Amino Acid Absorption by Tiger Grouper Fish (*Epinephelus fuscoguttatus*) Larvae

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Abstrak

Absorbsi Asam Amino oleh Larva Ikan Kerapu Macan (Epinephelus fuscoguttatus)

Ikan Kerapu merupakan salah satu ikan unggulan yang ditargetkan sebagai komoditi eksport Indonesia. Usaha budidayanya saat ini sangat terganggu dengan tingginya mortalitas pada stadia larva. Usaha untuk mengatasi masalah tersebut adalah dengan menggunakan obat seperti antibiotik yang ternyata tidak membuahkan hasil maksimal tetapi justru menimbulkan resistensi beberapa jenis bakteria. Salah satu aspek yang belum pernah dilihat dalam rangka mengatasi masalah ini adalah dengan mengusahakan percepatan pertumbuhan pada stadia larva sehingga akan lebih mampu menghindari dari beberapa penyebab mortalitas. Salah satu sumber energi yang terdapat dalam perairan namun dalam jumlah yang tidak besar adalah dissolved organic matter (DOM). Penelitian ini difokuskan untuk melihat kemampuan larva ikan Kerapu dalam memanfaatkan DOM (digunakan asam amino terlarut ;ATT) yang terdapat di air laut. Sebanyak 16 (enam belas) jenis asam amino yang terdiri dari tiga klas yakni neutral, basic, dan acidic digunakan dalam penelitian ini. Sedangkan larva ikan Kerapu yang digunakan berumur 2 hari. Hasil penelitian menunjukkan bahwa larva ikan Kerapu menyerap seluruh jenis asam amino baik neutral, basic, dan acidic. Namun jenis asam amino yang diserap adalah glutamat, histidin, lisin, serin, metionin, tritopan dan iso leusin. Sedangkan yang paling banyak diserap oleh larva ikan ini adalah lisin. Hal yang menarik dalam penelitian ini adalah terdapat beberapa jenis asam amino yang diduga justru dikeluarkan oleh larva ikan tersebut yakni glysin, alanin, tyrosin, valin, phenil alanin dan leusin. Penyerapan beberapa jenis asam amino ini diduga dimanfaatkan oleh larva ikan Kerapu dalam proses pertumbuhannya.

Kata kunci *: asam amino terlarut, larva, Kerapu Macan, Epinephelus fuscoguttatus*

Abstract

Kerapu (grouper fish) is known as an important and highly economic value fish and a good candidate for major export commodity for Indonesia. However, there is an important problem faced by its cultivation i.e. high mortality rate at larva stage. Many different efforts have been done to overcome this problem mainly by using drugs and antibiotics, which have caused another problem i.e. bacteria resitance. One aspect that has not been widely investigated is by increasing its growth rate so that the larvae will have the ability to avoid mortality, such as by utilising dissolved organic matter (DOM) which naturally occur in the environment. This research investigates the question whether Kerapu fish larvae have the ability to absorb DOM (in this case disolved free amino acids; DAA) as well as the preference and the rate of absorbsion. There were 16 species of DAA used in this experiment which consist of three classes i.e. neutral, basic, and acidic. Two days old larvae were used in the experimant. The results showed that Kerapu larvae absorbed all classes of amino acids, although not all amino acids given being absorbed but only glutamine, histidine, lysin, serine, triptophan, metionine and iso leusine. While the most absorbed amino acids was lysine. One interesting results showed that the larvae secrete several amino acids i.e. glysine, alanine, tyrosine, valine, phenil alanine and leusine. The absorbsion and secretion of amino acids were possibly related to its metabolic processes within the larvae in relation to growth processes.

Keywords*: dissolved free amino acids, DAA, larvae, Kerapu, Epinephelus fuscoguttatus*

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Diterima/Received: 13-09-2013
Disetujui/Accepted: 10-10-2013

Introduction

Grouper fish is known as having high economic value where demand is still increasing (Afero, 2012) and has the potential to increase foreign exchange earnings through exports of these products (Irianto and Susilo, 2007). The fact that some grouper fish products from Indonesia still depends on the catch at sea is quite alarming. As a result, natural fish populations is declining, this is compounded by the degradation of coral reef ecosystems in which species of fish live. Therefore, the development of grouper fish farming has become very important.

