

The Effect of Maggot Formulated Feed on Essential Amino Acids Composition, Digestibility and Growth of Dwarf Snakehead, *Channa gachua* Fry (Kesan Makanan Diformulasi Berenga pada Komposisi Asid Amino Penting, Kebolehceraan dan Pertumbuhan Anak Ikan Haruan Kerdil *Channa gachua*)

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ABSTRACT

This study was carried out to evaluate the influences of maggot formulated feed on growth, digestibility, feed utilization, and body composition of local snakehead *Channa gachua*. Five practical diets with iso-nitrogenous (40% protein) and caloric level (3.5 kcal g⁻¹) were determined as 10%, 20%, 30% 40%, and 50% substitution of maggot to fish meal protein. Results demonstrated that replacement of 30% protein from maggot in the diet produced in the highest specific growth rate (4.41 %BW day⁻¹). These results were in line with feed conversion ratio (FCR) and protein utilization (PER) at increasing dietary maggot level from 10% to 30%. Feed digestibility also attained the maximum level at 30% of maggot substitution. Thirty percent protein substitution from maggot meal exhibited the highest growth performance, nutrient utilization, and digestibility than other treatments. Dietary inclusion levels of maggot meal in diet above 30% remarkably influenced the growth, FCR, PER, and digestibility of *C. gachua*. Regarding feed price concern, the application of maggot meal may potentially be profitable for snakehead's fry culture.

Keywords: Aquaculture; fish nutrition; *haruan*; Indonesia; protein replacement

ABSTRAK

Penyelidikan ini dijalankan untuk menilai pengaruh makanan diformulasi berenga terhadap pertumbuhan, kecernaan, penggunaan makanan dan komposisi badan ikan haruan tempatan *Channa gachua*. Lima diet praktikal dengan iso-bernitrogen (40% protein) dan tahap kalori (3.5 kcal g⁻¹) ditentukan dengan 10%, 20%, 30% 40% dan 50% penggantian berenga kepada protein berasaskan ikan. Keputusan menunjukkan bahawa penggantian 30% protein daripada berenga dalam diet menghasilkan kadar pertumbuhan khusus tertinggi (4.41 %BW day⁻¹). Keputusan ini adalah selaras dengan nisbah penukaran makanan (FCR) dan penggunaan protein (PER) pada peningkatan tahap diet berenga daripada 10% kepada 30%. Kecernaan makanan juga mencapai tahap maksimum pada 30% penggantian berenga. Tiga puluh peratus penggantian protein daripada makanan berenga menunjukkan prestasi pertumbuhan tertinggi, penggunaan nutrien dan kebolehceraan berbanding rawatan lain. Tahap rangkuman diet makanan berenga dalam diet melebihi 30% sangat mempengaruhi pertumbuhan, FCR, PER, dan kebolehceraan *C. gachua*. Berkenaan keseimbangan harga makanan, penggunaan makanan berenga mungkin berpotensi menguntungkan untuk pengkulturan anak ikan haruan.

Kata kunci: Akuakultur; haruan; Indonesia; nutrisi ikan; protein gantian

INTRODUCTION

In aquaculture production, feed is the most expensive factor, especially for the protein component. Some cases

reported that more than 60% of the operational costs of fish culture come from a feed. Fish as monogastric livestock in aquaculture requires high quality protein. The need for

protein in fish is generally higher than in other livestock. Thus, this condition impacted the relatively high price of fish feed. In terms of intensive culture systems, the application of formulated feed plays a significant part in the fish growth and production. Many works have been done and found that the protein requirement for Snakehead (*Channa* sp.) reached 40% (Fang et al. 2019; Hien et al. 2018; Khaeriyah et al. 2018; Mithu et al. 2017; Wijianti et al. 2018).

Fish meal (FM) is the most widely source of protein in fish diet formulation (Paray et al. 2016; Sagada et al. 2017) due to its high protein content, high digestibility, and superb source of essential amino acid (EAA), essential fatty acids, vitamins, and minerals (Nguyen et al. 2009). The need of FM for most carnivorous fish accounts for 30-50% (Cheng et al. 2003). Increasing demand for the aquaculture industry and limited supply makes FM the most expensive protein source in fish feed (Amaya et al. 2007; Nguyen et al. 2009). Replacement of FM with a less expensive protein source without diminishing the fish growth would generate in more cost-effective fish production. Moreover, there is also an environmental concern related to FM production from capture fisheries.

