Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 27(3): 933 – 953 (2023)
www.ejabf.journals.ekb.eg

Effect of Fish Meal Substitution Using Maggot Flour (*Hermetia illucens*) on Growth Performance, Feed Utilization Efficiency, Protein Retention and Body Composition of the Milkfish (*Chanos chanos*)

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ARTICLE INFO

Article History:
Received: Dec. 12, 2022
Accepted: May 19, 2023
Online: June 30, 2023

Keywords:
Feed,
Growth,
Maggot flour,
Milkfish

ABSTRACT

Milkfish is a local Indonesian fish highly demanded by the public for its high protein. The feed quality in milkfish cultivation can affect aquaculture production's growth rate and success. The increased price of fish meals is due to more outstanding aquaculture production and the limited availability of fish meals. One alternative that can replace fish meals is maggot flour. Maggot flour has a crude protein content of 40-54% and a crude lipid content of 15-49%. This study aimed to examine the effect of substituting fish meal with maggot meal in artificial feed on the growth and survival of milkfish fry (*Chanos chanos*), determining the best dose for ultimate results. A completely randomized design (C.R.D.) was performed consisting of 5 treatments; namely, A: 0%, B: 30%, C: 35%, D: 40%, and E: 45%/100 g/feed. Milkfish specimens with weights 3- 3.5g and lengths ranging from 6- 7cm long were subject to study. Fish were reared in fiber tanks (10 fish/tubs) for 35 days. Based on the results of the study, the best dose was found in treatment C (35% maggot flour/100g feed), which was capable of producing TFC (90.40±1.93 g), FCR (1.53±0.21), FUE (57.32±0 .75%), SGR (2.64±0.05%/day), absolute weight (51.82±1.27 g) and absolute length (5,33±0.35 cm). Whereas, the best protein retention results were found in treatment B (76.57%). Based on the nutritional quality analysis results, treatment C recorded the highest yields of protein (49.95%), fat (18.54%), methionine (7.10%) and EPA (5.07%).

INTRODUCTION

Milkfish is a local Indonesian fish highly demanded by the public for its high protein **(Minarseh *et al.*, 2021)**. Milkfish have euryhaline properties; their growth is fast, and it is resistant to disease. This fish is one herbivore fish that tolerates various changes in environmental conditions **(Vasava, 2018)**. The advantage of this milkfish cultivation lies in its great demand and relatively high price **(Herawati *et al.*, 2020a)**. Milkfish contains 14.2% omega-3 and is a source of animal protein with high nutrition and low cholesterol levels **(Prabowo *et al.*, 2017)**.

The quality of feed given to milkfish cultivation can affect aquaculture production's growth rate and success. Fish meal plays a vital role as the primary source of protein in artificial feeds **(Pratiwi & Andhikawati, 2021)**. The easily digested protein content aligned with the good amino acid content qualify fish meal to be the main ingredient in the manufacture of feed **(Mamauag *et al.*, 2021)**. However, the price of fish meals is increasing to acquire more excellent aquaculture production, and the availability of fish meals is increasingly limited **(Nguyen *et al.*, 2021)**. Therefore, it is necessary to have other alternatives as a substitute for fish meals in artificial feeds. The flour used as an alternative to fish meal must have a protein content that can meet the needs of cultured fish. One alternative to the fish meal, which has a nutritional profile almost the same as a fish meal, is maggot flour **(Herawati *et al.*, 2020a)**.

Maggot flour is one of the raw materials with a high nutritional content **(Islam *et al.*, 2016)**, and it has the same essential amino pattern of the fish meal **(Henry *et al.*, 2015)**. Research has been conducted on substituting maggot flour for fish meals in artificial feeds. According to **Herawati *et al.* (2019a)**, maggot flour has a higher protein and amino acid contents than fish meal, with 40-50% protein and 30% fat. According to **Wang *et al.* (2020)**, maggot flour has a crude protein content of 40-54% and a crude lipid content of 15-49%. Crude protein and crude lipids in maggot flour vary depending on the feed given at the rearing time and the processing method. **Herawati *et al.* (2020a)** related the percentage of the protein content (60%) and fat content (10- 30%) in the dry maggot flour to the maggot growing medium, and the authors added that maggot flour has a balanced amino acid profile almost the same as fish meal. According to **Li *et al.* (2020)**, dried black soldier fly (B.S.F) larvae contains 42% protein, 35% lipid content, and an essential fatty acid profile closest to the fish meal's amino acid profile. In the research of **Belghit *et al.* (2019)**, the substitution of maggot flour in artificial feed was given to seawater phase salmon. In the study of **Herawati *et al.* (2020a)**, maggot flour was substituted on milkfish stadia 0.5 - 0.8 grams with a protein content of 33-34%. Therefore, in this study, the substitution of the fish meal using maggot flour with different doses in the artificial feed was carried out to determine the effect of growth and body composition of milkfish (*C. chanos*) fry.

MATERIALS AND METHODS

This research was conducted at the Center for Brackish Water Aquaculture (B.B.P.B.A.P.) in Jepara, Central Java. The test animals in this study were milkfish fry with a weight of 3 – 3.5g from the Center for Brackish Water Cultivation Fisheries (B.B.P.B.A.P.) Jepara. The stocking density for each treatment was ten fish/tub. According to **Pradika *et al.* (2021)**, the stocking density of milkfish is one fish / 3 L of water. The feed was artificial by substituting a fish meal with maggot flour. Fry maintenance was carried out for 35 days. The feeding frequency was three times/day with a fixed feeding rate of 5% of the total biomass **(Herawati *et al.*, 2020a)**.

The study used five treatments, and each treatment was repeated three times; the arrangement of the treatments was as follows:

Treatment A: 100% fish meal.

