FEED MANAGEMENT AND MOLASSES APPLICATION ON THE INTENSIVE MILKFISH CULTURE (*Chanos chanos* Forsk.) IN PONDS

Brata Pantjara[*)#], Erfan Andi Hendradjat[*)], and Anang Hari Kristanto[**)]

[*)] *Research and Development Institute for Coastal Aquaculture*
[**)] *Center for Aquaculture Research and Development*

(Received June 13th, 2013; Accepted November 29th, 2013)

ABSTRACT

The problems faced in the intensive milkfish culture were the high and expensive feeds requirement. To overcome the problem, it needs the optimization of feed management with application of molasses for producing of bioflocs in pond. Bioflocs were a biomass set of heterotrophic bacteria, protozoa, plankton, and organic particle. The purpose of this research was to know the influence of feed management and application of molasses on the intensive culture of milkfish in ponds. The study was conducted in brackishwater pond with 2,500 m^2 size; conducting at experimental pond installation, Research and Development Institute for Coastal Aquaculture, Maros. The treatments of this research were without reduction commercial feeds as control (A); 10% reduction of commercial feed and molasses application (B); and 20% reduction of commercial feed and molasses application (C). Reduction of commercial feed was as many as 10% and 20% of the feed given doses of each application. The results of this research showed that intensive milkfish culture with biofloc was not significantly increase of the growth and production of milkfish. The highest of milkfish production was obtained in A treatment with 7,260 kg/ha; followed by B (6,339 kg/ha) and the lowest was in C (5,780 kg/ha).

KEYWORDS: molasses application, feed management, milkfish culture

INTRODUCTION

Milkfish (*Chanos chanos* Forsk.) is the type of fish that is strategic to be developed, because the fish is well popular not only in domestic but also for export markets (FAO, 2008). The pond conditions that are less productive for shrimp culture, it can be used for milkfish culture which is a reliable alternative commodity for increased ponds productivity.

The problems of intensive milkfish culture are the use of highly commercial feed (Brune *et al.*, 2003; Liem, 2002). This condition can increase the operating costs and cause losses of fish farmers due to the imbalance of costs especially for feed costs that is higher than sales (selling price) of milkfish. It is necessary to reduce the feed cost. One such effort is to provide additional food (supplement) as bioflocs with molasses application in the pond (Crab *et al.*, 2009).

Providing of high feeding on intensive ponds can cause organic waste accumulation in bottom pond and influence of water quality in the ponds (Boyd, 1995; Buentello *et al.*, 2000). Utilization of bacteria In the pond, is expected to convert organic waste of residual feed and fish excretion into a bunch of heterotrophic bacterial biomass and others microorganism. Heterotroph bacteria together with other microorganisms such as protozoa,

* Corresponding author. Research and Development Institute for Coastal Aquaculture, Jl. Makmur Dg. Sitakka No. 129, Maros 90512, South Sulawesi, Indonesia.
Phone: +62 411 371544
E-mail:

phytoplankton, zooplankton, organic particles and others formed floc which contains high protein and good for fish nutrition (Caraco *et al.*, 1978; Garg *et al.*, 2007; Kuhn *et al.*, 2008). Decomposition of organic waste in the ponds occurs gradually starting from the decomposition of food organic waste (amonification process) by bacteria into ammonia and then in the nitrification process to nitrite and nitrate (Hargreaves, 1989; Jorand *et al.*, 1995; Kaiser, 1994). In ponds, the high content of ammonia (NH_3) and nitrite (NO_2^{2-}) were toxic to fish that could cause mass mortality (Halver & Hardy, 2002; Pantjara, 2008).

Bacterial growth in pond is influenced by the balance of carbon and nitrogen while comparison of carbon and nitrogen is good for heterotroph bacterial growth about 15-20 (Hargreaves, 1989). CN ratio in intensive pond is low, so that to increase of CN ratio is needed of molasses (Coyne, 1999; Moriarty, 1997; Pantjara, 2008). Therefore, utilization of feed and other organic waste into protein from a bunch flocs is expected as supplement for additional feed of milkfish and the reduction of organic waste in the ponds can improve water quality (Avnimelech, 1999; Brune *et al.*, 2003). Furthermore according Avnimelech (2009), molasses application to bioflocs in the pond can improve feed efficiency of red tilapia growth, but the use of biofloc for milkfish has not been much information.

