Original article

Hubungan jumlah plankton dalam saluran pencernaan terhadap akumulasi timbal (Pb) ke dalam tubuh ikan nila merah (O*reochromis* sp.) yang dibudidayakan di kolong tua pasca tambang timah Bangka Belitung

Relationships of plankton content in the digestive tract of a red tilapia (Oreochromis sp.) *to the accumulation of lead (Pb) into the fish muscle in the old lake of post-tin mining of Bangka Belitung*

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Abstract

The red tilapia were cultivated in kolong (above 30 years age of lake of post-tin mining) for four month; on the rate of lead (Pb) biomagnification in the organs (liver, kidney and muscle), given positif correlation from planktons in instestine composition, and its effects to the growth rate has been investigated. Results revealed that in the third month, accumulation of lead in the organs of both species were increased, especially in their livers, above the safe level to be consumed (62.14 mg/kg, 8.41 mg/kg and 15.39 mg/kg, 55.23 mg/kg, respectively), but then were decreased in the fourth month. Biomagnification of lead affects the growth rate of both species significantly.

Keywords : *Lead bio-magnification, old lake, growth rate, red tilapia.*

Introduction

Bangka Belitung Province to the present (2016) is still one of the biggest producer of tin (Sn) in the world. Mining activities was leaving holes dug as void or small lakes, which named *kolong* (lake of post-tin mining). In generally, the form of the lake of post-tin mining was a large basin filled by water, the length and width was 75-200 m along 2-50 m ranges of the depth (Henny 2007). The characteristics of the lake with no inflows and outflows water current resulting in the water condition very influenced by evapo-consentration process. It was making fluctuates enough in the dry season and concentrated of material. Henny and Evi (2009) has classified those kind of lakes into two part, the young lake (<10 years) and old lake (>10 years). Based on PT. Timah, Tbk findings, the lakes or *kolon*g left were 887 lakes with an total area about 1,712.65 ha. It was consisted of 544 lakes, which has 1,035.51 ha in the Bangka island, and the others 343 were 677.14 ha in the Belitung island. However, the reclaimed area was only a few parts, 108 lakes in Bangka Island,and 54 lakes located in Belitung island (Sujitno, 2007)

Department of Marine and Fisheries Babel province (2011) plans to take advantage of those post-tin mining lakes with developing freshwater aquaculture sector. One commodity that will be developed is the red tilapia (*Oreochromis* sp.), In addition, the red tilapia species has a high demand in local market, and suitable to be cultured in freshwater by floating net system. However, the problem was tilapia culture would face the poorwater, due to heavy metals polluted in the different age of lake. Brahmana *et al.* (2004) showing that, a young-age lake was indicates poor water quality with pH 2.9 to 4.5. The content of heavy metals such as Fe, Al, Pb, and Mn is very high. According to Lamidi (1997), by mining activities over 25 years, the concentration of heavy metals in the water decreased to safe levels for humans. Henny (2011) also demonstrated that there were several types of non-essential heavy metals in sediment under the old lake which the amounts indicates above quality standards (i.e.Pb), although the heavy metals is not scalable and is already rich in plankton. Nevertheless, the rich content of plankton in the old post-tin mining lake, lead Pb to enter the body of farmed fish, through the bio-magnification mechanism.

The development of freshwater aquaculture is not only focused on increasing production, but also on the quality and safety of products for human consumption. With these considerations, utilizing the old lake for fish farming by floating net system needs thorough in depth. The purpose of this study was to analyze the amount of plankton in the digestive track, and its correlation with the accumulation of Lead (Pb) in meats, liver and kidneys of red tilapia. The study expected for a safe consumption of fish through floating net farming system in the old post-tin mining lakes.

