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## Profile of Amino Acid and Fatty Acid from Different Type of Worm as Feed of Broodstock Candidates for Domesticated Uceng (*Nemacheilus fasciatus*)

Lik Anatus Sholikah<sup>1\*</sup>, Agoes Soeprijanto<sup>1</sup>, Yuni Kilawati<sup>2</sup>

<sup>1</sup>Department of Aquaculture, Faculty of Fisheries and Marine Science, University of Brawijaya

<sup>2</sup>Department of Management of Water Resources, Faculty of Fisheries and Marine Science, University of Brawijaya

\*Email Address : likanatusc@yahoo.com

### **KEYWORDS**

Uceng Fish  
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**Abstract** Uceng fish (*Nemacheilus fasciatus*) is a fish that lives wild on the river. All this time, the people have only relied on fishing from nature to consume, but the high market demand for fish will increase river fishing activities in excess. One of the steps to suppress the decline in fish populations is domestication because currently, the fish is not yet cultivated. The domestication process by feeding worms containing amino acids and fatty acids accelerates broodstock candidates' gonadal development. This research aims to determine the type of worms suitable as feed for the growth and gonadal development of broodstock candidates. The method that used in this research is Completely Randomized Design (CRD) with 3 treatments and 3 repetitions. Based on the analysis of amino acids and fatty acids in each type of worm, the best results were obtained on *Lumbricus rubellus* with an amino acid content of 50.91 gr/100gr and fatty acid 292.27%.

### **Introduction**

Uceng fish (*N. fasciatus*) is found mostly in Wlingi, Blitar, and lives wild on the river. This fish, *N. fasciatus* as the potential for consumption. It has a savory taste and contains many non-essential fatty acids, high in calories, and contains DHA-EPA (*Decosa Hexaenoat Acid - Eicosa Pentaenoat Acid*), which is very good for human health (Supangat, 1995). This has caused high market demand for *N. fasciatus* to increase. Attainment of need for *N. fasciatus* is still relying on catches from nature (Prakoso *et al.*, 2016). High market demand for *N. fasciatus* will increase overfishing activities in the river. One of the steps to reduce the decline of *N. fasciatus* populations in nature is by domestication. *N. fasciatus* until now, it has not been able to be cultivated.

Efforts made to help the domestication of fish are by providing quality feed and eating habits of wild fish in nature. Feeds with suitable

nutrient content will influence the growth and gonadal development of broodstock candidates. The selection of natural feed of worms is carried out to help the growth and gonadal development of broodstock candidates. Natural food is needed to develop fish, especially during spawning or before spawning, because the needs of essential amino acids and essential fatty acids can be fulfilled in the natural feed (Axelrod *et al.*, 1983). The natural feed used in this research is earthworms (*Lumbricus rubellus*), silkworms (*Tubifex sp.*), and blood worms (*Chironomus sp.*). Of the three, natural foods have different nutrients.

Earthworms are one organism that has a high protein content. Earthworms have a 64-76% protein content, and fat content was 7-10%. Moreover, earthworms also contain tocopherol and vitamin E, which function as antioxidants to spur fish reproduction (Susanti dan Mayudin,

2012). Silkworms (*Tubifex* sp.) has a good nutrient content feed for fish, including Protein, 57%, fat of 13.3%, Crude Fiber of 2.04%, and Ash content of 3.6%, and moisture of 87.7% (Bintaryanto dan Taufikurohmah, 2013). Blood worms are a type of worm that is popularly used as feed for ornamental fish and consumption fish. The protein content in bloodworms reaches 40% (Himawan et al., 2011).

This research was conducted to obtain information about the type of worms suitable for the feed of prospective brood using fish domestication products to help the growth and gonadal development of prospective brood fish.

## Materials and methods

### Instrument and Material

The instruments used in this research are aquariums with size 30 cm x 30 cm as much 12 pieces, aquarium heater, nets, thermometer, aerator, pH meter, DO meter, digital scales, calipers, film bottle, *sectio set*, *object-glass*, and *cover glass*, *microtome rotary*, *water bath*, small brush, tray, microscope, and camera. The materials used in this research are broodstock candidates of uceng fish as much 180, freshwater, Earthworms (*Lumbricus rubellus*), silkworms (*Tubifex* sp.) and blood worms (*Chironomus* sp.), formalin 10%, *acetone*, *xylol*, liquid paraffin, alcohol 96%, tissue and label.