Treatment B: 30% maggot flour and 70% fish meal.
Treatment C: 35% maggot flour and 65% fish meal.
Treatment D: 40% maggot flour and 60% fish meal.
Treatment E: 45% maggot flour and 55% fish meal.

Parameters

Total Feed Consumption (TFC)

TFC (g) = 1st day of feed (g) + 2nd day of feed (g) + .. + nth day of feed (g) … (1)

Feed Conversion Ratio (FCR)

$$\text{F.C.R.} = \frac{\text{Feed intake (g)}}{\text{Body weight gain (g)}} \quad \ldots (2)$$

Efficiency Feed Utilization (EFU)

$$\text{EFU (\%)} = \frac{\text{Final weight} - \text{initial weight}}{\text{Weight of diet consumed}} \times 100 \quad \ldots (3)$$

Absolute Length (LG)

L.G. (cm) = Final body length (cm) − Initial body length (cm) … (4)

Absolute Weight (WG)

W.G. (g) = Final body weight (g) − Initial body weight (g) … (5)

Specific Growth Rate (SGR)

$$\text{S.G.R. (\% per day)} = \left(\frac{\text{(in weight of biomass at the final} - \text{in weight of biomass at the begin}}{\text{ning) Time of experiment}}\right) \times 100 \quad \ldots (6)$$

Protein Retention

$$\text{P.R.} = \left(\frac{\text{Protein of final weight (g)} - \text{protein of initial weight (g)}}{\text{The total Protein consumed (g)}}\right) \times 100 \quad \ldots (7)$$

Survival Rate

$$\text{S.R.} = 100 \times \left(\frac{\text{Final count}}{\text{Initial count}}\right) \quad \ldots (8)$$

Proximate analysis

The sample's protein, fat, ash, carbohydrate, fiber and water content were determined using a proximate analysis **(A.O.A.C., 2005)**. The Kjeldahl technique was used to analyze the protein content, while the Soxhlet method was used to analyze the fat content. Utilizing gravimetric principles, water and ash contents were assessed. The carbohydrate content was manually determined based on the findings of the approximate analysis.

Amino acid analysis

An HPLC type 1100 system equipped with a Eurosphere 100-5 C18, 2504.6 mm column, and a P/N of 1115Y535 as the original sample were used to conduct the necessary analysis for the amino acid profile. T.H.F.>80:15:5 Fluorescence: Extra: 340 mm Em: 450 nm. After placing about 2.5 grams of the samples inside a closed glass, 15 mL of 6M hydrochloric acid was added to the mixture. The mixture was homogenized by vortexing and then hydrolyzed in an autoclave at 110^{o}C for 12 hours before being cooled to the surrounding air temperature and neutralized with 6M sodium hydroxide. After adding 2.5mL of lead acetate with a 40% concentration and 1mL of oxalic acid with a 15% concentration, 3mL of the mixture was filtered using a 0.45m Millex-HV filter (Merck KGaA, Darmstadt, Germany). To prepare the combination for injection into the HPLC system, the filtered mixture and the OPA anhydrase solution were combined and then incubated for three minutes. After that, the mixture was sent through the high-performance liquid chromatography (HPLC) system **(A.O.A.C. 2005)**.

Fatty acid analysis

Using a QP-2010 Gas Chromatograph - Mass Spectrophotometer (GCMS) (Shimadzu) with a 50m, 0.22mm Wall Coat Open Tubular CP-SIL-88 column, the fatty acid profile was measured (Agilent, Santa Clara, CA, U.S.A.). Analyses were conducted at column temperatures ranging from 120 to 200 degrees Celsius. *In-situ* transesterification was the technique adopted. A volume of 4mL of water was used to homogenize 100mg of the sample. The obtained homogenate (104 mg) was transferred to a test tube. An amount of 100L of methylene chloride and 1mL of 0.5M NaOH in methanol were added. After adding nitrogen, the tubes were sealed firmly and continued by heating for 10 minutes at 90°C and another 10 minutes at the same temperature. After cooling the test tubes, 1mL of 14% BF3 in methanol was added. After bringing the test tubes to room temperature, 1mL of water and 200- 500L of hexane were added. Stirring the mixture for one minute separated the methyl esters from the fatty acids. After centrifugation, the sample's uppermost layer was prepared for G.C. analysis **(A.O.A.C. 2005)**.

Water quality

The Environmental Quality Laboratory of the Center for Brackish Water Aquaculture (B.B.P.B.A.P.) in Jepara, Indonesia conducted tests on ammonia. A Water Quality Checker was used to test temperature, pH, salinity, and dissolved oxygen (D.O.) Temperature, salinity and pH were tested twice daily; oxygen was checked once daily, and ammonia was measured weekly.

Data analysis

The acquired data were evaluated using analysis of variance (ANOVA). A normality, homogeneity and additivity test was conducted to assess whether the data were typical, homogenous and additive. If significant differences were found ($P<0.05$), Duncan's Multiple Area Test was performed to identify the significant differences between sets. The data on water quality were descriptively evaluated.

RESULTS

The artificial feed and the composition of the feed formulation used in this study are presented in Table (1).