In the future research on feeding efficiency in intensive milkfish culture in the ponds by growing microorganisms (bacteria, plankton, algae, etc) in a semi-bioflocs through carbon source (molasses) application is required. The objective of this study was to know the influences of carbon sources (molasses) to feed efficiency in the intensive milkfish culture.

MATERIALS AND METHODS

The study was conducted in Experimental Pond Installation, Research and Development Institute for Coastal Aquaculture, Maros. The experimental ponds used for the research were six plots, each measuring 2,500 m^2. Each plot was equipped with two paddlel wheel, which each capacity has 1 and 2 house power (HP) to provide the sufficient oxygen requirement in the pond for microorganism flocs formation.

The combination of commercial pelleted feed and mollasses application was used as the treatment. Each treatment was a dosage of 3% of body weight/day (controls) without application of molasses, B. Dosage of 2.7% of body weight/day and application of molasses (10% reduction of feed standard dose) and C. Dosage of 2.4% of body weight/day and application of molasses (20% reduction of feed standard dose).

The rearing of milkfish in intensive pond was conducted for 7 months. Experimental animals tested in this study were young milkfish with measuring about 1-3 g/fish, and stocked at a density of 50,000 ind./ha. The milkfish did not provide a commercial pellet feed after stocking for 1 month, they only received a natural feed from given organic fertilizer.

The addition of commercial feed was conducted in the 3rd weeks and the 4th weeks that was expected of bioflocs growth in the pond. Commercial feed to milkfish contained about 20% protein. The bioflocs growth with molasses addition was conducted every day in the morning and in the afternoon by mixturing of molasses and pond water in a container with volume 5-10 L furthermore it was applicated to the pond. The molasses added was based on the amount of protein content in feed on a daily basis and estimated amount of nitrogen from protein in feed waste that accumulated in pond, moreover the total ammonia nitrogen (TAN) and C-organic molasses content.

The addition of water into the pond was conducted with minimum amount to replace water evaporation. The variables was observed including shrimp growth rate, amount of feed provided, survival rate, TAN, nitrite, nitrate, dissolved oxygen, temperature, pH, and total heterotrophic bacteria. Analysis of water quality refers to the APHA (2008). Histological analysis of the intestine of milkfish was carried in the Veterinary Diseases Investigation Center, Maros. To determine the feasibility of milkfish cultivation in each treatment was performed by economic analysis.

RESULTS AND DISCUSSION

Density and Composition of Flocs

Heterotroph bacteria plays an important role in reducing ammonia in the ponds to appropriate the environmental conditions and with other microorganisms form a floc (Bufford *et al.*, 2004; De Schryver, 2008; Jorand *et al.*, 1995; Schneider *et al.*, 2005). Observation of the bacterial population in pond waters on all treatments showed that the same relative growth with reaching about 10^3-10^7 CFU/mL. According Avnimelech (2009), a set of microorganisms in floc as a good categorized, if the total population heterotroph bacteria in the ranges of 10^7-10^9 CFU/mL and total vibrio population is less than 10^3-10^4 CFU/mL. While, flocks classified as good, if the bacterial population is rather low (10^4-10^5 CFU/mL) and the total vibrio population is more than 10^3 CFU/mL.

In this study, observation of floc bacteria under the microscopic showed that the composition of bioflocs consist of bacteria and various types of phytoplankton, zooplankton, and organic particles in the flocs. The composition of floc obtained in this study, showed the same as reported by Kuhn *et al.* (2008) and Garg *et al.* (2007), which the biofloc composition consists of a microorganisms variety (bacteria, plankton), algae, and organic matter from the waste of feed, feces shrimp, and others.

During the study, rains frequently occured, causing the pond water turbidity and low colloidal particles solubility and plankton abundance. When less sunlight and occured turbidity in the water, the phytoplankton could not grow well (Hargreaves, 2006). Plankton abundance in each treatment during the study are presented in Table 1.