### Research Methods

The method that used in this research is Completely Randomized Design (CRD) with 3 treatments and 3 repetitions for each treatment with different types of worms between Earthworms (*Lumbricus rubellus*), silkworms (*Tubifex* sp.), and blood worms (*Chironomus* sp.).

### Location and Time of Research

The research was conducted in April-Juni 2018. Maintenance and treatment activities of

test fish are carried out at Reproduction Laboratory, Fisheries and Marine Science, University of Brawijaya, Malang, according to published methods (Islamy et al., 2017; Kilawati and Islamy, 2019). Testing of amino acid profiles is carried out at Saraswati Indo Genetech Laboratory, Jakarta. Testing of fatty acid profiles is carried out at IPB, Bogor.

## Research Procedures

### Proximate Feed Analysis

Proximate analysis of the three types of worms consisted of Protein (%), Fat (%), Ash (%), Carbohydrate (%), Water content (%), Crude Fiber (%) carried out at the Feed Nutrition Laboratory, Faculty of Agricultural Technology, University of Brawijaya, Malang. The methods are modified according to published procedures (Pratama et al., 2019).

### Analysis of Feed Amino Acid Content

The method used in the amino acid analysis is the using Ultra Performance Liquid Chromatography (UPLC) method and modified according to published procedures (Pratama et al., 2019). Steps taken include the sample was weighed as much as 0.1 g was crushed and put into a closed test tube. The sample solution was added with 6 N HCl as much as 5-10 mL, hydrolyzed in an oven at 110 °C for 22 hours, then cooled at room temperature and transferred to a 500 mL measuring flask. Then, add distilled water to the boundary and filter with a 0.45 µL filter and piped 10 µL, adding 70 µL AccQ Fluoric Borate and divorce. Then 20 µL of the Flour Adan reagent was added to be cooked and left to stand for 1 minute and added for 10 minutes at 55°C and then injected into the UPLC as much as 1 µL with chromatographic conditions using ACCQ-Tag Ultra C18 column, temperature 49 °C, phase of system motion PDA composition gradient

detectors, flow rate 0.7 µL/minute and wavelength 260 nm.

*Analysis of Fatty Acid Content*

The method used in the fatty acid analysis is the using Gas Chromatography-Mass

Spectroscopy (GCMS) method and modified according to published procedures (Pratama et al., 2019). The GC/MS design stages include *Sample preparation, derivatization, injections, GC separation, MS detector, scanning.*

**Results and Discussion**

Based on the results of the proximate analysis of each type of worm shown in Table 1.

**Table 1. Proximate Feed Analysis Results**

Sample	Protein (%)	Fat (%)	Ash (%)	Carbohydrates (%)	Water Contain (%)	Crude Fiber (%)
Earth worms	58.09	15.13	9.53	12.53	2.68	2.04
Silk worms	57.20	13.30	11.32	11.42	3.56	3.20
Blood worms	60.50	10.40	13.50	9.53	3.53	2.54

**Table 2. Amino Acid Feed Content**

Amino Acids	Earth Worms ( <i>Lumbricus rubellus</i> )	Silk Worms ( <i>Tubifex sp.</i> )	Blood Worms ( <i>Chironomus sp.</i> )
Aspartic Acid (gr/100gr)	4.28	3.27	4.44
Glutamic Acid (gr/100gr)	5.58	4.72	7.00
Serine (gr/100gr)	3.05	2.64	1.92
Histidine (g/100gr)	1.99	1.90	0.86
Glycine (gr/100gr)	3.32	2.93	1.92
Threonine (gr/100gr)	3.03	2.85	2.15
Arginine (gr/100gr)	4.14	4.31	2.21
Alanine (gr/100gr)	3.76	2.62	2.96
Tyrosine (gr/100gr)	1.71	2.25	1.37
Methionine (gr/100gr)	2.09	1.63	0.87
Valine (gr/100gr)	3.25	2.73	2.65
Phenylalanine (gr/100gr)	4.60	3.67	2.11
I-leucine (gr/100gr)	2.83	2.33	2.52
Leusine (gr/100gr)	4.33	3.97	4.10
Lysine (gr/100gr)	2.95	2.50	3.22
Total (gr/100gr)	50.91	44.32	40.29