Development of grouper fish farming is started with seed supply capacity on a regular basis through artificial seeding. Although seed production methods already well known, however, it still leaves the issue of the high rate of mortality of fish larvae. According Widiastuti and Nismah (2006), as well as Suwiryo and Giri (2010), grouper larvae mortality rate can reach up to 98%, this is thought to be caused by environmental conditions, the availability of food or disease.

Several studies have been conducted to address this problem, but the success to suppress the mortality rate of the larvae is still very limited. These studies generally emphasizes in the use of antibiotics or vaccine development to cope with the disease. Research that led to the optimization of the water parameters fo the fish is also very limited. For example Widiastuti and Nismah (2006) using organic osmolit taurine against Tiger grouper larvae (*Epinephelus fuscoguttatus*), while Adriyanto *et al*. (2013) working on different water medium temperature setting for the fish.

One of the aspects that until now has not been much studied is the use of dissolved organic matter (DOM) as a source of energy that can improve the survival and growth of fish larvae. According to some researchers the absorption of DOM (in this case is DAA, dissolved amino acids, as well as several other dissolved organic material) is the source of nutrients of some marine species (Wright and Pajor, 1989; Peterson *et al.,* 1998). Rønnestad *et al.* (2003) stated that in its development after the egg yolk is absorbed, then the fish must meet the needs of amino acids contained in sea water for growth. Research using Tridacna clam larvae showed that the DOM is able to increase the growth rate significantly (Ambariyanto, 2000). Accelerate growth strategies to minimize such mortality occurs naturally in some marine animals. Many reports state that the amino acid is also an important component in the diet of fish, especially to support the growth of the fish (e.g. Wang *et al*., 2005; Zhou *et al.,* 2007). Applebaum and Rønnestad (2004) mentioned that larvae needs to absorb amino acids or other nutrient for growth especially since digestive tract has not developed. They found that absorption rates of alanine, glutamate, arginine, and lysine by larval Atlantic halibut (*Hippoglossus hippoglossus*) was not significantly different. This is supported by Rønnestad *et al.* (2004) who reported that larvae Senegal sole (*Solea senegalensis*) needs amino acids for growth.

Study on the effect of DOM on the growth rate and survival of fish, especially grouper larvae is still limited. On the other hand, the content of DOM in the ocean has been reported to increase (Ogawa and Tanoue, 2003). Dissolved amino acids known as one of the DOM which can be found in low concentrations in seawater. Therefore, this study is very important and strategic in order to see whether this condition can overcome high mortality rate in grouper larvae. Success in suppressing larval mortality would provide a good solution for the production of larvae and increase grouper production, therefore, high demand of the fish can be met as well as for export commodities. This study aims to investigate the ability of the grouper fish larvae in absorbing dissolved free amino acids which are thought to have positive impact on larval survival and growth rates.

Material and Methods

The study was conducted on two days old grouper (*Epinephelus fuscoguttatus*) fish larvae. Fish larvae at this age have reported to have very high mortality rate. These larvae were obtained from the Institute for Aquaculture Situbondo, East Java.

The experiments were conducted in beaker glass at a density of 2 larvae.ml^{-1}. Various types of amino acids (16 types, namely: glutamate, glutamine, serine, arginine, histidine, lysine, valine, asparagine, alanine, phenylalanin, tyrosine, leucine, glycine, threonine, isoleucine, methionine) were added into beaker glass containing 0.2 µ filtered sea water and the grouper fish larvae. The concentration of each amino acid used in the experiment was 400 UM.