Insects are recognized as an alternative ingredient to boost feed and food security in many studies (Henry et al. 2015; van Huis & Oonincx 2017). Protein from insects is more cost-effective and eco-friendly (van Huis & Oonincx 2017). Oonincx et al. (2015) also explained that insects have a high feed conversion ratio and can be produced on the large scale. They could convert organic refuse to body mass by reducing dry matter mass up to 58% (Sheppard et al. 1994) and associated 30-50% N and 61-70% P (Myers et al. 2008). The potential insects identified for protein replacement are such as *Tenebrio molitor* (Li et al. 2013), *Rhynchophorus* spp. (Elemo et al. 2011), *Musca domestica* (Awoniyi 2007; Calvert et al. 1969), and *Hermetia illucens* (Oonincx et al. 2015).

H. illucens larvae or maggot meal has several significant advantages exceeding other insects. This species can convert waste food material or manure into a high protein ingredient (Henry et al. 2015; Makkar et al. 2014). This species has elevated amylase, lipase, and protease activities in its gut extracts (Kim et al. 2011). *H. illucens* is rich in protein (47.6-51.8% in defatted meal), lipid (11.8-18.8%) and ash (9-28%) (Abd El-Hack et al. 2020; Barragan-Fonseca et al. 2017; Cullere et al. 2016; Kroeckel et al. 2012). Moreover, the EAA content in maggot meal is comparable with FM as a traditional

protein source in the fish diet (Abd El-Hack et al. 2020; Mustapha & Kolawole 2019). Research has found that maggot can be applied as an alternative protein source in several fish and shrimp species such as European sea bass (Magalhães et al. 2017), turbot (Kroeckel et al. 2012), Atlantic salmon (Bruni et al. 2019) (Lock et al. 2016; Belghit et al. 2019), yellow cat-fish (Dietz & Liebert 2018), Nile tilapia (Xiao et al. 2018), African catfish (Alegbeleye et al. 2012; Anvo et al. 2017; Fasakin et al. 2003), and white shrimp (Cummins Jr. et al. 2017). However, information about *H. illucens* used for *C. gachua* diet is still limited. For that reason, this work aimed to determine the use of *H. illucens* or maggot meal to replace FM protein in the diet on the growth response, nutrient digestibility, and EAA composition of *C. gachua*.

MATERIALS AND METHODS

MAGGOT PRODUCTION AND EXPERIMENTAL DIETS

Seventy-five (75) percent of kitchen waste and twenty-five (25) percent of palm kernel meal were mixed and placed on the container ($10 \times 10 \times 10 \text{ cm}^3$) up to 3 cm thick to form the substrate. Eggs from the house fly of 0.3 g per kg of substrate were distributed on the substrate provided. Eggs hatched into larvae within two days and the larvae were harvested after 9 days of cultivation. They were put in a sieve and immersed in clean water and thoroughly rinsed. They were immersed in hot water and oven-dried at 70 °C overnight. The dried maggots were milled into powder and used to replace fishmeal protein at 10, 20, 30, 40, and 50 percent in 40 percent crude protein diets. The diet formulations and nutritional profile of the experimental diets applied in the experiment are presented in Table 1. Feed ingredients after proper mixing were steamed for 10 minutes and after cooling was pelleted and stored in refrigerator at normal temperature conditions in the refrigerator and gradually used to feed the fish.

EXPERIMENTAL STUDY AND SAMPLING

The study was performed in the fish-rearing unit of the Department of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Brawijaya. Five treatment groups were administered in triplicate using a completely randomized method with a trial unit of a 20 liter volume aquarium (size $50 \times 30 \times 30 \text{ cm}^3$). Twenty fish seeds ($0.82 \pm 0.03 \text{ g}$) of test fish were stocked on each