Materials and methods

This study conducted in the PT Timah, Tbk old post-tin mining lake for four months (October 2011-february 2012). The Grasi lake had been chosen for the location with its coordinate was S $01^{0}52.464'$; E $106^{0}07.005'$, in Sungailiat, Bangka Regency, Bangka Belitung Province. The characteristics of Grasi lake are over than 30 years old, 2 ha area, 9-10 meters depth, the eutrophication average and lead concentration 16,50 mg/kg and < 0,030 mg/L in sediment and water contain, respectively. The location map as indicates by figure 1.

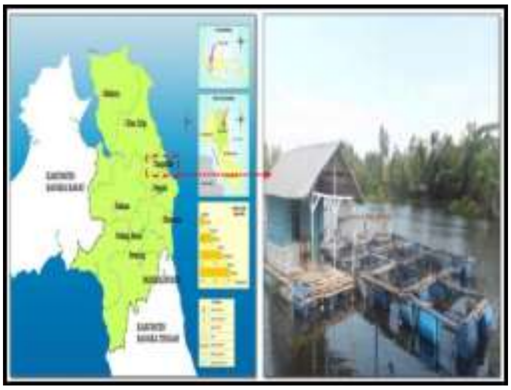

Fig 1 Location of study

Research Procedure

lead was measured in the water and sediment, then the water qualities (physical and chemical) were measured directly. Early data for Total Organic Matter and lead in sediement were measured in laboratory, then conducted directly in the lake of fish culture.

Cultivation Method

Four units of floating net with 3 m × 3 m × 2 m in size with a mesh size of 2.25 cm, a depth of 2 m used for the cultivation. One unit was used for lead test and three other units were used for test of growth. The initial weight of the 400 red tilapia was 6.8 ± 0.23 g/fish (100 individuals/ unit of floating net). Commercial feed (pellet) about 3% of the weight with a frequency of three times a day. During the maintenance period, feed, health and location safety were controlled.

Sampling methods

Measurement and sampling carried out each month from fish rearing maintenance period for four months. Samples were taken randomly from the red tilapia unit (lead treatment). Fish growth was calculated from the three units of the other floating nets with three replications on each treatment, the fish samples taken as many as 10 fish, then the total length and body weight were measured. For the measurement of heavy metals in samples of organs, 10 liver, kidney and meat of fish were taken from lead treatmnet's floating nets. Furthermore, each of the same organs was mixed into one. The organ was mashed with a blender (for meat) and frozen for laboratory test.

Pb analyze method

Pb contents were measured in the liver, kidneys and bacon. In the laboratory, each sample of fish organs were put into a beaker glass and been weighed using the analytical balance. 15 grams of the organ needed in AAS analysis followed by dry ashing to remove organic materials. the residue dissolved in dilute acid and then transferred into a flask. Add 5-6 ml HCN 6 N into the cup containing the ashes, heat on a hot plate with low heating until dry. add 15 ml HCN 3N and heat again to boiling and cooled then filtered using a 0.45 mm Millipore filter paper. The filtrate was put into 10 ml flask. Make a cup with water washing at least 3 times and then filtered washing water and put it into the flask.

After each organ was transferred to solution in a 10 ml flask, then the next lead content measurement using atomic absorption spectrometry (AAS) AA 300 P,Techtron Varian, Australia. AAS was calibrated according to the manual instructions to measure metal standard solutions, blank samples and measurement solution. During measurement, metal standard checked periodically to ensure that the default value is constant. The actual concentration of heavy metals counted by using the formula:

Composition Measurement Method of Intestinal Contents

Intestinal contents observations aimed to examine the composition of fish gut contents. The identifying of the number and types of plankton in the intestines of fish has done with a few steps. Firstly, take 10 of the 10 fish gut then preserved with 40% formalin. Second, measure the length of each of the intestine, and then clean the fish intestines samples by formalin. Scraped intestinal and the separation of intestinal contents then diluted into 10 cc or 1 bottle of film with aquadest. Next step is to take a single drop of intestinal contents that have been diluted with a pipette and then observed under a microscope. Observations were conducted three replications with five visual field. The final step, is to identify the type and record the number of food organisms that exist in every field of vision with algae identification book (Prescott 1970). Artificial feed crumbs and microorganisms found are recorded and calculated according to the formula on the Incidence and Frequency preponderance index (Effendie 1979). Model Incidence and Frequency formula preponderance index used:

1. Model of incidence Frequency :

$$N = \frac{Vd}{Vi} x \text{ n}$$

Description :
n: number of an individual species-if ound in sample
N: total number of alleged individual typesall i from fish to-i
Vd: volume dilution
Vi: volume drops were observed (1 drop : 0.05 ml)

2. Index of Preponderance Model:

Description :
Ii: indeks preponderance
Vi: Volume percentage of food-i
Oi: Incidence frequent of food -i

Methods of observation weight gain

Observation of the growth of red tilapia ascertained by measuring the body weight of the fish using a digital scale brand Osuka AJ 1000, the level of accuracy was 0.01. Initial weight (Wo), measured before the fish seed stocked to obtain accurate data measurement of the weight of fish every month. Final weight (Wt), measured after seed fish reared for 30 (thirty) days after the measurement of initial weight (Wo).

Fish growth, survival and feed conversion was calculated using the following equations (Effendie 1979) :

1. Absolute Growth (W)

$$W = Wt - Wo$$

Keterangan :
W : absolute weight gain (g)
Wo: initial weight of fish (g)
Wt : final weight of fish at the end of observation (g)

Observation Parameter

The parameters been measured were the composition of plankton in the digestive tract of the fish, the accumulated amount of lead in meats, liver and kidney by monthly observations, and growth rate of fish.

Data analysis

The data presented in tabular or graphical. Connectedness of all the parameters were described descriptively. The growth rate formula used (Effendi, 1987):

$$GR = Wt/Wo .$$

Result

Water quality analysis

The water quality of Grasi lake in Sungailiat District Bangka, Bangka Belitung province, during October-December 2011 to January-February 2012 is shown in Table 1. The average of physical and chemical parameters of water quality shows ideal conditions for red tilapia cultivation. Some parameters such as temperature, water clarity and increasing of TOM occurred in January 2012 and did not occur in previous months. This is due to the rainy season in January 2012.

The intestine compound of fish

There are several classes of microorganisms were identified in the intestine of red tilapia other than commercial feed (pellets) during four months maintenance. Using preponderance index method, the composition of the intestinal spectral range of red tilapia were obtained in November 2011.

Table 1 Measurement result of post-tin mining lake in Grasi during October 2011-February 2012

Month	Water quality parameters						Remarks
	Temp. (^{0}C)	Clarity (cm)	pH	DO (mg/L)	CO_2 (mg/L)	TOM (mg/L)	
October 2011	29,4	90	6	7,2	2,10	5,77	Dry seasons
November 2011	29,1	90	6,5	8	1,82	4,18	Dry seasons
December 2011	25,3	60	6	8	3,06	9,06	Dry seasons
January 2012	24,1	30	5	7,8	4,09	12,91	Wet seasons
February 2012	27,1	70	6	8	3,44	7,40	Dry seasons
Average/ month	**27±2,3**	**68±24,8**	**6,1±0,5**	**7,8±0,3**	**2,90±0,9**	**7,87±3,3**	
Standard values for red tilapia	25-30	20-30	6-8,5	≥3	< 5	-	SNI 2009

The type of material or organisms found in the intestines of red tilapia in November 2011 were the crumbs of pellet (commercial feed), Chlorophyceae and Chrysophyceae classes. Each value of the frequency of occurrence were 96.6%, 43.20% and 3.30% for pellets, Chlorophyceae and Chrysophyceae, respectively (Table 2).