Two types of controls were used in this study, namely: a) seawater that has been filtered and given the grouper fish larvae with the same density (10 larva.L^{-1}), as well as b) sea water which has been filtered without fish larvae. Water (1.5 ml) was sampled at 0, 5, 15, 30, and 60 minutes after addition of amino acids solutions by using filtered syringe. These samples were put in eppendorf tubes and stored in the freezer until ready to be analyzed. Analysis of the amino acid concentration was done by using HPLC. During this research process always use gloves to avoid contamination. Data on absorbtion preference were analyzed discriptively to describe the type, class and amount of amino acids absorbed by the grouper larvae.

Results and Discussion

Grouper fish is known as one of the fisheries and marine commodities that have high economic value and life in the reef ecosystem. This ecosystem is known as the ecosystems that have low nutrient concentrations. On the other hand, it is also known as ecosystem that have a high level of productivity. In addition, dissolved organic nutrients also play an important role in maintaining the high productivity of this ecosystem (Rabalais *et al*., 2002).

It has been reported that concentration of dissolved amino acids in coral ecosystems varied among several locations. Ferrier (1991) reported that the concentration of dissolved amino acids in Bermuda were between 0.05 - 0,22 UM, Mombasa were between 0.86 to 1.08 UM (Schlichter and Liebezeit, 1991) and the Great Barrier Reef were reported between 0.05 to 0.17 (Hoegh-Guldberg *et al.,* 1997).

This study showed that grouper fish larvae (*E. fuscoguttatus*) are capable of absorbing the entire class of dissolved amino acids either neutral, basic, and acidic. However, not all amino acids given were absorbed by the fish larvae. Figure 1 Absorption of the dissolved amino acids have also been reported in other marine organisms such as brittle star, *Amphipholis squamata* and *Ophiopholis aculeata* (Lesser and Walker, 1992); Mussel (*Mytilus edulis*) and *M. californianus* (Silva and Wright, 1992); Oyster (*Crassostrea gigas*) (Manahan 1989); Abalone, *Haliotis rufecens* (Jaekle and Manahan, 1989b); starfish, (*Achanthaster planci*) (Hoegh-Guldberg, 1994); and corals (Grove *et al*., 2008).

It appears that the rate of uptake of dissolved amino acids by fish larvae were between 0,024 to 0,286 Umol.larvae^{-1}.h^{-1} (Table 1). This result is higher than the capacity to absorb the dissolved amino acids by larval clams (*Tridacna squamosa*) with an average absorption rate of 0.017 Umol.larvae^{-1}.h^{-1} (Ambariyanto, 2000). This is presumably as a vertebrate animals, the fish larvae require more dissolved organic for growth compared with invertebrates.

Although the results of this study indicate that the grouper fish larvae absorb the entire class of amino acids given, but it appears that this fish larvae absorb only a few types of amino acids. Those include glutamate, histidine, lysine, serine, methionine, leucine and iso trytophan. Grouper larvae seem to have a preference for certain amino acids. Different behavior is found in the giant clam, *Tridacna squamosa* where larvae showed no preference for a particular type of amino acid (Ambariyanto, 2000). This preference is thought to be related to the biological needs in accordance with metabolisms and the degree of development or growth.