Table 1. Composition of feed formulation (% dry weight)

Types of feed ingredient	Composition (%/100 g Feed)				
	A (0%)	B (30%)	C (35%)	D (40%)	E (45%)
Fish flour	30.00	21.00	19.50	18.00	16.50
Maggot flour	0.00	9.00	10.50	12.00	13.50
Shrimp head flour	16.00	16.00	14.87	17.05	16.80
Soy flour	31.00	30.00	30.53	29.00	29.00
Bran flour	14.00	15.00	15.60	14.95	15.20
Fish oil	2.00	2.00	2.00	2.00	2.00
Corn oil	2.00	2.00	2.00	2.00	2.00
Vit min mix	3.00	3.00	3.00	3.00	3.00
CMC	2.00	2.00	2.00	2.00	2.00
TOTAL(%)	100.00	100.00	100.00	100.00	100.00
Protein (%)***	37.28	37.28	37.28	37.28	37.28
NFE (%) ***	20.51	20.35	20.65	19.99	20.03
Fat (%) ***	7.82	7.53	7.47	7.44	7.39
Energy (kkal/g)*	366.36	362.98	363.68	360.62	360.37
Ratio E/P**	9.83	9.74	9.76	9.67	9.67

Note: *It is calculated based on Digestible Energy according to Watanabe (1988), which states that one gram of protein contains 5.6 kcal/g; one gram of carbohydrates contains 4.1 kcal/g, and one gram of fat contains 9.4 kcal/g; **According to De Silva (1987), the E/P value for optimal growth of fish ranges from 8-12 kcal/g; ***Saraswanti Indo Genetech Laboratory, Bogor, West Java, Indonesia (2022).

The analysis of the nutritional quality of feed substituted with maggot flour showed that treatment C had the highest protein content (45.95%) and 21.54% of fat, while treatment A produced the lowest nutritional quality of feed. Table (2) presents the findings of the proximate analysis performed on milkfish feed.

Table 2. Results of proximate analysis of milkfish feed

Treatment	Water (%)	Content in 100% dry matter			
		Ash (%)	Fat (%)	Fiber (%)	Protein (%)
A (0%)	13.28	13.53	18.47	13.37	41.35
B (30%)	13.09	11.45	20.24	11.34	43.67
C (35%)	11.56	10.40	21.54	10.45	45.95
D (40%)	12.21	12.72	19.32	12.51	42.24
E (45%)	13.16	12.18	20.90	10.33	43.43

The highest results of the amino acid profile were in treatment C (5.80% methionine) and the lowest in treatment A (2.48% methionine). The amino acid profile of the feed with different treatments is displayed in Table (3).

Table 3. Amino acid profile of milkfish feed

Amino acid profile (%)	Treatment				
	A (0%)	B (30%)	C (35%)	D (40%)	E (45%)
Aspartic acid	1.20 ± 0.03[a]	2.06 ± 0.06[a]	3.19 ± 0.09[b]	1.98 ± 0.09[a]	2.98 ± 0.04[b]
Serine	1.66 ± 0.06[a]	1.10 ± 0.01[a]	3.03 ± 0.03[b]	1.46 ± 0.09[a]	1.23 ± 0.07[a]
Glutamic acid	1.16 ± 0.05[a]	2.26 ± 0.05[a]	4.19 ± 0.03[b]	2.16 ± 0.08[a]	2.20 ± 0.01[a]
Glycine	1.98 ± 0.04[a]	4.26 ± 0.07[b]	5.03 ± 0.06[b]	2.98 ± 0.05[a]	3.90 ± 0.09[b]
Histidine	3.79 ± 0.10[a]	2.80 ± 0.05[b]	3.90 ± 0.09[a]	3.79 ± 0.11[a]	2.75 ± 0.08[b]
Arginine	2.27 ± 0.03[a]	2.67 ± 0.07[a]	3.36 ± 0.08[b]	3.27 ± 0.02[b]	2.56 ± 0.02[a]
Threonine	1.43 ± 0.02[a]	1.89 ± 0.09[a]	1.78 ± 0.06[a]	3.43 ± 0.04[b]	1.96 ± 0.08[a]
Alanine	2.25 ± 0.09[a]	2.95 ± 0.08[b]	3.20 ± 0.09[b]	2.17 ± 0.03[a]	2.23 ± 0.09[a]
Proline	2.84 ± 0.07[a]	3.75 ± 0.09[b]	4.87 ± 0.07[b]	2.84 ± 0.03[a]	2.92 ± 0.01[a]
Valine	2.73 ± 0.05[a]	4.90 ± 0.04[b]	4.83 ± 0.03[b]	2.73 ± 0.08[a]	3.99± 0.08[b]
Methionine	2.48 ± 0.02[a]	3.99 ± 0.03[b]	5.80 ± 0.05[b]	2.20 ± 0.03[a]	3.48 ± 0.03[a]
Lysine	1.09 ± 0.01[a]	4.20 ± 0.04[b]	5.50 ± 0.03[b]	2.09 ± 0.03[a]	3.98 ± 0.05[b]
Isoleucine	2.16 ± 0.07[a]	3.09 ± 0.02[b]	4.23 ± 0.02[b]	1.16 ± 0.08[a]	3.99 ± 0.03[b]
Leucine	2.12 ± 0.02[a]	3.38 ± 0.01[b]	3.25 ± 0.03[b]	2.23 ± 0.02[a]	3.57 ± 0.01[b]
Phenylalanine	1.06 ± 0.05a	3.18 ± 0.09[b]	3.98 ± 0.01[b]	1.06 ± 0.05[a]	2.96 ± 0.06 [a]

The highest results of E.P.A. was in treatment C (4.97%), while the lowest was 1.05% in treatment A. The fatty acid profile of milkfish feed is shown in Table (4).