Table 1. Plankton abundance in intensive milkfish culture on biofloc systems at the trial ponds, Research Institute for Coastal aquaculture, Maros

Plankton abundance	Treatments		
	A	B	C
Range (ind./L)	15-156	10-247	15-387
Average (ind./L)	54.45	72.14	80.68
Plankton total (ind./L)	599	793.5	887.5

Description: The application of feed (A) dosage 3% of body weight/day without molasses, (B) dosage 2.7% of body weight/day and application of molasses, and (C) dosage 2.4 % of body weight/day and application molasses

The highest abundance of plankton during the study was obtained on the C treatment (887.5 ind./L), followed by B treatment (793.5 ind./L), and the lowest in A treatment (599 ind./L), it seems that the identified plankton dominated by phytoplankton. Observations of plankton identified as many as 28 species that consist of 18 species of phytoplankton and 10 species zooplankton. The identified phytoplankton was *Navicula* sp., *Oscillatoria* sp., *Protoperidinium* sp., *Sphaerellopsys* sp., *Gleotrchia* sp., *Eutreptia* sp., *Thallasionema* sp., *Noctiluca* sp., *Gleotrichia* sp., *Spirulina* sp., *Ceratium* sp., *Coscinodiscus* sp., *Chaetoceros* sp., *Favella* sp., *Gyrosigma* sp., *Gymnodinium* sp., *Pleurosigma* sp., and *Thalasiosira* sp. While identified zooplankton was *Brachionus* sp., copepod nauplii, *Oithona* sp., *Schemackeria* sp., *Tortanus* sp., *Onychocamptus* sp., *Acartia* sp., *Temora* sp., *Labidocera* sp., and *Echinocamptus* sp., with different species every month. *Coscinodiscus* sp. which is cellular uni plankton formed floc along with bacteria. While, *Nitzschia* sp., produces of biotoksin.

Water Quality

The water temperature in the ponds can affect the metabolic process, growth and the survival rate of milkfish. In Figure 1, it appears that the water temperature fluctuates daily throughout the study.

The water temperature in April was in the range of 26.4°C to 31.0°C in May (25.2°C to 30.9°C), June (25.2°C to 31.0°C), July (24.6°C to 30.8°C), August (28.6°C to 31.2°C), September (26.5°C to 31.0°C), October (26.2°C to 30.0°C) and October (26.2°C to 30°C). According Halver & Hardy (2002), the increase in temperature caused a decrease in the water gas (O_2, CO_2, N_2, and CH_4), an increase of metabolic rate in the respiration of aquatic organisms and in oxygen consumption. The temperature increase also caused an increase in the decomposition of organic matter by microbial (Coyne, 1999). The optimum temperature for the growth of phytoplankton ranges from 20°C-30°C. According Buentello *et al.* (2000), at low water temperatures can lead to impaire metabolic processes.

The availability of oxygen in water is an important factor for biofloc formation and occurrence of chemical processes and their effect on milkfish in ponds. According to Calle-Delgado *et al.* (2003), floc formation required oxygen in ponds and strong water streams.