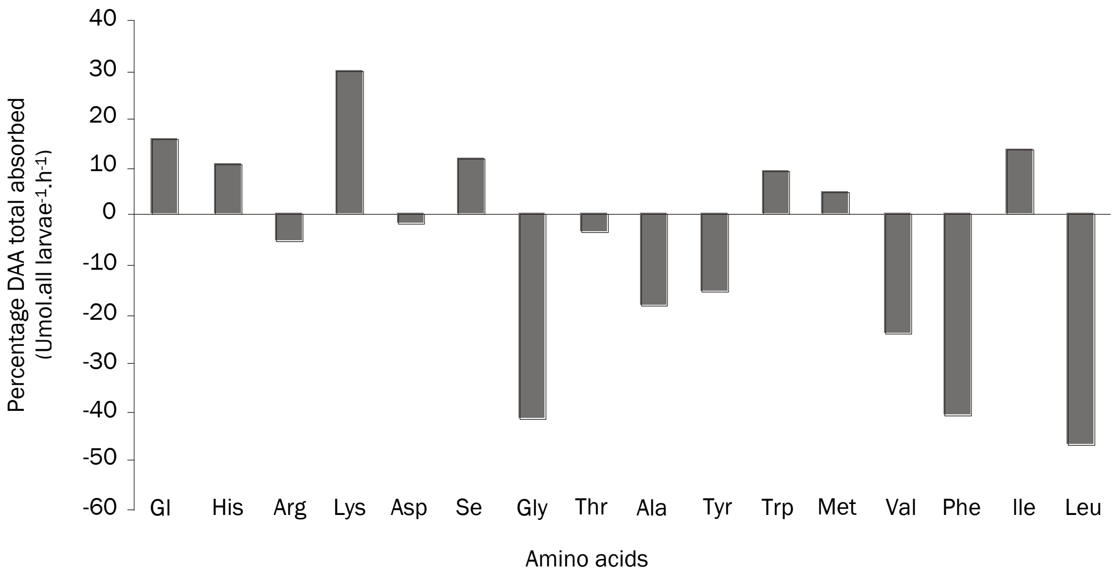

Figure 1. Dissolved amino acids absorption by the larvae of tiger grouper (*Epinephelus fuscoguttatus*) after correction with control

Table 1. The rate of absorption of various dissolved amino acids by tiger grouper larvae (*Epinephelus fuscoguttatus*)

No	DAA	Class of DAA	Umol.all larvae^{-1}.h^{-1}	Umol.larvae^{-1}.h^{-1}
1	Aspartate	Neutral	1,34	0,134
2	Glutamine	Asidic	1,11	0,111
3	Serine	Neutral	0,88	0,088
4	Histidine	Basic	0,89	0,089
5	Arginine	Basic	0,24	0,024
6	Glysine	Neutral	-0,24	-0,024
7	Threonine	Neutral	0,34	0,034
8	Alanine	Neutral	1,11	0,111
9	Tyrosine	Neutral	-0,53	-0,053
10	Trytophan	Neutral	0,50	0,050
11	Metionine	Neutral	0,36	0,036
12	Valine	Neutral	-0,34	-0,034
13	Phenil alanine	Neutral	-0,63	-0,063
14	Iso leusine	Neutral	0,95	0,095
15	Leusine	Neutral	0,64	0,064
16	Lysine	Basic	2,86	0,286

The results also showed that there has been an increase in the concentration of several other amino acids. It is suspected this fish larvae actually releasing several types of amino acids which include glysine, alanine, tyrosine, valine, alanine and leucine phenil alanine. This finding is supported by Thorsen and Fyhn (1996) who reported the occurrence of hydrolysis of the protein from the yolk and become DAA. Similarly, Kawasaki and Benner (2006) also reported the presence of DOM that has been released by certain bacteria in the growth process.

Suspected that the absorption of certain amino acids is related to the needs of the amino acids in the metabolism of fish larvae is particularly associated with the absorption of nutrients. Reported by some researchers that the absorption of DAA and some other dissolved organic matter by some species of marine organisms is related to the nutritional needs of these animals (Wright and Pajor, 1989; Rice, 1999). One thing that need to get the attention of this study is this fish larvae absorb much more lysine (0.286 Umol.larva^{-1}.h^{-1}) compared with other amino acids.

These results are in line with the report of Zhou *et al.* (2007) who showed that the highest number of types of amino acids that are needed for the growth of juvenile cobia (*Rachycentron canadum*) compared with other types of amino acid is lysine. It is also consistent with the reports Wang *et al*. (2005) on juvenile fish *Ctenopharyngodon idella*. Absorption of dissolved amino acids in this study was thought to affect the rate of growth of this Tiger Grouper fish larvae. Some reports indicate that the dissolved amino acid plays an important role as an energy source for marine life, for example, larvae of marine worms (*Urechis caupo*) is able to absorb the amino acid leucine which is able to provide 51% of the metabolic energy requirements of the larvae (Jaekle and Manahan, 1989a). The same researchers also reported that the amino acid (1.6 UM) able to provide 39-70% of the metabolic energy requirements of larval abalone Haliotis rufecens (Jaekle and Manahan, 1989b).

