Enhancement of Omega-3 DHA content by Feeding *Hermetia illucens* and Microalgae *Aurantiochytrium* sp. on Catfish

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Abstract

The growing demand for sustainable and high-quality animal feed has led to exploring alternative sources of protein and other essential nutrients. The black soldier fly (BSF) maggots <u>Hermetia illucens</u> and microalgae Aurantiochytrium sp. are promising candidates. Both have shown significant potential due to their nutritional profiles and environmental benefits. This study aimed to compare the fatty acid content of two groups of Sangkuriang catfish. One group was given commercial animal feed, while the other group was fed with a combination of <u>Aurantiochytrium</u> sp. microalga biomass feed, <u>H. illucens</u> maggot, rice bran, and corn flour. The catfish fed with <u>Aurantiochytrium</u> sp. microalga biomass, <u>H. illucens</u> maggot, rice bran, and corn flour had a different fatty acid composition than the control group. This alternative diet led to an increase in the omega-3 docosahexaenoic fatty acid (DHA) content in the catfish. The study results suggest that fish feed containing <u>H. illucens</u> maggots and microalgae Aurantiochytrium sp. can enhance fish's beneficial nutrition after being fed this particular feed mixture.

Keywords: <u>Aurantiochytrium</u> sp., Fish Feed, <u>Hermetia illucens</u>, Omega-3 DHA, Sangkuriang Catfish.



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INTRODUCTION

The global aquaculture industry has seen substantial growth over the past few decades, with fish feed playing a crucial role in this expansion. As the demand for seafood continues to rise, driven by increasing population, health consciousness, and declining wild fish stocks, the fish feed industry has evolved to meet the nutritional requirements of farmed fish. This trend is evident worldwide, including in Indonesia, one of the leading aquaculture producers (Tacon & Metian, 2015). The global fish feed market has been growing steadily, driven by the increasing demand for aquaculture products. According to a report by Allied Market Research, the global fish feed market size was valued at \$98.20 billion in 2020 and is projected to reach \$162.56 billion by 2027, growing at a compound annual growth rate (CAGR) of 6.6 % from 2021 to 2027 (Allied Market Research, 2024).

Indonesia has abundant natural resources, including vast water areas and high biodiversity. With the growing population, there is an increasing demand for protein, which can be met by fish. Catfish is a top cultivated fish product, contributing more than 10 % of national aquaculture production (Muntafiah, 2020), and is expected to boost it to 38 % (Fauzi & Sari, 2018). The high demand for protein in Indonesia has led to an increase in the market for aquaculture (Lestari, 2020; Hutari et al., 2024). As a result, the government is planning to develop the fisheries sector (Zaenuri et al., 2014). However, providing artificial feed is expensive and can make up to 60 - 70 % of the production cost component (Murni, 2013). Researchers are looking for ways to produce economical feed with the required nutritional content for fish (Audila et al., 2021).

Maggots of the Black Soldier Fly (BSF) *Hermetia illucens* are a sustainable protein source (Makhrojan, 2019). The nutritional composistion of maggot Hermetia illucens L. is very good as a potential source of animal protein as an alternative to natural fish feed (Meitiyani et al., 2020). Maggots BSF are a sustainable protein source (Ridho et al., 2020; Budhi et al., 2020; Nurhayati et al., 2022). They can be combined as artificial feed pellets and grown in media that contains nutrients that suit their living needs (Berampu et al., 2021). On the other hand, *Aurantiochytrium* sp. microalgae has been found to enhance the growth of Tilapia (Nobrega et al., 2019). This microalgae is known to contain omega-3 DHA, squalene, and astaxanthin, and is usually found in mangrove forests (Hutari et al., 2022; Suhendra et. al. 2022; Leyland et al., 2017). Omega-3 docosahexaenoic acid (DHA) obtained from microalgae is highly beneficial for maintaining cardiovascular, brain, and vision health (Bradbury, 2011). The use of microalgae *Aurantiochytrium* sp. in fish feed provides an ideal ratio of omega-3 and omega-6, which is beneficial for human consumption (Nobrega et al., 2019).

Currently, reports on the use of maggots *Hermetia illucens*, and microalgae *Aurantiochytrium* sp as fish feed ingredients are still very limited. Therefore, this study aims to investigate the effect of administering both maggots *Hermetia illucens* and microalgae *Aurantiochytrium* sp. on the omega-3 DHA content of catfish (*Clarias* sp.).

METHODS

Time and Location of Research

The research was conducted between September 2023 and January 2024 in the Biology Education laboratory of the Faculty of Teacher Training and Education at Muhammadiyah University, Prof. Dr. Hamka (UHAMKA) Jakarta. Fatty acids analysis is carried out at the National Research and Innovation Agency (BRIN) by ordering analysis through the E-Layanan Sains (ELSA) system.

Tools and Materials

The tools and materials used consist of a Laminar Air Flow Cabinet, UV lamp, orbital shaker (DLAB), tube needle, bunsen, Erlenmeyer flask, alcohol, autoclave, gas chromatography, rotary evaporator, GC-vials, n-Hexane (Merck), acetyl chloride (Sigma-Aldrich), dried methanol (Merck), bacteriological agar (Oxoid), glucose (Himedia), yeast extract (Himedia), distilled water, reef salt (Aquaforest), aluminum foil, cotton gauze, falcon tube, and Sangkuriang catfish.