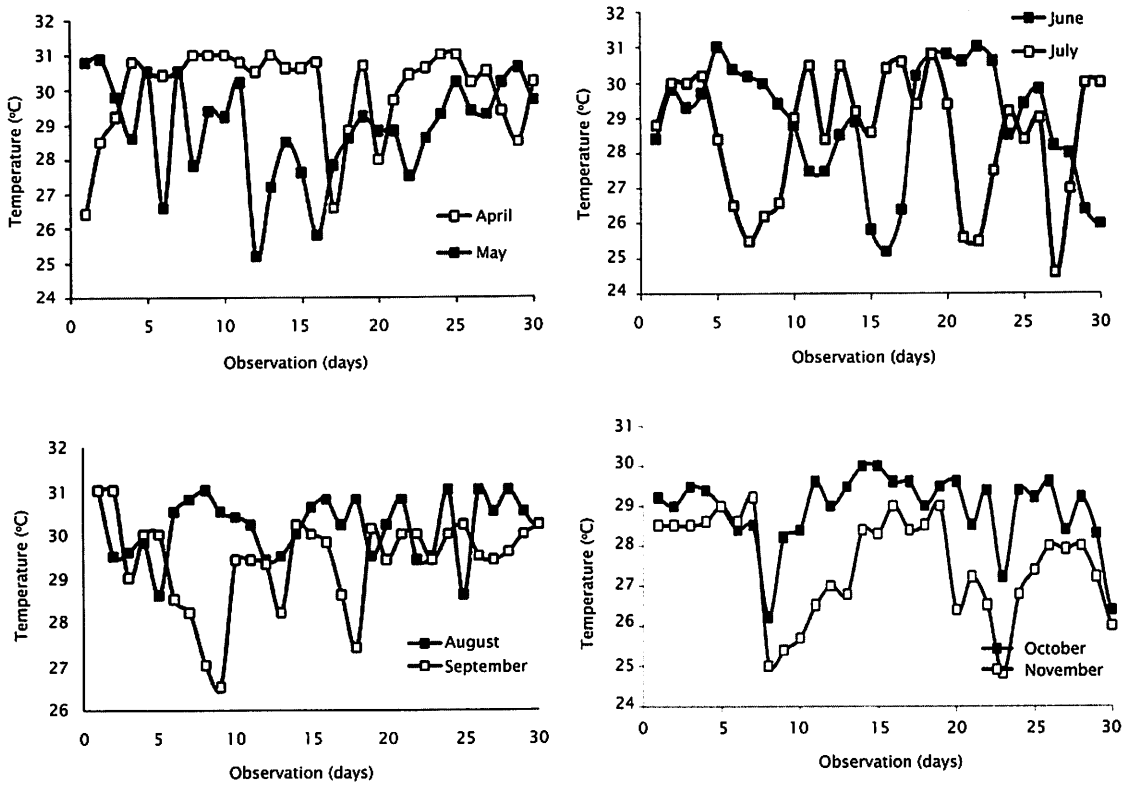


Figure 1. Daily water temperature fluctuation in the pond every month during experiment

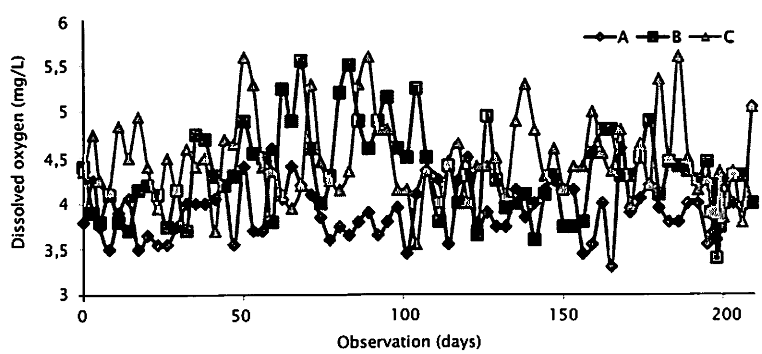


Figure 2. Dissolve oxygen fluctuation to all treatment of feed management (A) dosage 3% of body weight/day without molasses, (B) dosage 2.7% of body weight/day and application of molasses, and (C) dosage 2.4% of body weigh/day and application molasses

Strongly rotated water in ponds, both horizontally and vertically was very important so that the microorganisms and organic particles produced optimum flocs (Scheneider *et al.*, 2005). Oxygen fluctuates each observation, but in general concentration of oxygen during the study more than 3 mg/L and still quite decent for milkfish (Figure 2).

Table 2. The effect of feed management in intensive milkfish culture to the water quality at the ponds during the experiment

Variables	Units	Treatments		
		A Range (average SD)	B Range (average SD)	C Range (average SD)
Total organic matter	mgL-1	13.62-38.38 (31.14±6.773)	10.04-36.78 (32.04±7.596)	13.76-36.25 (31.72±7.199)
Total ammonia nitrogen	mgL-1	0.34-0.45 (0.43±0.088)	0.05-0.72 (0.37±0.319)	0.05-0.54 (0.32±0.212)
NH3 -N	mgL-1	0.006-0.593 (0.135±0.309)	0.010-0.5326 (0.157±0.4336)	"0.033-0.601 (0.126±0.4613)"
NO2-N	mgL-1	0.009-0.035 (0.023±0.0109)	0.014-0.044 (0.024±0.0104)	0.014-0.061 (0.026 ±0.0152)
NO3-N	mgL-1	0.003-0.458 (0.094±0.1658)	0.001-0.499 (0.066±0.1528)	0.062-0.335 (0.058±0.1093)
PO4-P	mgL-1	0.029-0.514 (0.184±0.1607)	0.102-0.938 (0.266±0.2475)	0.059-0.573 (0.191±0.1438)
Fe2+	mgL-1	0.0045-0.0070 (0.005±0.0008)	0.065-0.010 (0.006± 0.0017)	0.005-0.016 (0.007±0.0035)
SO42-	mgL-1	326.81-870.87 (628.37±240.008)	309.17-950.42 (601.66±244.996)	352.07-968.18 (627.01±241.526)

Description: The application of feed (A) dosage 3% of body weight per day without molasses, (B) dosage 2.7% of body weight per day + application of molasses, and (C) dosage 2.4% of body weight per day + application molasses

Water quality during the study was still within the limits of tolerance for milkfish culture in ponds. The results of the analysis of water chemical during the study are shown in Table 2.