**Table 3. Fatty Acid Feed Content**

Fatty Acids	Earth Worms ( <i>Lumbricus rubellus</i> )	Silk Worms ( <i>Tubifex sp.</i> )	Blood Worms ( <i>Chironomus sp.</i> )
Kaprat (%)	0.98	0.92	0.22
Laurat (%)	5.92	1.24	3.88
Miristat (%)	11.34	10.06	6.50
Palmitat (%)	21.27	21.57	28.80
Stearat (%)	6.58	5.82	3.94
Oleat (%)	28.38	28.20	32.68
Linoleat (%)	13.38	13.16	12.56
Linolenat (%)	1.05	0.84	0.54
EPA (%)	70.23	68.97	70.04
DHA (%)	133.14	116.16	123.60
Total (%)	292.27	266.94	282.76

The importance of proximate analysis of amino acid content has been noted previously (Pratama et al., 2020) Based on the amino acid profile analysis results of each type of worm shown in Table 2. The best results of amino acid profiles are found in treatment A (earthworm feed). The highest non-essential amino acid profile is glutamic acid, with a value of 5.58 gr/100gr. Functions of Glutamic acid are an energy source for the brain and are found in serum, muscle, and brain fluids, but 60% is found in the body in free amino acids (Greenwell, 1999). In comparison, the highest essential amino acid is lysine of 2.95 g/100g. The great function of lysine in an animal's body is to deposit protein tissue because its needs are not affected by other metabolic roles and depend on fish species and the type of raw material. Lysine usually has a major role in limiting amino acids because it is known that the need for lysine is much greater than for other amino acids (Miles dan Chapman, 2007). Based on the results of the fatty acid profile analysis of each type of worm shown in Table 3.

The highest results of fatty acid profiles were found in earthworms (*Lumbricus rubellus*) of 292.27%. This fat can function as a source of energy and essential fatty acids, and fat is used as a constituent material for the structure of fat grains and egg yolk granules. Fat is significant for the survival of fish eggs because this component is a major component of the process of vitellogenesis (Frengky et al., 2012). However, Environmental conditions also play an important role in the condition of fish (Islamy and Hasan, 2021)

### Discussion

The growth and gonadal development of prospective brood fish can be improved by improving feed quality by providing high protein foods. The three types of worms obtained the highest results in earthworms (*Lumbricus rubellus*). In principle, amino acids are needed for two purposes: growth and maintaining the body's metabolic processes. Amino acid requirements for growth are more evident in small size fish whose growth

is relatively fast (Cowey, 1994). According to Lante *et al.* (2015), Protein a vital role in fish growth. Because Protein is a builder and constituent of new tissue for growth, replacing damaged tissue, regulating substances in the formation of enzymes and hormones, regulating various metabolic processes in the body, and as an energy source when carbohydrates and fat cannot meet fish's energy needs. Until the mature stage, fish's gonadal development will experience an increase in body weight, which can reach 10% -25% of the original fish's body weight. The influence of gonadal development causes a specific growth rate (SGR) (Effendi, 1979).

From the analysis of fatty acids, the highest content is in earthworms (*Lumbricus rubellus*), with the highest type of fatty acids found in DHA of 133.14%. DHA is needed for cell membranes' function from neural networks and, as a precursor for eicosanoid formation, several types of hormones (Tocher, 2003). The highest fat requirement during vitellogenesis (egg yolk formation). The need for EPA starts in the previtellogenesis period until the ovulation process. Thus EPA and DHA are crucial for normal larval growth and development (Sargent *et al.*, 2002). Deficiency of essential fatty acids will disrupt fish health, including reduced fecundity and ability to form embryos, death of abnormal larvae and growth, defective pigmentation, inability to eat at a low light intensity, abnormal behavior, and decreased membrane function at temperatures low (Tocher, 2003). We recommend that further researchers conduct similar research using tested fish that are often cultivated and have economic value such as tilapia (Insani et al., 2020); Genggehek (Valen et al., 2019); Gobiidae (Hasan et al., 2021a); Beardless barb (Hasan et al., 2021b).

Another suggestion, comparison of the test material with other protein sources such as snails (Islamy and hasan, 2020) can be a consideration in developing Feed of Broodstock Candidates.

### Conclusions and Suggestion

Based on the analysis of amino acids and fatty acids from each type of worm, it was found that earthworms (*Lumbricus rubellus*) had the highest content in the study of amino acids and fatty acids, the acceleration of growth and gonadal development of prospective brood fish. This shows that earthworms can be used as feed for domesticated broodstock.

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