25.3-28.2
pH	6.8-7.2	6.5-7.1	6.3-7.0	6.7-7.2

TAN in each treatment was found to fluctuate (Figure 1). TAN levels in all treatments increased on day 15 but on day 30 they all decreased considerably to the levels closed to those in the beginning of the trial period. In the end of the trial period, TAN levels in all treatment groups rebound but the levels were not as high as those on day 15. The increase in TAN level in control group was found to be the smallest. The highest TAN level of 1.50 mg/L was found in C/N ratio 20 on day 15 while the lowest of only 0.15 mg/L was found in C/N ratio 16 in the beginning of the trial period. TAN levels in all treatment groups were still within the acceptable ranges for fish growth.

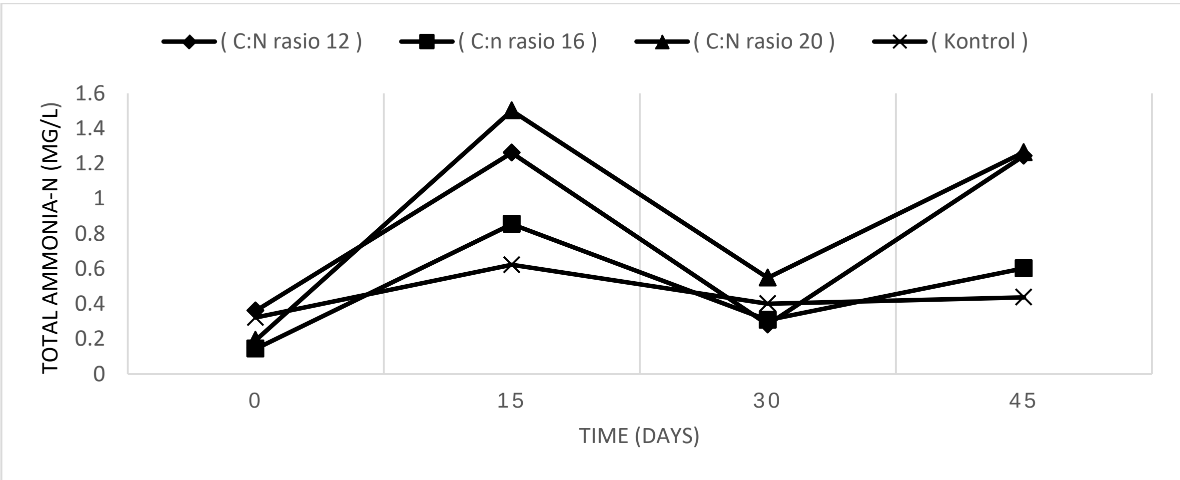

Figure 1: Total ammonia-N in growing media with different C/N ratios during 45 day trial period