Range of water quality (NH_3-N, NO_2-N, NO_3-N, BOT, PO_4-P, Fe^{2+}, and SO_4^{2-} in all treatments of feed management was still tolerated for milkfish growth. Nevertheless, it seems that the application of molasses can reduce the concentration of total ammonia nitrogen (TAN). The average of TAN concentration on the B and C treatment was better than without molasses (A) (100% of feed).

Ammonium and nitrate play an important role as a source of nitrogen for phytoplankton growth though the ions have different roles on the phytoplankton species (Hargreaves, 2006; Moriarty, 1997). Reported by APHA (2008) and Sumagaysay-Chavoso (2003), that NH_3 is a compound that does not have ions, but it is more toxic to fish than NH_4^+. The concentration of ammonia is safely for fish less than 1.0 mgL^{-1}.

Further reported by Herbert (1999); Kennedy & Gelwin (1997), that the nitrification process produced nitrite (NO_2^-) performed by several genera of bacteria, including *Nitrosospira* and *Nitrosomonas.* The concentration of nitrite for fish farming should be less than 1 mgL^{-1}. Meanwhile, nitrate is often considered harmless to fish. In ponds, nitrate is a nutrient that can be utilized for the growth of phytoplankton (Caraco *et al.*, 1978). However, excessive nitrate (more than 100 mgL^{-1}) can be dangerous, because in certain condition, denitrification process occurs from nitrate to ammonia that is toxic to fish (Avnimelech, 1999). At the time of the research activity coincided with the rainy season, causing natural food (klekap) could not grow optimally in spite of fertilizer N and P. High rainfall conditions can cause pond water turbidity and lower water temperatures.

Milkfish Production

The growth of milkfish in each treatment for 7 months was relatively slow, reaching an average range of 225.75 g/fish in C treatment and 230.50 g/fish in B treatment. While on A treatment, reached an average weight of 242.30 g/fish (Table 3).

The slow growth of milkfish in all treatments was caused by the environmental conditions, especially water temperature (Figure 3).

Table 3. The effect of molase applicate to weight gain, survival rate, and intensive milkfish production

Variables	Treatments		
	A	B	C
Densities (ind./ha)	50,000	50,000	50,000
Initial weight (g/ind.)	4.1	4.1	4.1
Final weight	242.30	230.50	225.75
Survival rate (%)	60.0	55.0	52.0
Production (kg/ha)	7,260	6,339	5,870
Food conversion ratio	1.58	1.48	1.36

Description: The application of feed (A) dosage 3% of body weight per day without molasses, (B) dosage 2.7% of body weight per day and application of molasses, and (C) dosage 2.4% of body weight per day and application molasses

At this study, the growth of milkfish was influenced by pelleted feed and has not been able to fully accept additional food in the form of floc. The results of histological analysis of intestinal milkfish that reached a body weight of 245 g/ind. (4 ind./kg) in A treatment showed an increase in goblet cells (figure 4). This phenomena indicates that the milkfish can perform to its full potentiality for growth metabolism (Barman *et al.*, 2012). On the B treatment, despite an increase in goblet cells, but it was found the infiltrating and fibrin lymphocytes, while on C treatment, which was dominant of smaller milkfish, its intestine occured deplesia ephitel in the lamina propria and undergo necrotic.

The results of the proximate biofloc analysis in this study, showed that the biofloc composed of protein (26%), fat (3%), crude fiber (6.5%), and ash (16.2%), this composition was not much different from the composition of the floc identified in the gut milkfish that consisting of protein (35.17%), fat (3%), crude fiber (7.97%), ash content (16.59%), and other extract materials without N reaches 39.97%. Reported by Ekasari (2008), Verstraete *et al.* (2008) and Wilson (2000), that microorganism in flocs contained high nutrients i.e; protein (19-32%), fat (17%-39%), carbohydrates (27%-59%), and ash (2%-7%) and was quite good for the growth of fish. Biofloc is very good for fish as a food supplement, because it contains nutrients for the growth of fish (Bolliet *et al.*, 2002; Borlongan *et al.*, 2003; and Jana *et al.*, 2012).