Table 4. Fatty acid profile of milkfish feed

Fatty acid profile (%)	Treatment				
	A (0%)	B (30%)	C (35%)	D (40%)	E (45%)
Myristic	0.81 ± 0.05[a]	2.51 ± 0.02[b]	3.68 ± 0.09[b]	1.50 ± 0.05[a]	2.05 ± 0.03[b]
Steraic	1.09 ± 0.06[a]	1.77±0.304[b]	2.25 ± 0.08[b]	1.08 ± 0.02[a]	1.49 ± 0.01[a]
Lauric	2.14 ± 0.09[a]	3.97 ± 0.08[b]	4.79 ± 0.04[b]	2.77 ± 0.01[a]	3.82 ± 0.03[b]
Palmitic	1.71 ± 0.07[a]	1.52 ± 0.03[a]	3.11 ± 0.09[b]	2.08 ± 0.05[b]	2.08 ± 0.02[b]
Oleic/ω9	1.07 ± 0.02[a]	2.89 ± 0.08[b]	3.91 ± 0.01[b]	2.55 ± 0.03[b]	2.55 ± 0.07[b]
Linoleic/ω6	0.83 ± 0.09[a]	3.49 ± 0.07[b]	3.37 ± 0.02[b]	3.75 ± 0.02[b]	3.75 ± 0.08[b]
Linolenic/ω3	0.54 ± 0.05[a]	2.39 ± 0.03[b]	3.32 ± 0.01[b]	2.56 ± 0.07[b]	2.56 ± 0.05[b]
Arachidic	1.30 ± 0.08[a]	2.02 ± 0.04[b]	3.05 ± 0.03[b]	2.25 ± 0.05[b]	2.25 ± 0.04[b]
Arachidonic	0.07 ± 0.02[a]	1.15 ± 0.02[a]	2.13 ± 0.08[b]	0.96 ± 0.08[a]	1.06 ± 0.03[a]
DHA	1.33 ± 0.05[a]	2.87 ± 0.01[b]	3.50 ± 0.03[b]	1.59 ± 0.08[a]	1.93 ± 0.02[a]
EPA	1.05 ± 0.02[a]	3.68 ± 0.02[b]	4.97 ± 0.08[b]	2.33 ± 0.05[a]	3.33 ± 0.01[b]

The results of the growth performance, feed utilization efficiency, protein retention and body composition of milkfish are presented in Table (5).

Table 5. Value of total feed consumption (TFC), feed conversion ratio (FCR), feed utilization efficiency (FUE), absolute length (LG), absolute weight (WG), specific growth rate (SGR), protein retention (PR) and survival (SR) during the study

Variable	Treatments				
	A	B	C	D	E
Total feed consumption (TFC) (g)	59.28±2.24[a]	87.00±0.57[bc]	90.40±1.93[c]	64.40±2.87[a]	82.41±7.66[b]
Feed conversion ratio (FCR)	2.37±0.22[c]	1.54±0.15[a]	1.53±0.21[a]	1.99±0.13[b]	2.31±0.07[bc]
Feed utilization efficiency (FUE) (%)	30.32±2.62[a]	55.36±3.66[d]	57.32±0.75[d]	34.95±4.62[ab]	40.68±4.83[c]
LG (cm)	3.33±0.21[a]	4.37±0.25[bc]	5.33±0.35[c]	3.63±0.21[a]	4.30±0.20[b]
WG (g)	17.98±1.72[a]	48.15±2.90[d]	51.82±1.27[e]	22.45±2.50[b]	33.28±1.16[c]
SGR (%/day)	1.32±0.12[a]	2.52±0.14[d]	2.64±0.05[d]	1.54±0.16[b]	1.93±0.08[c]
PR (%)	75.26[a]	76.57[b]	75.56[a]	75.78[a]	76.44[b]
S.R. (%)	83.33±11.55[a]	86.67±5.77[a]	90.00±310.00[a]	76.67±35.77[a]	96.67±5.77[b]

Note: Values with different *superscripts* showed a significant difference (P<0.05)

Based on Table (5), the best treatment results were obtained in treatment C (35% maggot flour) with the results of TFC, FCR, FUE, absolute length, absolute weight and SGR as follows: 90.40 ± 1.93 g, 1.53 ± 0.21, 57.32 ± 0.75%, 5.33±0.35 cm, 51.82±1.7 grams 2.64±0.05%/ day. Meanwhile, the best protein retention results were found in treatment B with the addition of 30% maggot flour (76.57%), and the best survival rate was in treatment E (96.67±5.77%).

Total feed consumption (T.F.C.)

The result of the graph of the Orthogonal Polynomial test is presented in Fig. (1).

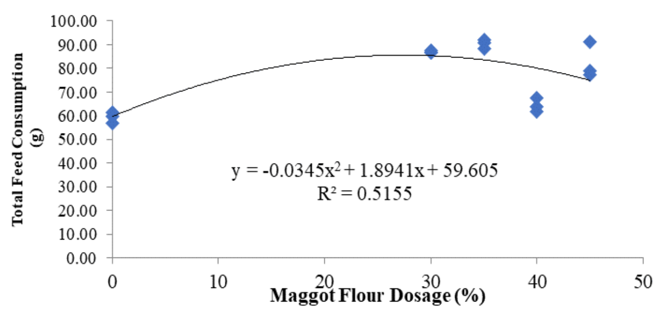

Fig. 1. Relationship between maggot flour substitution in feed with T.F.C. of milkfish (*C. chanos*)

Based on the Orthogonal Polynomial test, a quadratic patterned relationship (y = -0.0345x2 + 1.8941x + 59.605) and R^2 = 0.5155 and the optimum point in treatment C (35% maggot flour in artificial feed) obtained the dose from the equation was 27.45 % able to produce a maximum crime scene of 85.59 grams. The R^2 value indicates that substituting maggot flour influences 51.55% of T.F.C. in artificial feed, and other unknown factors influence 48.45%.

Feed conversion ratio (F.C.R.)

The result of orthogonal polynomials is presented in Fig. (2).