Experimental Steps

The research method involves feeding two groups of Sangkuriang catfish (app. 10 cm in length) different diets: one group (Group A) with commercial catfish pellets, and the other group (Group B) with 25 % dried maggot biomass, 5 % wet microalgae *Aurantiochytrium* sp., and 70 % rice bran & corn mix. After the feeding period, the catfish meat will be processed into filets for fatty acid analysis.

Analysis of Fatty Acid

The fatty acid omega-3 DHA analysis involved resuspending approximately 1 gram of catfish fillet in 1 mL of dried methanol and mixing it with 4 mL of a fresh 10 : 1 mixture of dried methanol and acetyl chloride. The samples were then incubated at 50 °C for 16 hours. Subsequently, the methyl esters were extracted two times with 5 mL n-hexane, the hexane was evaporated, and the remaining fatty acid was dissolved in 1.5 mL of n-hexane. Finally, the solution was transferred to GC vials for analysis by gas chromatography according to the method described by Hillig et al., (2014).

RESULTS AND DISCUSSION

The chromatograms show a comparison of fatty acid profile between a control sample (Figure 1A) and a treatment sample (Figure 1B) of Sangkuriang catfish after analysis via GC-MS. From Figure 1, it can be seen that there are differences in the fatty acid profiles contained in the meat of control catfish fed commercial catfish feed and treatment catfish fed maggot and *Aurantiochytrium*-based feed. The chromatogram peaks correspond to different fatty acid methyl esters (FAMEs) identified in the samples. From this image, it can be seen that after finishing feeding, the results of GC analysis showed an increase in omega-3 DHA levels from what was previously not visible in the chromatogram of the control group.

Based on Table 1, the DHA content is present only in the treatment sample, indicating a significant improvement in the nutritional profile with the treatment. The results of this study are by research conducted by Effiong & Fakunle (2010), where the dietary lipid sources significantly influenced the fatty acid composition of the fish. According to Effiong & Fakunle (2010) added the primary saturated fatty acid in the fish fed with coconut oil was lauric acid, while fish fed olive oil had the highest concentration of monounsaturated fatty acids (73.55 %), particularly oleic acid. Fish fed sunflower oil exhibited high levels of docosahexaenoic acid (DHA) and

arachidonic acid (ARA). According this a report in the fatty acid analysis of silver catfish, the omega-3 DHA content of the fish depends on the area where the fish is found and the part of the fish's body that is analyzed. On average, the omega-3 fatty acid DHA content in silver catfish is between 1 - 2.8 %.

No.	Retention	Percentages area (%)		
peak	time	Control	Treatment	Components
	(min)	(A)	(B)	
1	3.02	12.42	48.74	Furan, tetrahydro-3-methyl
2	37.42	1.61	1.03	Methyl tetradecanoat
3	42.30	2.58	0.84	9-Hexadecanoic acid, methyl ester
4	42.94	30.81	24.93	Hexadecanoic acid, methyl ester
5	47.04	8.37	2.67	Methyl 10-trans, 12-cis-
				octadecadienoate
6	47.23	30.27	13.35	9-octadecanoic acid, methyl ester
7	47.33	2.92	0.73	11- octadecanoic acid, methyl ester
8	47.76	10.00	3.87	Methyl stearate
9	50.99	0.53	-	Cyclononasiloxane, octadecamethyl
10	51.73	0.49	-	cis-Methyl 11-eicosenoate
11	54.84	-	1.03	Methyl 4,7,10,13,16-docosapentaenoic
				acid
12	55.03	-	2.81	4,7,10,13,16-19-docosahexaenoic acid,
				methyl ester
	Total	100.0	100.0	

Table 1. Fatty acids composition of control and treatments

Microalgae *Aurantiochytrium* is known for its high content of docosahexaenoic acid (DHA) and other omega-3 fatty acids. Feeding fish with *Aurantiochytrium* can enrich their fatty acid profile, particularly in terms of DHA and eicosapentaenoic acid (EPA). The presence of these fatty acids can enhance the nutritional value of the fish. Research Batista et al., (2021) shows that providing biomass *Aurantiochytrium* sp. feed can increase omega-3 levels in fish.

The practice of enhancing the omega-3 DHA percentage in catfish by feeding them maggots *Hermetia illucens* and microalgae *Aurantiochytrium* sp. aims to enrich the catfish with these essential fatty acids, known for their numerous health benefits such as aiding in brain function and promoting heart health (Cederholm et al., 2013). It has been found that incorporating maggots *Hermetia illucens* and microalgae *Aurantiochytrium* sp. in the diet of Catfish can significantly improve their omega-3 DHA percentage (Table 1; Figure 1). This approach could be beneficial in enhancing the nutritional value of catfish and ensuring better health for consumers. It may also be of interest to those in the aquaculture industry.

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Figure 1. Fatty acids profile of control group (A) and treatment group (B)

CONCLUSION

Omega-3 DHA is known for its health benefits, particularly in supporting brain function and heart health. This research describes enhancing omega-3 DHA percentage in catfish by feeding them *Hermetia illucens* and microalgae *Aurantiochytrium* sp. This feeding strategy is likely used to increase the nutritional value of the catfish by enriching them with omega-3 DHA fatty acids derived from these particular sources.

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