A comparison of the drying techniques for maggot of the black soldier (*Hermetia illucens*) as an insect-based protein source for the feed of juvenile tilapia fish (*Oreochromis niloticus*)

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Abstract. The identification of an appropriate method for drying black soldier fly (BSF) (*Hermetia illucens*) maggots used as fish feed is essential to improve the quality of the final product. This study compares maggot-drying techniques to formulate a feed to obtain the best growth results for juvenile tilapia (*Oreochromis niloticus*). The study was conducted from July to September 2021 in the Laboratory of Building 4, Faculty of Fisheries and Marine Sciences, Padjadjaran University. A completely randomized design was used for the experiment, with four treatments, each with four repetitions: a control treatment of only pellet feed; a combination of 50% pellets and 50% oven-dried maggots; 50% pellets and 50% sand-roasted maggots; and 50% pellets and 50% sun-dried maggots. Juvenile tilapia were fed the different diets for 70 days of observation. Growth and hematologic parameters of tilapia were similar between the three combination diets containing dried maggots and the control. The combination diets resulted in an absolute weight gain of 12.34-13.24 g, absolute length increase of 5.0-5.4 cm, daily growth rate of 3.025-3.16%, feed conversion ratio of 1.34-1.36 with feed efficiency of 73.7-74.4%, total erythrocyte count of 9.97-10.0.6 × 10^{-5} cells mm^{-3}, and total leukocyte count of 6.82-7.0 × 10^{4} cells mm^{-3}.
Key Words: drying, fish growth, maggot of black soldier fly, tilapia fish.

Introduction. Fish production, particularly aquaculture, has rapidly progressed worldwide. Minister of Marine Affairs and Fisheries of Indonesia had a target for fishery production of 19.47 million tons in 2021 (Marine and Fisheries Ministry Republic of Indonesia 2021). This is an approximately 5.5% increase in the production target compared to 18.44 million tons in 2020. The Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) is an important commodity in fishery cultivation development, which aims to encourage industrialization programs (Hadie et al 2018). Based on the Food and Agricultural Organization's 2017 ranking, Indonesia is the second-largest exporter of tilapia after China, with a production of 1.2 million tons in 2017 (Hadie et al 2018).

The rapid growth in aquaculture production has led to an increased need for fish feed. One of the costliest commodities in fish cultivation is feed, which is extremely expensive and responsible for around 50% of the total production cost (Rostika et al 2019). Meanwhile, an important source of protein in feed is fish flour, whose production has remained stagnant since the 1990s (Fahmi 2015). This has encouraged several researchers to find an alternative ingredient for fish flour (fishmeal replacement). Although some researchers have successfully developed fishmeal replacements, the availability of their ingredients is too limited for bulk production (Priyadi et al 2009).

The use of insects as a source of protein can be more economical than the use of fish meal. Insects have an important role in feed because they are natural and environmentally friendly (Van Huis 2013). The cultivation of maggot of the black soldier fly (BSF) (*Hermetia illucens*), uses the bio-waste treatment system. Thus, it can reduce organic waste, which has the potential to pollute the environment. Organic waste management is expected to cut maggot production costs leading to a decrease in the price of fish feed. Another benefical factor arising from the use of maggots in feed is that they are an insect-based protein source that does not compete with sources for humans; therefore, maggots are a convenient raw material for animal feed, including for fish and poultry (Veldkamp et al 2012).

Wachira et al (2021) investigated the profitability of maggot-based tilapia feed and Tippayadara et al (2021) studied its impact on the growth, hematology, and mucus production of tilapia. However, the information on an effective way to dry maggots is still limited. The drying process is part of the preparation (precondition) of raw material for fish feed. A better understanding of the effects of the drying process on BSF maggots is highly recommended to contribute to improvements in the quality of the final product. The aim of this study was to compare the BSF maggot drying technique that gave the best growth performance for tilapia juveniles.