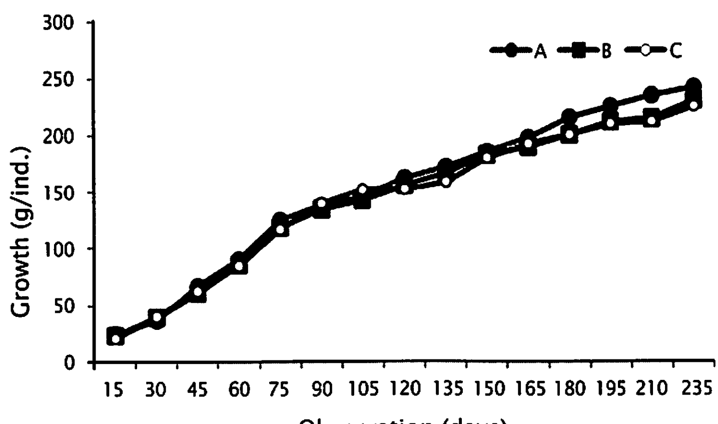
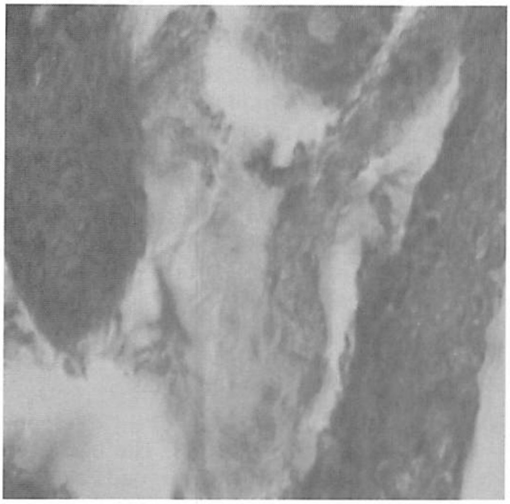


Figure 3. The effect of feed management on the growth of milkfish (A) dosage 3.0% of body weight/day without molasses, (B) dosage 2.7% of body weight/day and application of molasses, and (C) dosage 2.4% of body weight per day and application molasses



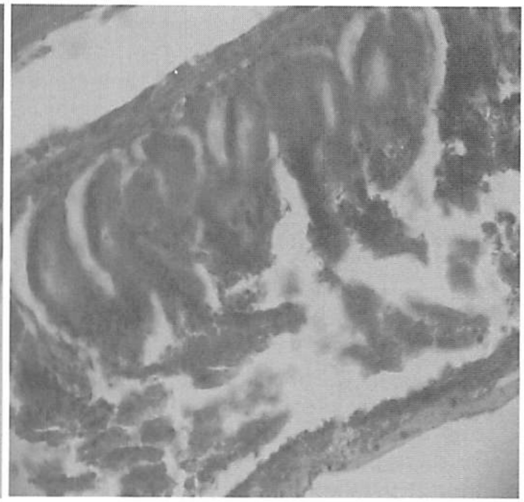


Figure 4. The increase in goblet cells (UA), an increase in goblet cells, infiltration of lymphocytes, fibrin (UB), epithelial lamina propria deplecya, necrotik (UC)

The highest survival rate of milkfish obtained on the A treatment was 60%, followed by B treatment (55%), and C treatment (52%), while, the highest milkfish production was obtained on the A treatment (7,260 kg/ha), followed by B (6,339 kg/ha), and the lowest production in C (5,780 kg/ha). The survival rate and milkfish production were lower in C treatment because of the decrease of feed dosage although the B and C treatment received an additional food or supplement from bioflocs after molasses application but It was not able to enhance the fish growth (milkfish has not been able to receive nutrients in the form of floc). In contrast, it caused a slow-growing fish and most vulnerable to stress.

The results of economic analysis on all treatments still gave the advantage. The benefit and cost (BC) ratio is more than 1. In general, the benefits of A treatment was Rp 35.044.500,-/cycle/ha followed by B treatment Rp 29.709.000,-/cycle/ha, and C treatment Rp 22.098.500,-/cycle/ha. However, on A and B treatment had the same value of BC ratio (BC ratio of 1.47 and 1.45) and were higher than in C (1.37). It shows that the intensive cultivation of milkfish on biofloc system was able to reduce amount of 10% given feed.