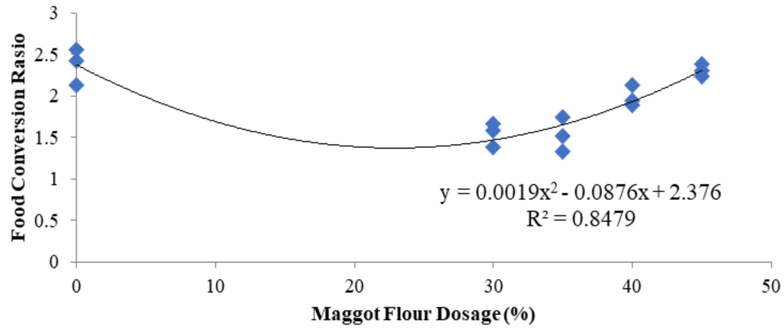

Fig. 2. Relationship between maggot flour substitution in feed with F.C.R. of milkfish (*C. chanos*)

Based on the Orthogonal Polynomial test, a quadratic patterned relationship was obtained (y = 0.0019x2 - 0.0876x + 2.376), and R^2 = 0.8479 and the optimum point in treatment C (substitution of fish flour using 35% maggot flour in artificial feed) obtained the dose from the equation; 23.05% was proved to be capable of producing a maximum F.C.R. of 1.37. The value of R^2 indicates that 84.79% of F.C.R. was influenced by maggot flour in artificial feed, while other factors influenced 15.21%.

Feed utilization efficiency (F.U.E.)

The result of the orthogonal polynomial test is presented in Fig. (3).

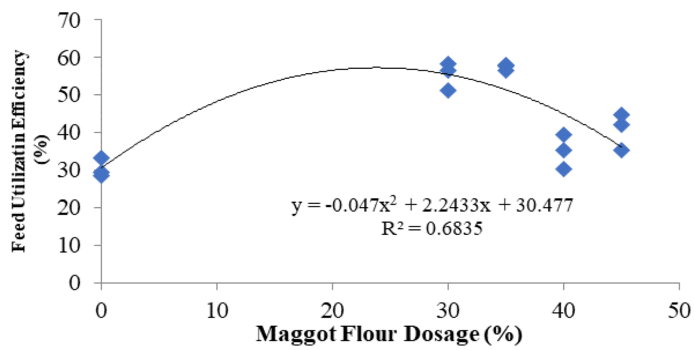

Fig. 3. Relationship between maggot flour substitution in feed and F.U.E. of milkfish (*C. chanos*)

Based on the Orthogonal Polynomial test, a quadratic patterned relationship was obtained (y = -0.047x2 + 2.2433x + 30.477) and R^2 = 0.6835 and the optimum point in treatment C (35% maggot flour in artificial feed) obtained the dose from the equation, which was 23.85 % that was able to produce a maximum F.U.E. of 57.25%. The R^2 value showed that 68.35% of F.U.E. was influenced by maggot flour in artificial feed, and other factors influenced 31.65%.

Absolute length

The result of the orthogonal polynomial test is presented in Fig. (4).

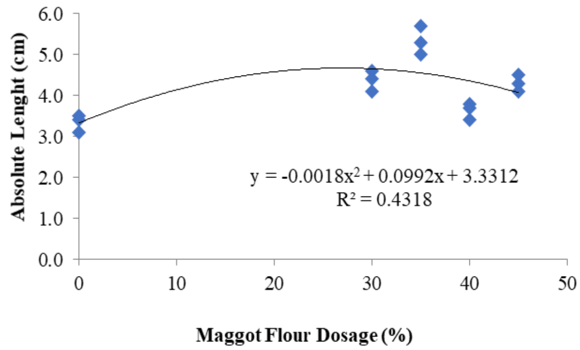

Fig. 4. Relationship between maggot flour substitution in feed with absolute length of milkfish (*C. chanos*)

Based on the Orthogonal Polynomial test, a quadratic patterned relationship (y = -0.0018x2 + 0.0992x + 3.3312) and R^2 = 0.4318 and the optimum point in treatment C (35% maggot flour in artificial feed) obtained the dose from the equation. It shows that 27, 56% was able to produce a maximum absolute length of 4.7cm. The R^2 value showed that substituting maggot flour influenced 43.18% of absolute length in artificial feed, and other factors influenced 56.82%.

Absolute weight

The result of the orthogonal polynomial test is presented in Fig. (5).

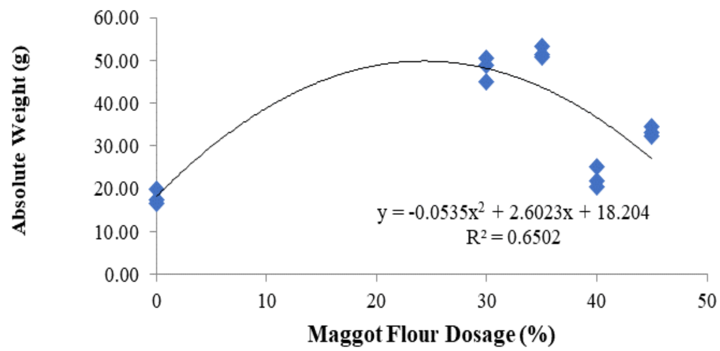

Fig. 5. The relationship between maggot flour substitution in feed with absolute weight of milkfish *(C. chanos)*

Based on the Orthogonal Polynomial test, a quadratic patterned relationship (y = -0.0535x2 + 2.6023x + 18.204) and R^2 = 0.6502 and the optimum point in treatment C (35% maggot flour in artificial feed) obtained the dose from the equation. 24, 32% can produce an absolute maximum weight of 49.85 grams. The R^2 value indicates that substituting maggot flour influences 65.02% of absolute weight in artificial feed, and other factors influence 34.98%.

Specific growth rate (S.G.R.)

The results of the orthogonal polynomial test are presented in Fig. (6).