TABLE 1. Diet formulations and nutritional compositions of experimental diets

	Fish meal protein replacement by maggot (%)				
	10	20	30	40	50
Ingredient composition (%)					
Fish meal	44.6	39.3	33.9	28.5	23.2
Soya cake	24.0	24.0	24.0	24.0	24.0
Maggot meal	5.4	10.7	16.1	21.5	26.8
Rice bran	14.0	14.0	14.0	14.0	14.0
Wheat flour	10.0	10.0	10.0	10.0	10.0
Vit mix	0.2	0.2	0.2	0.2	0.2
Min mix	0.9	0.9	0.9	0.9	0.9
CMC	0.4	0.4	0.4	0.4	0.4
Cr ₂ O ₃	0.5	0.5	0.5	0.5	0.5
Total	100.0	100.0	100.0	100.0	100.0
Proximate analysis (% DM)					
Dry Matter	90.2	90.0	89.8	89.6	89.4
Protein	40.9	40.7	40.5	40.3	40.1
Fat	10.8	11.2	11.5	11.9	12.2
Fiber	7.4	7.4	7.3	7.2	7.1
Ash	10.2	10.1	10.1	10.1	10.0
NFE	29.5	29.5	29.5	29.5	29.5
Total Energy (Kcal g ⁻¹)	3.5	3.5	3.5	3.5	3.5

Note: total protein in each diet was formulated from fish meal (90.9%DM, 57.0%Protein, 17.0%Fat); Soya cake (92.0%DM, 38.9%Protein, 1.7%Fat); maggot meal (87.0%DM, 53.1%Protein, 23.3%Fat); Rice bran (89.2%DM, 13.2%Protein, 11.0%Fat); Wheat flour (86.0%DM, 14.4%Protein, 0.5%Fat)

of the 15 aquaria according to treatment. Before placing the seeds in a randomly selected aquarium among the five treatments, the average seed biomass of each aquarium stocked was recorded. The feed was given three times daily as much as 5% biomass and adjusted every sampling period. The aquaria were cleaned on daily basis after the last feeding through siphoning. The 10% of the aquaria water was replaced with drilled well water.

The number of dead fish was monitored and counted daily. Cannibalism was estimated from the difference between the initial number of stocked fish and the number of live fishes. Feces were collected every day to be used for the nutrient digestibility analysis using

Cr₂O₃ (chromium (III) oxide) as an indicator. Sampling fish weight was done every week by taking 10 fish each aquarium and adjusting the amount of feed according to the growth. All fish were harvested after 42 days. They were measured for fish weight and 10 samples from each replication packed in polythene and stored in the freezer (-20°C) for proximate analysis.

The proximate composition of diets and feces of fish was analyzed following the AOAC method. All samples were analyzed in three replications. Dry matter was calculated by drying in an oven at 105 °C for 24 hours, crude protein (NX 6.25) by Kjeldahl method, and crude fat with ether extraction. The nitrogen-free extract

was determined by the reduction method. The profile of essential amino acids (EAA) of fish meal, maggot meal, and the fish carcass fed with experimental diets were determined by using HPLC based on AOAC (2005).

CALCULATIONS AND DATA ANALYSIS

The following formulas were used to evaluate the survival rate, growth performance, and nutrient digestibility among treatments.

$$\text{Feed intake (FI, g)} = \frac{\text{total dry feed given}}{\text{number of fish}}$$

$$\text{Survival rate (SR, \%)} = \frac{\text{total fish harvested}}{\text{total fish stocked}} \times 100$$

$$\text{Specific Growth Rate (SGR, \%B/d)}$$

$$= \frac{\ln(\text{final body weight}) - \ln(\text{initial body weight})}{\text{number of feeding day}} \times 100$$

$$\text{Feed Conversion Ratio (FCR, g/g)} = \frac{\text{feed consumption}}{\text{body weight gain}}$$

$$\text{Protein efficiency Ratio (PER, g/g)} = \frac{\text{net weight gain}}{\text{protein fed}}$$

Apparent digestibility coefficients (ADC, %) of protein, fat and energy

$$= \left[1 - \frac{(\text{dietary chromium (III) oxide level} \times \text{feces nutrient or energy level})}{(\text{feces chromium (III) oxide level} \times \text{dietary nutrient or energy level})} \right] \times 100$$

Data were demonstrated as mean \pm standard deviation. Analysis of variance (One-way ANOVA) was utilized to evaluate the level of significance between different growth parameters with SPSS version 20.0. Significant levels were considered at $p < 0.05$ and means were analyzed using Duncan's multiple range test. The regression graph was plotted to compare the relationship between fish meal protein substitution by maggot with digestibility parameters.