CONCLUSION

The highest milkfish production obtained on the A treatment (dosage feed 3% of body weight/day) produced 7,260 kg/ha. Feed reduction on the Intensive milkfish culture by 10% of the dose and molasses application produced 6,339 kg/ha while 20% reduction of the dose of feed and molasses application produced 5,780 kg/ha. Feed management in intensive culture of milkfish with biofloc system by the addition of molasses has not provided a satisfactory results on the growth of milkfish. The A treatment generated (Rp 35.044.500,-/cycle/ha), B (Rp 29.709.000,-/cycle/ha), and C treatment (Rp 22.098.500,-/cycle/ha).

REFERENCE

American Public Health Association (APHA). 2008. Standard methods for examination of water and waste-water. 20th edition. APHA, AWWA, WEF, Washington, 1,085 pp.

Avnimelech, Y. 1999. Carbon nitrogen ratio as a control element in aquaculture systems. *Aquaculture*, 176: 227–235.

__________.2009. Biofloc technology, A practical guide book. World Aquaculture Society. 182 pp.

Barman, U.K., Garg, S.K., & Bhatnagar, A. 2012. Effect of different salinity and ration levels on growth performance and nutritive physiology of milkfish, *Chanos chanos* (Forsskal) - Field and Laboratory Studies. *Fisheries and Aquaculture Journal*, 53: 1-11.

Bolliet, V., Azzaydi, M., & Boujard, T. 2002. Effect of feeding time on feed intake and growth. *In* Houlihan, D. & Jobling, M. (Eds.). Food intake in fish, Oxford, Blackwell Science. p. 233–249.

Borlongan, I.G., Eusebio, P.S., & Welsh, T. 2003. Potential of feed pea (*Pisum sativum*) meal as a protein source in practical diets for milkfish (*Chanos chanos* Forsskal). *Aquaculture*, 225: 89-98.

Boyd, C.E. 1995. Bottom soils sediment and pond soil aquaculture. Chapman and Hall. Auburn University, Alabama, 347 pp.

Brune, D.E., Schwartz, G., Eversole, A.G., Collier, J.A., & Schwedler, T.E. 2003. Intensification of pond aquaculture and high rate photosynthetic systems. *Aquaculture Engineering*, 28: 65-86.

Burford, M.A., Thompson, P.J., McIntosh, R.P., Bauman, R.H., & Pearson, D.C. 2004. The contribution of flocculated material to shrimp (*Litopenaeus vannamei*) nutrition in a highly-intensity zero-exchange system. *Aquaculture*, 232: 525-537.

Buentello, J., Gatlin, D., & Neill, W. 2000. Effects of water temperature and dissolved oxygen on daily feed consumption, feed utilization, and growth of channel catfish (*Ictalurus punctatus*). *Aquaculture*, 182: 339–352.

Calle-Delgado, P., Avnimelech, Y., Mc Neil, R., Bratvoldd, D., Browdy, C.I., & Sandifer, P. 2003. Physical, chemical, and biological characteristics of distinctive regions in paddlewheel aerated shrimp ponds. *Aquaculture*, 217: 235-248.

Caraco, N., Tamse, A., Boutros, O., & Valiela, I. 1978. Nutrient limitation of phytoplankton growth in brackish coastal ponds. *Canadian Journal Fish Aquatic Science*, 44: 473-476.

Crab, R., Kochba, M., Verstraete, W., & Avnimelech, Y. 2009. Bioflocs technology application in over-wintering of tilapia. *Aquaculture Engineering*, 40: 105-122.

Coyne, M. 1999. Soil microbiology: *An exploratory approach*. Delmar Publisher. 62 pp.

De Schryver, P., Crab, R., Defoirdt, T., Boon, N., & Verstraete, W. 2008. The basics of bioflocss technology: The added value for aquaculture. *Aquaculture*, 277: 125-137.

Ekasari, J. 2008. *Biofloc technology: The effect different carbon source, salinity, and the addition of probiotics on the primary nutritional value of the bioflocs*. Thesis. Ghent University, Belgium, 72 pp.

FAO. 2008. The state of the world fisheries and aquaculture. FAO, Fisheries Departement, Food, and Agriculture Organization of the Unites Nations. Publishing Management Service, Rome, Italy.