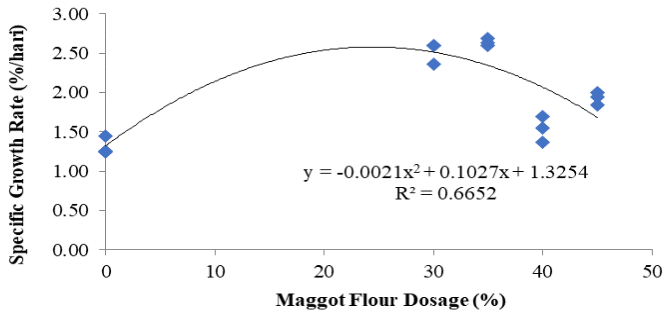

Fig. 6. Relationship between maggot flour substitution in feed and S.G.R. of milkfish (*C. chanos*)

Based on the Orthogonal Polynomial test, a quadratic patterned relationship (y = -0.0021x2 + 0.1027x + 1.3254) and R^2 = 0.6652 and the optimum point in treatment C (35% maggot flour in artificial feed) obtained the dose from the equation. 24.45 % proved its potential to produce a maximum S.G.R. of 2.58%/day. The R^2 value indicates that the substitution of maggot oil influenced 66.52% of S.G.R. in artificial feed, and other factors affected 33.48%

Proximate analysis

Table (6) displays the findings of the proximate analysis performed on milkfish feed. The highest result of the nutritional quality (proximate analysis) of milkfish was treatment C, resulting in 49.95% of protein and 18.54% of fat, while the lowest was treatment A (46.35% of protein and 16.07% of fat).

Table 6. Results of proximate analysis of milkfish with different feeds for 35 rearing days

Treatment	Water (%)	Content in 100% Dry Matter			
		Ash (%)	Fat (%)	Fibre (%)	Protein (%)
A	12.68	11.53	16.07	13.37	46.35
B	11.09	11.45	17.24	11.34	48.67
C	10.56	10.40	18.54	10.45	49.95
D	11.21	11.72	16.32	12.51	47.24
E	12.16	11.18	17.90	10.33	48.43

The highest analysis results of the E.P.A. of milkfish was treatment C (5.07%), while treatment A (1.95% E.P.A.) was recorded the lowest. The result of the amino acid profile of milkfish fed different treatments is presented in Table (7).

Table 7. Amino acid profile of milkfish after being fed

Amino Acid	Treatment				
(%)	A (0%)	B (30%)	C (35%)	D (40%)	E (45%)
Myristic	1.75 ± 0.05 a	2.99 ± 0.02 b	4.08 ± 0.09 b	2.50 ± 0.05a	2.75 ± 0.03 b
Steraic	1.09 ± 0.02 a	1.77±0.304 a	2.25 ± 0.08 b	1.08 ± 0.02a	1.49 ± 0.01 a
Lauric	2.64 ± 0.08 a	4.47 ± 0.08 b	5.15 ± 0.04 b	3.77 ± 0.01a	4.52 ± 0.03 b
Palmitic	1.71 ± 0.07 a	1.52 ± 0.03 a	3.11 ± 0.09 b	2.08 ± 0.05b	2.08 ± 0.02 b
Oleic/ω9	1.07 ± 0.02 a	2.89 ± 0.08 b	3.91 ± 0.01 b	2.55 ± 0.03b	2.55 ± 0.07 b
Linoleic/ω6	1.13 ± 0.09 a	3.49 ± 0.07 b	3.37 ± 0.02 b	3.75 ± 0.02b	3.75 ± 0.08 b
Linolenic/ω3	1.24 ± 0.05 a	2.39 ± 0.03 b	3.32 ± 0.01 b	2.56 ± 0.07b	2.56 ± 0.05 b
Arachidic	1.30 ± 0.08 a	2.02 ± 0.04 a	3.05 ± 0.03 b	2.25 ± 0.05 a	2.25 ± 0.04 a
Arachidonic	1.07 ± 0.02 a	2.75 ± 0.02 b	3.13 ± 0.08 b	1.56 ± 0.08a	2.56 ± 0.03 b
DHA	1.55 ± 0.06 a	3.57 ± 0.01 b	3.96 ± 0.03 b	2.59 ± 0.08a	2.93 ± 0.02 b
EPA	1.95 ± 0.02 a	4.68 ± 0.02 b	5.07 ± 0.08 b	3.33 ± 0.05b	3.93 ± 0.01 b

The amino acid profile analysis showed that treatment C was the best, with milkfish fed 35% of maggot meal containing 7.10% methionine. The results of the fatty acid profile of milkfish fed different treatments are presented in Table (7).

Table 8. Fatty acid profile of milkfish after being fed

Fatty Acid	Treatment				
(%)	A (0%)	B (30%)	C (35%)	D (40%)	E (45%)
Aspartic acid	2.20 ± 0.04 a	2.76 ± 0.05 a	4.19 ± 0.03 b	1.98 ± 0.08 a	2.98 ± 0.02 a
Serine	2.66 ± 0.06 a	3.10 ± 0.04 b	4.03 ± 0.07 b	2.46 ± 0.02 a	2.23 ± 0.05 a
Glutamic acid	2.16 ± 0.09 a	2.26 ± 0.08 a	5.19 ± 0.06 b	2.16 ± 0.05 a	2.20 ± 0.06 a
Glycine	2.98 ± 0.02 a	4.26 ± 0.09 b	5.23 ± 0.05 b	3.98 ± 0.05 b	3.90 ± 0.08 b
Histidine	3.99 ± 0.08 b	2.80 ± 0.04 a	3.95 ± 0.07 b	3.79 ± 0.11 b	2.75 ± 0.03 a
Arginine	2.77 ± 0.02 a	2.67 ± 0.07 a	3.66 ± 0.08 b	3.27 ± 0.02 b	2.56 ± 0.02 a
Threonine	2.43 ± 0.01 b	1.89 ± 0.09 a	1.88 ± 0.06 a	3.43 ± 0.04 b	1.96 ± 0.08 a
Alanine	2.75 ± 0.04 a	2.95 ± 0.08 b	3.25 ± 0.09 b	2.17 ± 0.03 a	2.23 ± 0.09 a
Proline	2.94 ± 0.07 a	3.75 ± 0.09 b	4.97 ± 0.07 b	2.84 ± 0.03 a	2.92 ± 0.01 a
Valine	2.73 ± 0.05 a	4.60 ± 0.04 b	4.85 ± 0.03 b	3.73 ± 0.08 b	3.99± 0.08 b

Water quality

Water quality is one factor that supports fish's growth and survival. The results of water quality are presented in Table (9).