RESULTS AND DISCUSSION

The growth performance and nutrient utilization of snakehead fed diets with maggot meal replacing fishmeal are presented in Table 2. The survival rate of snakehead fish was not significantly different among maggot feed treatments ($p > 0.05$) ranged from 81.42% to 84.12%. There were not any disease-related mortalities found during this study. This result is in accordance with Ogunji et al. (2008) who reported that the survival rate of Tilapia was not affected significantly after being fed with a 30% maggot meal.

Visual observations during the study showed that fish actively swam to all experimental diets. There was no significant effect ($p > 0.05$) of treatments on observed feed intake by *C. gachua*. However, FI by *C. gachua* was higher in feed containing maggot levels of more than 30% compared to that in other treatments. This indicated that the maggot meal was palatable for *C. gachua*. This is in agreement with Olaniyi and Salau (2013) who reported that increasing maggot meal levels in the diet led to an increase in the feed intake of *Clarias gariepinus*.

There was no significant difference ($p > 0.05$) in the initial body weight of *C. gachua* allocated to the experimental feed. However, there was a significant difference ($p < 0.05$) of final mean body weight gain and SGR in *C. gachua* group fed different experimental diets. The significantly highest body weight was noticed in fish fed with replacement of 30% maggot meal, while the lowest was recorded in those fed with replacement of 50% maggot meal. The SGR of *C. gachua* experienced an upward trend with increasing levels of maggot meal up to the level of 30%. The *C. gachua* SGR then decreased with the higher inclusion of maggot meal in their diets. A similar trend was also true for FCR. The FCR and PER values were significantly different ($p < 0.05$) with the different levels of maggot meal in the diet. The significantly highest FCR (1.34) was observed in *C. gachua* fed with the highest portion of maggot meal in their diet. While the lowest one was recorded in *C. gachua* fed with 10 and 30% maggot meal in the diet. Protein utilization efficiency (PER) decreased significantly when the maggot meal level in the feed exceeds 30%. All these results indicated that the protein of maggot meal could successfully substitute a portion of the protein of fishmeal in *C. gachua* diet. In line with these results, the replacement of FM by maggot meal was limited to certain levels in several fish species. Maggot meal successfully replaces fish meal up to 22.5% without any adverse effect on growth performance, feed utilization, or digestibility of European seabass *Dicentrarchus labrax* juveniles (Magalhães et al. 2017). In turbot, incorporation of maggot in the fish diet was possible but limited to 33% (Kroeckel et al. 2012). Replacement of 25% fish meal protein in yellow catfish resulted in the best performances (Xiao et al. 2018). Replacement of FM by maggot meal was limited to less than 25% in the diet white shrimp (Cummins Jr. et al. 2017). In contrast to our results, several studies have shown that maggot meal protein can be successfully used as a complete replacement for FM. A study on *Atlantic salmon* showed

that replacement of fish meal up to 100% with maggot meal had no unfavorable impacts on fish growth, histology, and sensory testing of fillets such as odor, flavor, and texture (Belghit et al. 2019; Lock et al. 2016). These varied results in growth performances between

different species showed the differences in tolerance levels toward maggot meal in their diets. In addition, the various stages of life of fish species used in trials also influenced the results (Belghit et al. 2019).

TABLE 2. Growth performance and nutrient utilization of *C. gachua* during experimental study

Parameters	Fish meal protein replacement by maggot (%)				
	10	20	30	40	50
Initial mean body weight (g)	0.82±0.04 ^a	0.81±0.05 ^a	0.81±0.03 ^a	0.83±0.04 ^a	0.82±0.01 ^a
Final mean body weight (g)	4.72±0.46 ^b	4.76±0.39 ^b	5.07±0.14 ^b	4.51±0.47 ^b	4.36±0.26 ^a
SR (%)	84.12±1.08 ^a	83.22±2.05 ^a	83.35±2.02 ^a	83.52±3.03 ^a	81.42±2.05 ^a
FI (g)	4.47b±0.05 ^a	4.48b±0.05 ^a	4.46b±0.18 ^a	4.77a±0.15 ^a	4.84±0.04 ^a
SGR (%.bwd ⁻¹)	4.37±0.02 ^c	4.39±0.02 ^c	4.41±0.02 ^c	4.10±0.02 ^b	3.88±0.05 ^a
FCR (g/g)	1.14±0.01 ^c	1.15±0.01 ^c	1.14±0.01 ^c	1.28±0.01 ^b	1.34±0.01 ^a
PER (g/g)	2.25±0.05 ^b	2.52±0.04 ^b	2.54±0.06 ^b	2.25±0.06 ^a	2.14±0.02 ^a