Garg, S.K., Kumar, A., Arasu, A.R.T., Bhatnagar, A., Jana, S.N., & Barman, U.K. 2007. Effect of periphyton and supplementary feeding on growth performance and some aspects of nutritive physiology of nile tilapia, *Oreochromis niloticus* and Pearl spot, *Etroplus suratensis* under polyculture. *Journal of Applied Aquaculture*, 19(3): 19-45.

Hargreaves, J.A. 1989. Nitrogen biogeochemistry of aquaculture ponds. *Aquaculture*, 166: 181-212.

_____________. 2006. Photosynthetic suspended growth systems in aquaculture. *Aquaculture Engineering*, 34: 344-363.

Halver, J.E. & Hardy, R.W. 2002. Nutrient flow and retention. *In* Halver, J.E. & Hardy, R.W (Eds.). Fish Nutrition. Academic Press. New York, p. 755-770.

Herbert, R.A. 1999. Nitrogen cycling in coastal marine ecosystems. *FEMS Microbiology Review*, 23(5): 563-590.

Jana, S.N., Garg, S.K., & Patra, B.C. 2006. Effect of inland water salinity on growth performance and nutritional physiology in growing milkfish, *Chanos chanos* (Forsskal): field and laboratory studies. *Journal of Applied Ichthyology*, 22: 25-34.

__________. Rani, S., Garg, S.K., Sabhlok, V.P., & Bhatnagar, A. 2012. Nutritive evaluation of lysine-and methionine-supplemented raw vs heat-processed soybean to replace fishmeal as a dietary protein source for Grey Mullet, *Mugil cephalus*, and milkfish, *Chanos chanos*. *Journal of Applied Aquaculture*, 24(1): 69-80.

Jorand, F., Zartarian, F., Thomas ,F., Block J.C., Betteru, J.V., Villemin, G., Urbain, V., & Manen, J. 1995. Chemical and structural (2nd) linkage between bacteria within activated-sludge flock. *Water Res.*, 29(7): 1,639-1,647.

Kaiser, E.A. 1994. Significance of microbial biomass for carbon and nitrogen mineralization in soil. *Z. Pfanzenernahr. Bodenk*, 157: 271-278.

Kennedy, A.C. & Gewin, V.L. 1997. Soil microbial diversity: Present and future considerations. *Soil Science*, 162(9): 607-617.

Kuhn, D.D., Boardman, G.D., Craig, S.P., Flick, G.J., & Mclean, E. 2008. Use of microbial flocs generated from tilapia effluent as a nutritional supplement for shrimp, *Litopenaeus vannamei* in recirculating aquaculture systems. *Journal of the World Aquaculture Society*, 39: 72-82.

Lim, C., Borlongan, I.G., & Pascual, F.P. 2002. Milkfish, *Chanos chanos*. *In* Nutrient requirements and feeding of finfish for aquaculture. Webster, C.D. & Lim, C.E. Cabi Publishing. New York, USA, p. 172-183.

Martinez, F.S., Mei-Chen, T., & Sin-Ping, Y. *2006*. Milkfish (*Chanos chanos*) culture: situations and trends. *J. Fish. Soc. Taiwan*, 33(3): 229-244.

Moriarty, D.J.W. 1997. The role of microorganisms in aquaculture ponds. *Aquaculture*, 151: 333-349.

Pantjara, B. 2008. Efektivitas sumber C terhadap dekomposisi bahan organik limbah tambak udang intensif. *Prosiding Seminar Nasional Kelautan IV*. II: 195-199.

Schneider, O., Sereti, V., Eding, E.H., & Verreth, J.A.J. 2005. Analysis of nutrient flows in integrated intensive aquaculture systems. *Aquaculture Engineering*, 32: 379-401.

Sumagaysay-Chavoso, N.S. & San Diego-McGlone, M.L. 2003. Water quality and holding capacity of intensive and semi-intensive milkfish (*Chanos chanos*) ponds. *Aquaculture*, 219: 413-429.

Tacon, A.G.J. & Forster, I.P. 2003. Aquafeed and the envinronment: policy implications. *Aquaculture*, 226: 181-189.

Verstraete, W., Schryver, P.D., Defoirdt, T., & Crab, R. 2008. Added value of microbial life in flock. Laboratory for Microbial Ecology and Technology, Ghent Univeristy. Belgium, 43 pp. http://labmet.ugent.be.

Wilson, R.P. 2000. Amino acids and proteins. *In* Halver, J.E. & Hardy, R.W. (Eds.), Fish Nutrition. New York. Academic Press. p. 143-179.