Table 9. Water quality data during maintenance

Variable	Unit	Result	Reference
Temperature	^oC	24.1-26.5	23 – 32*
DO	Mg/L	3.53-5.98	>3**
pH	-	7.9-8.2	8 – 9***
Salinity	ppt	24-27	15-35**

Note: *: Kuang *et al.* (2012), **: Herawati *et al.* (2020a), ***: Andrila *et al.* (2019).

DISCUSSION

Growth performance

Fish growth is an increase in weight and length, which can be seen from weight and length changes caused by the entry of nutrients into the body. The best results were found in treatment C, with the average values of T.F.C., F.C.R., F.U.E., total length, total weight S.G.R., and protein retention: 90.40±1.93 grams, 1.53±0.21, 57,32±0.75%, 5.33±0.35 cm, 51.82±1.7 grams 2.64±0.05%/day, 75.56%, respectively. Fish meal substitution using maggot flour (*H. illucens*) in artificial feed with a dose of 35% (C) was able to optimize the growth of milkfish fry in terms of the observed variables and was thought to be better than other treatments; this was because the protein content of maggot flour ranged between 30 & 50%. According to **Ebeneezar *et al.* (2021)**, the crude protein content in B.S.F. larvae was 41.44 ± 0.17g/ 100g, and crude lipid content was 35.69 ± 0.24g/ 100g. The nutritional content of maggot flour is 40-50% protein and 30% fat **(Herawati *et al.*, 2020b)**. This flour contains antimicrobial and antifungal properties so that if consumed by fish, it will cause resistance to diseases caused by bacteria. In addition, maggots also have a storage organ called trophocytes, which function as storage of nutrient content in the culture media they eat **(Ebeneezar *et al.*, 2021)**. The high feed consumption in treatment C also resulted in increased growth. This took place since the feed in treatment C has a slightly fishy aroma; the texture of the feed is softer than other treatments, thus it attracts the attention of fish to eat the feed.

The specific growth rate (S.G.R.) results showed that the best results were found in treatment C with a dose of 35%. Substitution of maggot flour with an appropriate dose will have a good effect on the cultivar; feed substituted with maggot flour can meet the nutritional needs of milkfish that are kept **(Mulyani & Rangga, 2021)**. The results of the calculation of the growth rate in this study produce different values since the growth rate is directly proportional to the level of feed consumption. Fish feed consumption is also high if the growth rate value is high. The quality and quantity of feed, water quality and internal fish conditions, such as health and stress levels in fish can influence the growth rate of milkfish. According to **Kuang *et al.* (2012)**, the difference in fish-specific growth is thought to be due to the physiological response of fish caused by external conditions (quantity and quality of feed, temperature and water quality) as well as the status of internal fish conditions (health, stress and reproductive status). Protein is a chemical compound needed by the fish body as an energy source and is required for the growth and maintenance of damaged body tissues, the formation of enzymes and steroid hormones. The feed's protein content is the most critical energy source for fish. **Masriah and Alpiani (2019)** explained that providing protein with appropriate levels will increase fish growth.

Substitution of maggot flour in feed for milkfish with different doses has different results. The highest total feed consumption value in the study was found in treatment C with an amount of 35% maggot flour. The total feed consumption in fish is closely related to the availability of sufficient nutrients that can be a source of energy, supporting fish needs. If the total feed consumption is high, it will affect the growth rate of fish. Several factors affect the total consumption of milkfish feed, including the nutritional content in the feed, the amount of feed given and internal factors such as age and size. This is reinforced in the study of **Wang *et al.* (2019)** who elucidated that, the growth rate depends on the amount of feed consumed, the amount of protein in the feed, water quality, and other factors such as heredity, age and endurance in addition to the ability of the fish to utilize feed. The amount of feed consumed is also influenced by palatability (digestibility); the feed's protein and fat content determine the feed's palatability. The increase in feed consumption indicates a good palatability value in feed **(Rolland *et al.*, 2015)**. According to **Rakhfid *et al.* (2020)**, the feed's energy level influences feed consumption. If the energy level of protein exceeds the needs of fish, it will reduce feed consumption, resulting in decreased absorption of other nutrients including protein. Metabolic processes in the fish's body will spur them to consume more feed. The more feed consumed and the more efficient feed used, the more protein will be retained so that fish growth will increase. The increase in the total feed consumption of milkfish given maggot flour at different doses can be due to the longer time the fish individual takes in digesting the feed and the nutrients contained in the feed to meet its needs as well as the ability of the fish to utilize the feed provided, which subsequently increase fish weight. According to **Didyawati *et al.* (2019)**, feed consumption is an organism's preference level for the feed given. The amount of feed consumed and the ability of organisms to utilize feed will affect the growth rate of fish.

The value of feed utilization efficiency is a comparison between the growth obtained during the study and the amount of feed given to milkfish. The highest efficiency value was 57.32±0.75% (treatment C 35% magot flour); this FUE value is good because it exceeds 50%. This percentage is reinforced in the work of **Puspasari *et al.* (2015)** reporting that, good feed efficiency is between 50%-100%. According to **Marwan *et al.* (2022)**, the level of feed consumption is influenced by the energy level in the feed. If the protein energy level is a high feed efficiency value, it indicates an efficient use of feed so that only a few nutrients are remodeled to meet energy needs, and the rest is used for the growth of the fish. The value of the efficiency of feed utilization will be related to the growth rate of milkfish. If the efficiency value of feed utilization is high, then the growth rate obtained is also high for fish use the feed provided efficiently for growth. According to **Adriansyah *et al.* (2022)**, the value of feed efficiency shows how adequate the level is in using feed used by fish for metabolic and growth processes.