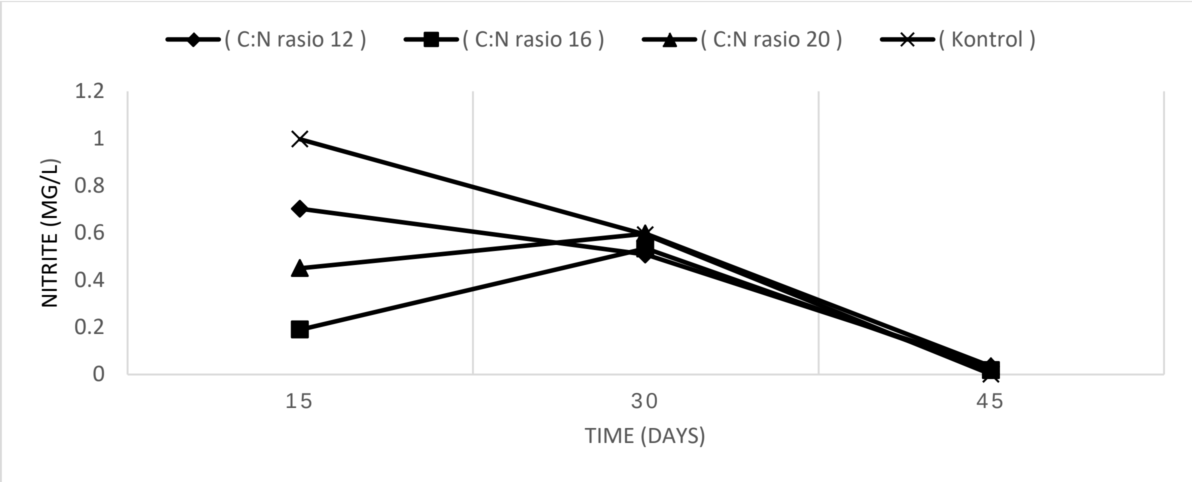

Figure 2: Levels of nitrite (mg/L) in growing media with different C/N ratios during 45 day trial period

On day 15, levels of nitrite (Figure 2) in all treatment groups were different. The highest level of nitrite was found in control group (1.00 mg/L) followed by those in C/N ratio 12 (0.70 mg/L), C/N ratio 20 (0.45 mg/L), and C/N ratio 16 (0.19 mg/L). In the end of the trial period, nitrite levels in all parameters were found to be decreased to almost similar levels of 0.02-0.03 mg/L, although on day 30 those of C/N ratio 16 and 20 were found to be increased. In control and C/N ratio 12 groups, nitrite levels were found to decrease steadily from day 15 to the end of the trial period. However, nitrite levels in all treatment groups, except that in control group on day 15 which was fairly high, were still within the acceptable ranges for fish growth.

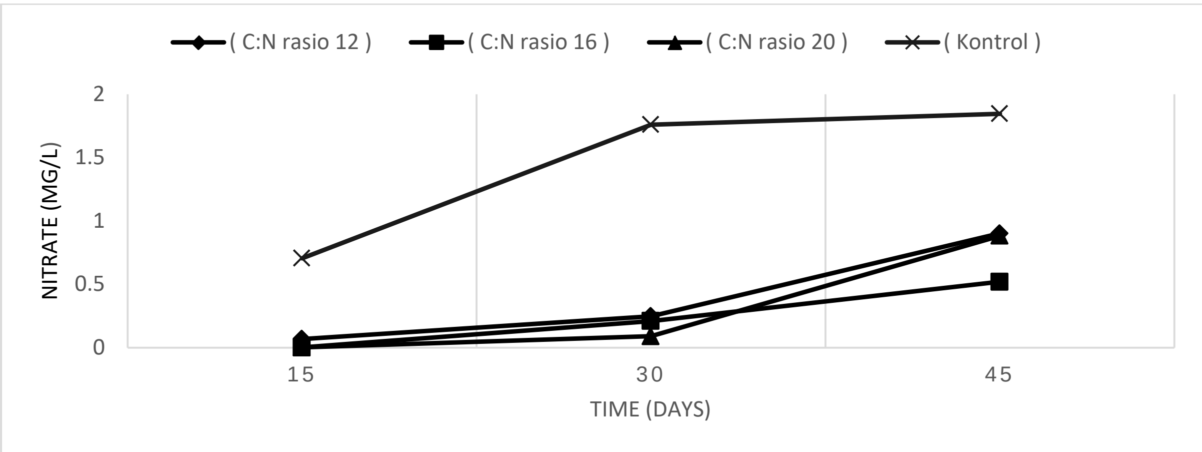

Figure 3: Levels of nitrate (mg/L) in growing media with different C/N ratios during 45 day trial period

Nitrate levels in all treatment groups steadily increased until the end of trial period (Figure 3). Nitrate levels of control group on day 15 to the end of the trial period were always higher than those of other treatment groups. The highest nitrate level of 1.85 mg/L was found in control group in the end of the trial period. Nitrate levels of all treatment groups were still within the acceptable ranges for fish growth.