Tabel 2 Frequency of occurrence (FO), and IP value of red tilapia feed in November 2011

Feed	FO (%)	Volume (%)	IP (%)
Pellet	96,60	53,53	83,82
Chlorophyceae	43,20	43.43	15,99
Chrysophyceae	3,30	3,03	0,16

Table 3 (December 2011) shown that the intestines material found as same as the result on November 2011. However, the Bacillariophyceae was appearance. Each value of the frequency of

occurrence were 10% and 20% for Chrysophyceae and Bacillariophyceae, respectively.

Feed	FO (%)	Volume (%)	IP (%)
Pellet	90	21,35	42,15
Chlorophyceae	100	59,87	51,53
Chrysophyceae	10	8,85	1,90
Bacillariophyceae	20	9,89	4,33

Tabel 3 Frequency of occurrence (FO) and IP value of red tilapia feed in Desember 2011

In addition, the frequent value of intestine of red tilapia in Januari 2012 dominated by Chrysophyceae and Chlorophyceae classes, as well as few pellet (Table 4). The material found in the intestines were 53% pellet, 100% Chlorophyceae, 83% Chrysophyceae and 46,60% Bacillariophyceae (Table 4).

Feed	FO (%)	Volume (%)	IP (%)
Pellet	56,6	18,77	21,10
Chlorophyceae	100	61,95	66,71
Chrysophyceae	3,30	0,93	0,06
Bacillariophyceae	33,30	18,30	12,10

Tabel 4 Frequency of occurence and IP value of red tilapia feed in Januari 2012

The frequent value of intestine of red tilapia in Februari 2012 were found pellet crumble (commercial feed), Chlorophyceae, Chrysophyceae and Bacillariophyceae classes. Each value was 56,6% pellet, 100% Chlorophyceae, 3,30% Chrysophyceae and 33,30% for Bacillariophyceae (Table 5).

Feed	FO (%)	Volume (%)	IP (%)
Pellet	53,3	19,71	18
Chlorophyceae	100	36,6	23,19
Chrysophyceae	83	32,09	48,14
Bacillariophyceae	46,60	11,59	9,73

Table 5 Frequency of occurrence and IP value of red tilapia feed in February 2012

Using preponderance index method, the composition of the intestinal spectral range of red tilapia in November 2011 to February 2012, (figure 2 A-D).

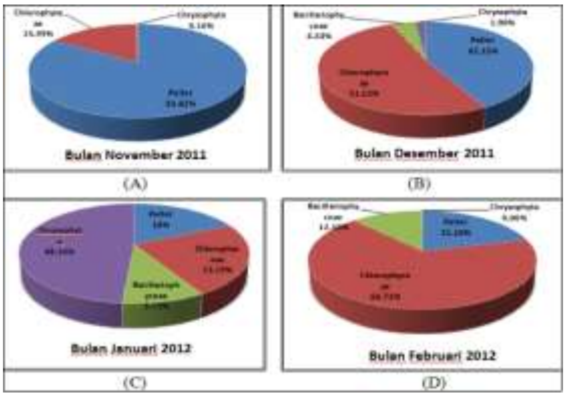

Figure 2 Spectrum range of Red Tilapia Intestine Composition

Total Pb Accumulation

The number accumulation of heavy metals Pb measured in each organ of red tilapia, can be seen in Table 3. The amount of Pb that accumulate in the heart of red tilapia was 0.085 mg/kg in November 2011.On January 2012, Pbwas found in all of organ. However, the kidney of red tilapia was exceed of the safe level for consumption, 93,98 mg/kg. Heart was 62,14 mg/kg, and no Pb concentration found in the meat. On February 2012, the meat was contaminating by Pb 0,188 mg/kg.

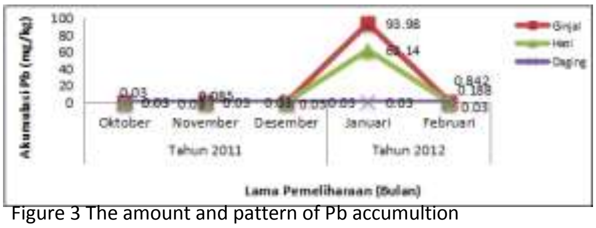

Figure 3 The amount and pattern of Pb accumultion

The increasing of body weight and growth rate of fish

The results of this study showed weight gain test fish were relatively slow, but still close to normal (Figure 4). The results show that the growth rate of red tilapia in every month was increased. (Figure 5).