Material and Method

Research methodology. This study used an experiment with a completely randomized design, including four treatments, each with four repetitions. The treatments were carried out for 70 days from October 2021 to December 2021, consisting in a combination of pellet and dried BSF maggot: treatment of 100% pellet (A), treatment of 50% pellet + 50% oven-dried maggots (B), treatment of 50% pellet + 50% sand-roasted maggots (C), and treatment of 50% pellet + 50% sun-dried maggots (D). The observed parameters included absolute weight growth, absolute length growth, daily growth rate, feed efficiency, feed conversion ratio (FCR), and total erythrocyte and leukocyte.

Maggot culture. BSF eggs were used to initiate maggot culture. Organic waste, which was the incubation and growth medium for maggots, was placed in a container. BSF eggs were deposited on a pad before placing them on the organic waste to prevent their death from exposure to water from the organic waste. The eggs hatched within 1-5 days. The organic waste was replenished as required. Maggots can be nurtured until 20 days after the hatching process before they turn into black prepupae. The skin of the prepupa hardens and turns black, a sign of its high chitin content, which renders it unsuitable as fish feed. Maggots used in this study were harvested when they were 15 days old.

Maggot blanching. Before being used, the maggots were first washed with clean water to remove the dirt from the cultivation medium and to prevent them from being lost during the drying process. The success of dry maggot production is determined by the process of killing maggots. According to Larouche (2019), blanching for 40 seconds at a temperature of $\pm 100^{o}$C is the best procedure for killing maggots.

Drying. Drying was performed by using oven and wok according to the following methods: oven-drying at a temperature of $\pm 55^{o}$C for ±24 hours, sand-roasting at a temperature of $\pm 100\text{-}180^{o}$C for ±15 minutes, and sun-drying for ±4 days.

Fish handling. Tilapia juvenile (N = 160) measuring 3-5 cm (standard length), with a weight of ±3 g, were obtained from the Ciparay Fish Seed Center, Bandung District, Indonesia, and were raised for 70 days in 16 aquaria. The fish were sampled every ten days to measure length and weight, and determine the feed given. The aquaria were 40 × 25 × 25 cm and filled with 20 liters of water. They were equipped with continuous aeration and the water was changed by siphoning every day. The fish were fed based on treatment at the same dose, i.e., 5% g^{-1} day^{-1} (Sepang et al 2021). The frequency of feeding was twice per day.

Red and white blood cell counts. Red and white blood cell counts was performed based on the Klont (1994) method. Briefly, fish blood was taken by inserting a tuberculin needle, moistened with 10% EDTA, into the caudal aorta (below the lateral line, at the base of the tail). The blood thus obtained was placed in an Eppendorf tube with 10% EDTA and analyzed further.

Red blood cells were drawn with a Thoma pipette up to the 0.5 mark and diluted with Hayem's solution up to the 101 mark. White blood cells were drawn with a Thoma pipette up to the 0.5 mark and diluted with Türk's solution up to the 11 mark. Blood cell samples were then observed under a microscope.

Data analysis. The data were analyzed using analysis of variance (ANOVA) to identify significant differences between feed treatments. If a significant *F*-statistic was obtained, the analysis continued with Duncan's test at the 95% confidence level.

Results

Growth. Growth is the change in length or weight in a time unit of time and is divided into two categories: absolute growth, i.e., average total size; and daily growth, i.e., the percentage of every day growth (Aliyas et al 2016). The results of ANOVA of average absolute weight growth and daily growth rate are shown in Table 1.

Table 1

Absolute weight growth and daily growth rate of juvenile tilapia

Treatment	*Absolute weight (g)*	*Absolute length (cm)*	*Daily growth rate (%)*
A	10.03	5.45	3.081
B	13.24	5.40	3.160
C	12.34	5.00	3.025
D	12.92	5.03	3.057

The results of the proximate test (Table 2) show an increase in protein and fat content of the dried maggots compared to fresh maggots, possibly caused by the decrease in moisture content, which leads to a relative increase in other constituents. The sun-dried maggots had the highest protein content, compared to the other treatments, because of the drying temperature of ±30°C, while the sand-roasted maggots had the lowest protein content because of protein denaturation and a drying temperature of 100-180°C.