4. Discussion

4.1 Giant gourami performance

In this study, C/N ratios in biofloc media did not give significant effects (P>0.05) on daily growth rate of gourami fish (Table 1). This indicated that biofloc media with different C/N ratios could still support the growth of fish although during the trial period no water changes were done in treatment containers and only 20%. Water volume was changed in control containers in order to maintain good water quality[17]. Fish growth is affected by the environment (water quality) where fish is reared[18]. Minimal water change was also found to increase shrimp production [20]. The utilization of microbial activity in biofloc technology reduced organic material in aquaculture medium [21] allowing water quality to be well maintained. Biofloc technology lowered ammonium concentration and improved water quality in aquaculture ponds [19,20]. Studies on *Litopenaeus vannamei* [24] and *Labeo victorianus* [25], and *Carassius auratus* [21] reared in different media C/N ratios found that no different daily weight gain was found which was similar to the results of this study. Different results were found by [22,23,24,25], and [26] where the use of biofloc technology gave higher daily growth rates than the control group did. Biofloc technology was also found to give better performance in vannamei shrimps [27]; [28], *Farfantepanaeus brasiliensis* shrimps [29;30], and giant freshwater prawns [31].

Results of this study showed that C/N ratio treatments gave significant effects on feed efficiency with the highest feed efficiency was found in C/N ratio 12 group. It is shown in Table 1 that feed efficiency in biofloc media was higher than that in control group. Similar results were shown in vannamei shrimps [27]. Higher feed efficiency in biofloc medium indicated that fish reared in this medium was able to use the feed for its growth better. Biofloc could trigger the activity of digestive enzymes which was beneficial for fish growth [24]. The positive effect of bioflock medium on feed efficiency was attributed to the role of bacteria found in fish rearing media. It was known that bacteria in rearing media would form flocks which served as supplementary protein feed for fish allowing it to increase its feed efficiency [37]. Nutrient contents in flocks were high and fish could immediately use the flocks as its feed [32;33;34;35].

C/N ratios treatments were found to give different effects on feed conversion ratio. The best feed conversion ratio was found in C/N ratio 12. Results of this study showed that feed conversion ratio in biofloc media was better than that in control group. Biofloc technology was found to lower feed conversion ration [41]. Different from feed conversion ratios, no different growth rates were found in this study. Similar results in which feed conversion ratio was smaller in biofloc media than in control group were confirmed by [24,28,23]. Feed conversion ratio indicates the amount of feed (kilogram) required in order to produce a kilogram of fish. Low feed conversion ratio found in C/N ratio treatment groups in this study indicated that the amount of feed was lower than that in control group. Similar growth rate with lower feed given in bifloc media indicated that there was bacterial contribution serving as supplementary feed to support fish growth. Bacteria in fish rearing medium could serve as feed source for fish [36;15].

It was shown in this study that C/N ratio treatments gave significant effects on fish survivability. Fish reared in biofloc media had better survivability rate than fish in control group. Water quality is an important factor affecting fish survivability and biofloc was found to be able to control water quality. This was in line with the results of this study where 100% survivability rate was found in fish reared in media with C/N ratio 12 and 73% survivability rate was found in fish reared in control group. Higher survivability rates of 90-100% were found in previous biofloc studies [26;21].

Mortality rate was the percentage of dead fish in the end of the trial period. Mortality rates of gourami fish used in this study was significantly different (table 1). The best mortality rate of 0% (no dead fish) was found in C/N ratio 20 treatment. It was also shown that mortality rate in biofloc medium was lower than that in control group. Fish mortality could be caused by bad water quality or pathogens. In this study, water changes were done in control group only but in biofloc media. However, water quality in all rearing media was considered to be similar except that nitrite value in control medium was considerably high (measurement on day 15). Low mortality rate in biofloc media was attributed to bacterial roles. Adding carbon to a fish rearing medium improves bacterial abundance. In this study, bacterial abundance in the end of trial period in C/N ratio treatment media (biofloc) was higher than in control group. Bacteria found to live in bioflock media were heterotrophic and beneficial (Table 2) which would attach pathogenic ones. In addition, biofloc containing *poly-β hydroxybutirate* improved fish immune system [37;38]. High mortality rate of fish reared in control might be caused by high nitrite value (1.00 mg/L). Nitrite value of 0.66-200 mg/L could cause death in freshwater fish [44].