The report on the important role of amino acids to provide the energy needed by marine organisms above only occurs during larval stage. In adult organisms, form example ini giant clams, it turns out the role of dissolved amino acids and nitrogen in providing the required energy is very small (Hoegh-Guldberg and Ambariyanto, 1999). This is due to the fact that in the adult clams almost all the energy required is obtained from the process of filter feeding and nutrition from the zooxanthellae obtained through the process of translocation of a portion of photosynthesis, therefore, the role of dissolved amino acids were not significant. On the other, hand dissolved amino acid plays an important role in providing the energy needs of giant clams larvae. After fertilization takes place, the energy required for larvae development mainly is obtained from egg yolk only. It is possible that it also occurs in other marine organisms such as fish, including groupers. Rønnestad *et al*. (1999, 2003) states that amino acids play an important role in the development of the larvae of various species of fish, especially after the egg yolk is used. Before the fish reach the stage where they are able to actively seek and obtain food as a source of energy from various sources in the vicinity, then the energy needed larvae obtained from egg yolk and then the energy obtained from the surrounding sea water. Energy source for larval clams from the sea water probably derived from the DOM (dissolved organic matter), including amino acids (Klumpp and Grifith, 1994). Baines *et al.* (2005) reported the uptake of DOM by zebra mussel *Dreissena polymorpha,* which is related to the metabolic requirments of the organisms. In species grouper fish hatchery operations, the larvae begin to be given additional food after 3 days old. High mortality rate of grouper fish larvae is suspected due to the lack of energy sources required by larvae after egg yolk has been used. Some researchers state that dissolved amino acids in sea water plays an important role as a source of nutrition (Peterson *et al.*, 1998). Rønnestad *et al*. (2000) stated that the DAA is more easily absorbed by fish larvae compared with the protein. Therefore, enrichment of amino acids in the water will be absorbed by fish larvae and used as energy intake. Growth rate of the fish larvae is expected to increase and also decrease the mortality rate of the larvae. The importance of DAA will also affect the development of adult fish. According Aragao *et al.* (2004) the feed given to the fish Seabream gilthead (*Sparus aurata*) and Senegalese Sole (*Solea senegalensis*) in hatchery has not met the amino acid requirements of the fish.

Strategy to cope with high mortality rate during larval stages can be done by accelerating growth rate. Increased larval growth resulting from the addition of DOM has been demonstrated in the larvae of clams *Tridacna squamosa* (Ambariyanto, 2000), larvae of the marine worm, *Urechis caupo*, (Jaekle and Manahan, 1989a), larval abalone of *Haliotis rufecens* (Jaekle and Manahan, 1989b). Reports from Rønnestad *et al*. (2000) showed that dissolved amino acids apparently can be absorbed more efficiently than the protein by fish larvae Senegal sole (*Solea senegalensis*). Therefore, DAA is a better source of energy than protein. *Skjærven et al.* (2003) also confirmed that the developmental stage of larval fish plaice (*Pleuronectes platessa*) require energy derived from DAA contained in the water. It is thought that its also occur in grouper larvae in which the uptake of dissolved amino acids contained in the water will accelerate growth and improve survival.

Conclusion

Grouper larvae *Epinephelus fuscoguttatus* is able to absorb the amino acid, and lysine was the most absorbed. On the other hand, the fish larvae were also secrete several types of amino acids that is glysine, alanine, tyrosine, valine, alanine and leucine phenil alanine. Absorbed amino acids is thought to be used for the growth of grouper larvae.

Acknowledgments

This study was funded by Due Like Project to the Marine Science Program, FPIK, Diponegoro University. Thanks are also extended to three students namely Arif Suryolelono, Diyan Novita, and Ismu Dewantara, as well as the management and all staff Balai Budidaya Situbondo for all their help.

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