Table 2

Proximate analysis of maggot at the age of 14 days

Sample	*Protein (%)*	*Fiber (%)*	*Fat (%)*	*Carbohydrate (%)*
Fresh	38.95	7.06	9.32	35.09
Oven-dried	40.56	3.65	28.58	18.94
Sand-roasted	35.11	5.72	20.90	26.67
Sun-dried	47.41	5.88	22.86	16.19

Feef efficiency and FCR. Feed efficiency is a comparison between the amount of feed consumed and the resulting weight gain (Saputra et al 2018). The ANOVA results for feed efficiency of juvenile tilapia are shown in Table 3. The FCR is the amount of feed consumed, which results in a body weight gain of 1 kilogram. The ANOVA results for the FCR of tilapia showed that the treatments were not significantly different. Sepang et al (2021) found that tilapia given maggots had an FCR of ±1.2-1.8. This may be because some of the feed given was not consumed by the fish, resulting in differences in FCR. According to Saputra et al (2018), the lower FCR the higher feed efficiency, and conversely the higher the FCR, the lower the efficiency.

Table 3

Feed efficiency and feed conversion ratio (FCR) of juvenile tilapia

Treatment	*Feed efficiency (%)*	*FCR*
A	74.12	1.35
B	74.42	1.34
C	73.73	1.36
D	74.30	1.35

Fish hematology. The hematological analysis of the fish provided parameters that indicated whether the use of maggots as fish food had a negative impact or caused disease. The red and white blood cell counts can indicate the health status of fish (Table 4).

Table 4

Average red blood cell and white blood cell counts of juvenile tilapia

Treatment	*Average red blood cells ($x\ 10^5$ cell mm^{-3})*	*Average white blood cells ($x\ 10^5$ cell mm^{-3})*
A	11.16	6.52
B	9.98	6.90
C	10.06	6.82
D	9.97	7.00

Discussion. The result showed that the absolute weight gain and daily growth rate were not significantly different between treatments. This means that dried maggots can replace 50% of the commercial pellet feed and provide nutrients for the growth of juvenile tilapia, as indicated by the lack of difference in growth between the 100% commercial pellet treatment and the other treatments including 50% dried maggots. Insects are an alternative food, which contains proteins, macro- and micronutrients, and vitamins that are the same as those in fish flour (Basto et al 2020). Besides, maggots are rich in essential amino acids, particularly lysine, methionine, and leucine, and lack anti-nutritional substances (Shumo et al 2019).

Tippayadara et al (2021) found that tilapia fed with pellets and maggots had a growth rate of approximately 1.29-1.44% day^{-1}, and there was no significant difference between feed with maggots and the control treatment. However, the growth rate they obtained is different from that of this study, i.e., 3.025-3.160% day^{-1}. This disparity may be due to the difference between the studies in the initial weight of the test fish and fish density in the aquaria. In the study by Sepang et al (2021), tilapias fed with maggots were capable of a daily growth rate of 2.7-3.7% day^{-1}. The combination of several additional feeds during fish growth did not significantly affect the length and weight of the test fish.

In this study, the absolute length increase was highest in treatment A (control; Table 1), possibly due to the control feed having a nutrient composition suitable to meet the nutritional needs of tilapia for growth and other activities. However, the differences between treatments were not significant. The protein content in the feed formulation must be in accordance with the needs of fish. Kardana et al (2012) suggested that increased protein content in feed does not always result in an increase in growth. However, a higher protein content in feed, without a non-protein energy balance, will make protein a source of energy for activities (NRC 1983). The fat content of the dried maggots in the three treatments was approximately 20%, with the highest in the oven-dried maggots at 28.58% (Table 2). Fat is usually a better energy source than carbohydrates in fish feed (Wachira et al 2021). Therefore, fat should be considered as a constituent in feed to make fish growth optimal. The energy obtainable from carbohydrates and fat in fish feed minimizes the need to include protein, which is quite expensive (Ali & Al-Asgah 2001).