4.2 Abundance and types of bacteria

Carbon addition into an aquaculture medium increases C/N ratio which is an important factor affecting the growth of heterotrophic bacteria [6]. In this study, measurements done throughout the trial period showed carbon (molasses) addition increased the value of bacterial abundance in C/N ratio 20 only. Bacterial abundance in C/N ratio 12 and 16 was found to be stable. In control group, there was a reduction in bacterial abundance throughout the trial period. This was a result of water changes that might reduce the concentration of bacteria in fish rearing media. Bacterial abundance in the beginning of trial period in control medium was resulted from the initial bacterial growth following the availability of organic materials from uneaten feed, feces, and metabolic wastes. Bacterial growth rate in control group was lower than that in C/N ratio treatments as there was no carbon addition into the control medium. Bacterial abundance depended on nutrient availability in water [45] and carbon was found to be a bacterial growth limiting nutrient in aquaculture environment. Heterotrophic bacterial would have their maximum growth at increased C/N ratio [7]. C/N ratio treatment ensured bacterial abundance as a result of daily carbon addition. The patterns of bacterial abundance in C/N ratio 12 and C/N ratio 20 were similar with an increase in the middle of trial period and a decrease in the end of trial period. The addition of carbon resulted in improved bacterial abundance while decreased bacterial abundance might be caused by the utilization of bacteria as fish feed. This was reflected in growth rate, feed efficiency, and feed conversion ratio that were better in C/N ratio 12 and 20 treatments (Table 1).

Types of bacteria in all treatments were found to be varied. In the end of trial period, 6 types of bacteria were found in control, C/N ratio 16, and C/N ratio 20 groups and 4 types of bacteria were found in C/N ratio 12 group. Types of bacteria found to live in all treatments were similar (Table 2). All bacteria found were heterotrophic bacteria which required organic carbon as their carbon source. Heterotrophic bacteria used organic materials as the source of carbon for their growth [46].

4.3 Water quality

Oxygen content in all gourami fish rearing media was found to be similar and fairly higher (5.1-7.2 mg/L) than the minimum requirement level for good fish growth (3 mg/L) [47]. Temperature and pH levels were also found to support good fish growth in all rearing media (Table 3). DO and temperature were important for metabolic activities of bacteria [48}. In biofloc technology, DO also affects floc structure. Floc size was found to be bigger and dense at high DO concentration [49]. It was shown in Figure 1 that TAN values in all treatments fluctuated and shared similar patterns. TAN values were low in the beginning of trial period, increased on day 15, dropped on day 30 before they rebounded on day 45. In the beginning of trial period, TAN vales were low as there were no accumulated organic materials from uneaten feed, feces, and metabolic wastes. Increased TAN values on day 15 in C/N ratio treatments indicated the accumulation of organic materials which were utilized by bacteria for their growth as indicated by lowered TAN values. For their growth, bacteria used nitrogen and this reduced ammonium concentration in water [23]. Organic material accumulation was found to be reduced as a result of bacterial activities [21]. In control group, TAN values were relatively lower and less fluctuated than those in C/N ratio treatment groups. Low TAN values in control group were caused by daily water changes (20% volume) and nitrification and denitrification processes by bacteria changing TAN into nitrite and nitrate. This was reflected by higher nitrite and nitrate values in control group than in C/N ratio groups (Figures 2 and 3). In control group, nitrite value on day 15 was high and kept decreasing to the end of trial period. Reducing nitrite value and increasing nitrate value indicated that there was a conversion of nitrite into nitrate.

5. Conclusions

Increased C/N ratio up to 20 did not affect the growth of gourami fish but resulted in better feed efficiency, feed conversion rate, survivability, and mortality rate. The best feed efficiency (76.29%) and feed conversion ratio (1.31) were found in fish reared in C/N ratio 12. The best survivability (100%) and mortality rate (0%, no dead fish) were found in fish reared in C/N ratio 20. C/N ratio increment in fish rearing media reduced nitrogen content although no water changes were done and increased bacterial abundance. *Bacillus* sp. was found as the dominant type of bacteria in this study.

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