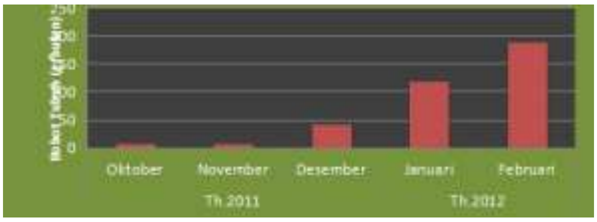

Figure 4. The increasing of body weight of fish during cultivation

Figure 5. The growth rate of fish during cultivation

Discussion

The differences weather conditions during study have led to changes in water quality parameters. It will also affect to the abundance of plankton into the water. Plankton found in the gut of red tilapia in January 2012 was more vary. It percentage was higher than artificial feed. This condition is the impact of the declining quality of the water in January 2012, as reduced levels of brightness. An increasingof the type and amount of plankton in the red tilapia intestines positively correlated to the increase ofPb accumulated in every organ of red tilapia.

The improvement of water quality (February 2012), such as increasing the brightness value, followed by the reduced value of the planktondominance in fish intestines, both quantity and types. This condition accompanied by decreasing the amount of Pb measured in every organ of the fish. Increasing the amount of plankton (quantity and type) gradually in fish intestines test during the month of October 2011 until December 2011, always followed by an increase of the amount of accumulated Pb in every organ. This phenomenon reinforces that, Pb accumulation in organs of red tilapia was going through by the food chain (bio magnification).

The processes of metals taking in aquatic autotrophic organisms (phytoplankton) based on Bryan (1976 b) was through the mechanism of ion exchange.It was quickly absorbed on the surface of cells, from where they diffuse into the cell membrane, the last absorbed and bound by proteins (where ion exchange) inside the cell. The amount of plankton in the water that was contaminating by Pb will going through inside fish body. Morphologically, red tilapia have an ability to filter natural feed (plankton) through the gills (Wittmann, 1979). in addition, the length of Intestinal morphology red tilapia allow this fish to store food in large quantities and longer. Those were the reason of planktonhave Pbaccumulating into the body of red tilapia.

Measurability of Pb in liver and kidney organ of fish indicate the inclusion of Pb in the blood of red tilapia (Manahan, 1997). This suggests that, there has been a Pb heavy metal accumulation in the body of red tilapia through the food chain.

During four-month maintenance of fish, the rates of fish growth werefoundsignificant accumulation of Pbin every organ.The Pb accumulation was gradually changes from the first moth of cultivation to the last month (fourth month). It was not effect on the first and second month. However, at the third to fourth month the Pb accumulation showing rapidly increases. On the other hand, the decline in the growth rate continues to occur from the third to four months of maintenance. It is clear that, the increasing of Pb accumulation in several organs becausethe body's metabolismof red tilapia was disrupt.Thus, the energy of the feed, which designed for growth, was using to defend the body from pollutants (Wilson, 1988).

Conclusion

Pb was bound in the sediment and are not measurable in the cultivation lake (grasi). Accumulation of Pb was found to occur in the liver, kidneys and red tilapia meat. The highest accumulation occurred in the third month of maintenance (January 2012) which is the rainy season. The entrance of Pb accumulation in red tilapia was through the food chain and have a significant impact on the growth rateduring the four months of maintenance. Depurasi naturally occur very quickly on the fish, along with the improvement of water quality of post-tin mining lake.

Recommendation

Based on these results, further research needs to be done with the full span of a year to see the effect of accumulation based on the season. Preferably fish kept on the post-tin mining lake by the floating net method and suggests not harvested in the transition season or rainy season.

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