Note: means with different superscript letters are significantly different ($P < 0.05$)

The profile of EAA of fish meal, maggot meal, and *C. gachua* carcass fed with experimental diets are presented in Table 3. The EAA of maggot meal was observed lower than that of fish meal. The EAA profile of insect meal is taxon-dependent (Henry et al. 2015). In contrast to our study, Barroso et al. (2014) noted that the EAA profile of Diptera was quite similar to that of the FM profile, while the EAA profile of Coleoptera and Orthoptera were close to soybean with potential lysin or methionine deficiencies. The EAA profile of *C. gachua* fed with experimental diets experienced a downward trend with the higher inclusion of maggot meal in their diet. Moreover, the growth of *C. gachua* in this study was highly related to the EAA content in the feed. Liao et al. (2014) and Hien et al. (2018) reported that weight gain increases along with the increase in EAA levels, especially methionine and lysin diets at optimal conditions of 1.1 and 3.1% dry matter, respectively. Lysin is known to stimulate the synthesis of digestive enzymes in the pancreas and also plays a role in the secretion and

release of digestive enzymes in fish (Li et al. 2013). It has been found that the content of essential amino acids in fish can be used as an indicator of their needs (Bicudo & Cyrino 2009).

The Apparent Digestibility Coefficient (ADC) of protein, fat, and energy in this study are presented in Figure 1. The ADC of protein, fat, and energy of *C. gachua* were affected with the inclusion of maggot meal in their diet ($p < 0.05$). ADC of protein and fat of *C. gachua* in this research tended to increase when the maggot meal levels in the diet enhanced from 10 to 30%. Inclusion of maggot meal more than 30% in the diet would then decrease the ADC of protein and fat. In this study, the relative value of protein and fat ADC was more than 77%. The same pattern of protein and fat ADC also was found in energy ADC. However, the energy ADC value was relatively lower (69-72%) compared to that of protein and fat. These results agreed with Ogunji et al. (2008) who explained the ADC of protein, lipid, and energy was decreased with enhanced levels of maggot meal in the diet of *O. niloticus*.

TABLE 3. Essential amino acid profile of fish meal, maggot meal and *C. gachua* fed with experimental diets

EAA (% DM)	FM	Maggot meal	EAA of <i>C. gachua</i> fed with diet containing maggot meal				
			10%	20%	30%	40%	50%
Thr	1.50	1.43	2.11	2.14	2.05	2.02	1.99
Val	2.47	1.39	2.05	2.08	1.99	1.96	1.94
Met	1.37	1.03	1.51	1.53	1.46	1.44	1.42
Ileu	2.15	1.39	2.04	2.07	1.98	1.95	1.92
Leu	3.51	2.37	3.48	3.54	3.38	3.34	3.29
Phe	1.89	1.33	1.96	1.99	1.90	1.88	1.85
His	1.11	0.81	1.18	1.20	1.15	1.13	1.12
Trp	nd*	nd	nd	nd	nd	nd	nd
Lys	3.58	2.36	3.47	3.53	3.37	3.33	3.28
Arg	2.60	2.11	3.11	3.15	3.02	2.97	2.93

*nd = not determined

The decreased nutrient and energy digestibility when the maggot meal levels exceed 30% is probably related to maggot meal chitin and could also depress protein digestibility in the fish gut. Sánchez-Muros et al. (2016) also described the excessive chitin content in feed can decrease the growth and nutrient utilization of fish. As a monogastric animal, fish cannot digest chitin (Rust 2002). The chitin in insect cuticles is a matrix

of protein, lipids, and other compounds (Kramer et al. 1995). The matrix form of chitin reduces the accessibility of related enzymes (chitinases or proteinases) to digest and then prevent the absorption process by the intestine (Tanaka et al. 1997). It then reduced not only chitin digestibility but also protein and lipid digestibility which subsequently affected the fish growth performance and nutrient utilization (Henry et al. 2015).