According to Kardana et al (2012), when feed efficiency is higher, fish can make better use of the feed consumed. In Tippayadara et al's (2021) study, tilapia fed with pellets and maggots had a feed efficiency of 44.92-48.72%. This is a lower feed efficiency than that obtained in our study, which was in the range of 73.7-74.4%. This difference may be caused by the size of test fish, method of feeding, and nutrients in the different feeds. In our study, maggots were given in combination with pellets, while Tippayadara et al (2021) provided them as an alternative to fish flour. However, both studies showed that the use of maggots as fish feed has a positive outcome.

The FCR indicates the amount of feed used to grow 1 kg in body weight of fish. A low FCR means a higher feed efficiency, while a higher FCR means lower efficiency (Saputra et al 2018). The result of the ANOVA of FCR of juvenile tilapia shows that the four treatments were not different (Table 3). In Sepang et al (2021) study, tilapia fed with maggots had an FCR of 1.2-1.8, possibly because some feed was not consumed by the fish, which led to the range of FCRs.

From an economic perspective, the profit from fish cultivation can be increased by replacing the fish feed with dried maggot. The fish maggot was possible to be combined with the pellets by proportion to 50%. According to Wachira et al (2021), complete substitution of fish flour in pellets with maggots had the lowest production cost at USD 0.172 per fish, compared to the control treatment, which cost USD 0.201 per fish, while the cost saving did not affect the growth parameters of the test fish.

The results of the ANOVA of the average total red blood cell count in juvenile tilapia show that there were no differences between treatments (Table 4). Therefore, the use of dried maggot in the diet does not significantly affect total erythrocyte count. Tippayadara et al (2021) also found that the use of up to 100% maggot flour in feed did not affect the red and white blood cell counts in tilapia. The ANOVA of average total white blood cell count in juvenile tilapia also did not show treatment differences (Table 4). Total white blood cells were in the normal range. According to Rastogi (1977), the normal range of leukocytes in fishes, in general, is 20,000-150,000 cell mm^{-3}. In this study, the leukocyte count in all combination treatments was higher than that in the control treatment of commercial pellets. This may be due to an increased immune system response in the fish. Similar results were observed with the addition of prebiotics by Hartika et al (2014). Leukocyte number increased in the prebiotics addition treatment; the total leukocyte count in tilapia was 2.65×10^4 in the control treatment, while it was $\pm 3.58\text{-}8.04 \times 10^4$ in the prebiotics treatment. The increased leukocytes play a role in the immune response of fish to disease and infection. It is likely that the use of dried maggots as feed could increase the immune system response in fish. Maggots have antimicrobial and antifungal properties; consequently, fish consuming them may be resistant to diseases caused by bacteria and fungi (Margolin & Gialanella 2010). BSF larval extracts possess a broad spectrum of antibacterial activity, suggesting that secretions of BSF larvae may be useful in the fight against methicillin-resistant *Staphylococcus aureus* (MRSA) and could be a potential source of novel antibiotic-like compounds for infection control (Park et al 2014).

Conclusions. Maggots dried using three different methods were fed to juvenile tilapia over 70 days of observation. All growth and hematologic parameters of tilapia-fed dried maggots were the same as those of the control fed commercial pellets. The combination of 50% commercial pellets and 50% maggots dried using the three methods led to an absolute weight gain of 12.34-13.24 g, absolute length increase of 5.0-5.4 cm, a daily growth rate of 3.025-3.16%, FCR of 1.34-1.36 with a feed efficiency of 73.7-74.4%, total erythrocyte count of $9.97\text{-}10.06 \times 10^5$, and total leukocyte count of $6.82\text{-}87.0 \times 10^4$. The combination of 50% commercial pellets and 50% dried maggots is recommended as tilapia feed.

A reduction in production cost may be achieved when maggots are independently produced because dried maggot distributed in the market is still quite expensive. Further research should be conducted on the percentage of maggots that can be used, up to 100%, for making fish feed to find the best formula and on reducing the high fat content of dried maggots.

Conflict of interest. The authors declare that there are no conflicts of interest.

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