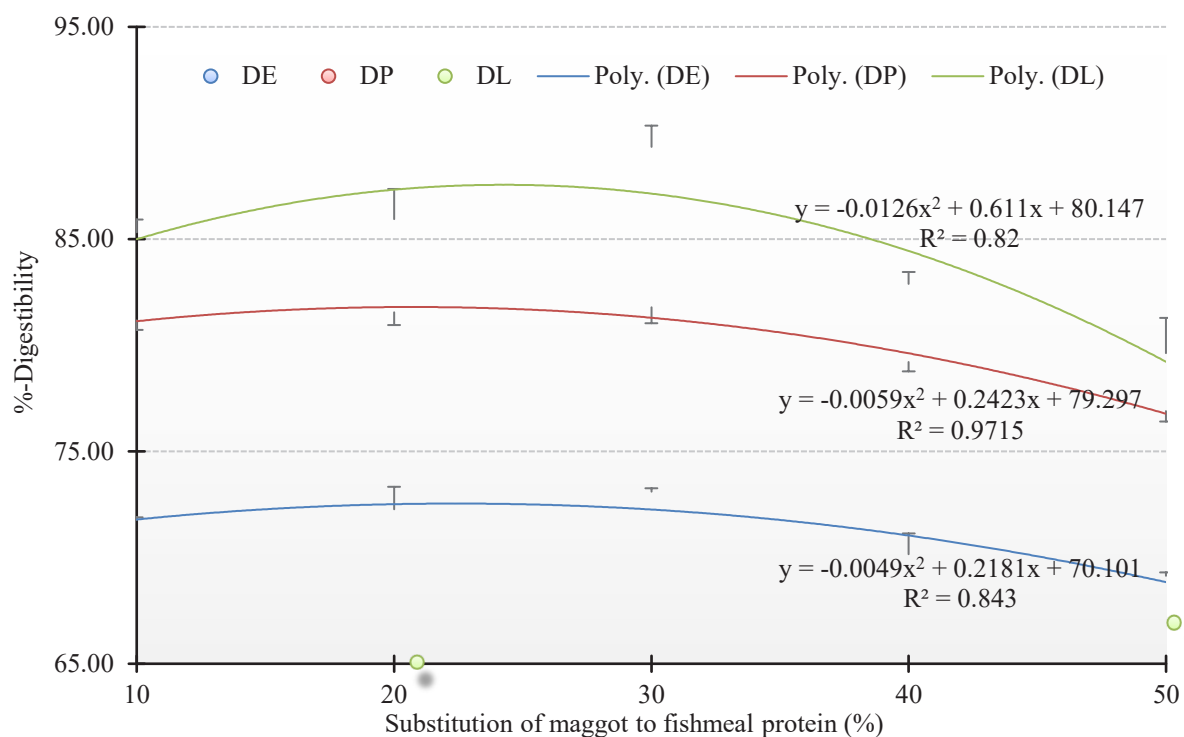


FIGURE 1. Nutrients and energy digestibility of *C. gachua* fed maggot containing diet (DE = Energy Digestibility, DP = Protein Digestibility, DL = Lipid Digestibility)

Our findings suggested that maggot meal protein was well utilized by *C. gachua* thus enhancing good growth. In this study, feeding *C. gachua* fingerlings with maggot-containing diets did not result in any form of physiological stress at all inclusion levels. Hence, maggot protein can therefore partly replace fish meal in the diet of snakehead, *C. gachua* fingerling since it can meet the nutrient requirements of this species. With the increase in nutrients available from the maggot levels and with the low cost, it is possible to increase the maggot value relative to the diet for fish. Therefore, maggot can be effectively used as a low-cost feed for high-quality fish production and promotion of the growth performance of *C. gachua* culture in Indonesia. In this study, 30% substitution of maggot protein to the fish meal is the optimal level recommended for *C. gachua*.

CONCLUSIONS

Based on availability, biological value, essential amino acids profile, growth, and nutrient digestibility, maggot meal is an excellent alternative protein source to fish meal for *C. gachua* diets up to 30 % without compromising the growth.

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