The value of the feed conversion ratio is closely related to the feed efficiency value. The higher the feed efficiency value, the lower the feed conversion ratio value. The lower the feed conversion ratio value, the more efficiently the fish utilize the feed provided. Judging from the calculated variables, treatment C (35% maggot flour) gave the best F.C.R. in line with a good FUE value, compared to other treatments. One of the indicators that can affect the difference in the F.C.R. value in each treatment is the quality of feed; if the feed given has good quality, the feed will be effectively utilized by the fish for growth. **Pradika *et al.* (2021)** explained that, the composition of ingredients and protein content in feed according to fish needs would increase feed digestibility. If feed protein digestibility rises, it will increase fish growth.

Different doses of fish meal substituting for maggot flour in the artificial feed will produce different F.C.R. values. In this study, the highest F.C.R. value was not > 3. The F.C.R. value of the milkfish obtained was categorized as good. **Sonavel *et al.* (2020)** stated that, a good feed conversion value is less than 3; the smaller the feed conversion value, the better the feed quality and the increasing growth rate.

Fish meal substitution using maggot flour in artificial feed at 35% produced better growth than treatment without maggot flour. Based on the efficiency value of feed utilization and the specific growth rate in this study, the highest result was recorded with treatment C (35% maggot flour), with values of 57.32±0.75% and 2.64±0.05%/ day. The results of this study are higher than those of **Herawati *et al.* (2020a)** who postulated that, using maggot flour in artificial feed of milkfish at the best dose of 50% can produce a relative growth rate value of 2.34±0.10% and utilization efficiency feed amounted to 27.51±0.77%. The results of the efficiency value of feed utilization in this study were also higher than those of **Hussain *et al.* (2021)**, who used fish meal substituted with maggot flour in the feed made of milkfish, which resulted in the highest feed utilization efficiency value at a dose of 100% maggot flour; the FUE value obtained was 31, 67 ± 2.92%. The lowest F.C.R. value in this study was 1.53±0.21, and the highest feed efficiency value was 57.32±0.75%.

Substitution of the fish meal using maggot flour in artificial feed affects the nutrition of feed. Based on the proximate analysis results, the best treatment was treatment C, which produces 45.95% crude protein, 21.54% crude fat, 10.45% crude fiber, 10.50% ash, and 11.56% water content. The lowest treatment in this study was treatment A (control), which produced 41.35% crude protein, 18.47% crude fat, 13.37% crude fiber, 13.53% ash content and 13.28% water content. It is suspected that the high protein content in artificial feed can optimize growth in milkfish. Protein is one of the nutrients fish need for growth. Protein absorbed by the fish body will help repair damaged cells and can used for daily metabolic processes **(Wicaksono *et al.,* 2018)**. Fish growth occurs when the energy in the feed consumed meets basic needs such as swimming, metabolism and maintenance **(Masriah & Alpiani, 2019)**. The protein requirement for milkfish fry feed size (0.25-4.43 grams) is 30-40% (**Hussain *et al.,* 2021)**. The 0.5-0.8 g milkfish size requires 40% protein **(Herawati *et al.,* 2020a)**. The protein content in the proximate analysis results of this study was excessive but not balanced by the availability of carbohydrate content. Therefore, protein will act as a protein-sparing effect to meet carbohydrate needs so that using protein as an energy source can be minimized through non-protein energy sources, carbohydrates and fats. According to **He *et al.* (2019)**, the process of protein sparing effect by carbohydrates and fats can balance the use of protein as an energy source for metabolic activities and maintenance of the body so that the protein in feed can be used for growth.

Most energy sources for fish are those provided from protein, carbohydrates and fats in the feed. Fish uses most of the energy obtained from the feed in metabolic processes. Metabolic processes require energy, and catabolic processes require oxygen. Thus, fish need energy for maintenance, physical activity and growth **(Hussain *et al.* 2021)**. Growth in the fish's body will occur if there is excess energy from the feed consumed after the basic energy needs have been met, such as breathing, swimming, metabolic processes, and maintenance. The energy requirement for catabolism must be completed first, and the excess energy will be used for anabolism. The extra energy will be used to build new networks resulting in growth **(Masriah & Alpiani, 2019)**. It is anticipated that most fish energy requirements would be covered by non-protein foods such as fat and carbs. If fish can get enough energy from non-protein sources, most protein content will be utilized for development. However, if energy and non-protein foods are lacking, protein will be used as an energy source.

During the study, the nutritional content of milkfish feed with maggot flour substitution for fish meal ranged from 41.35- 45.95% protein, 18.47-21.50% fat, and the best treatment was treatment C. The high nutritional content is likely attributable to the tremendous growth rate in treatment C, resulting from sufficient nutrients and feed quality. The fat supply in the feed may also be utilized as a source of energy and growth. This statement is based on the nutritional content of milkfish; the protein was 46.35-49.95%; the fat was 16.07-18.54%, and the best milkfish nutrition was treatment C. The fair growth rate shows that the feed quality corresponds to the nutritional requirements of fish to boost its nutritional content **(He *et al*., 2019; Herawati *et al*., 2019a; Herawati *et al*., 2019b)**. The fat level required by milkfish is 10- 18% (**Nguyen *et al.*, 2012)**, and fat is a critical nutrient for developing shrimp ovaries **(Andrila *et al.*, 2019)**.

Compared to fish meal substituted with 0% maggot flour (A), which had the lowest methionine yield (2.88%), the amino acid profile of milkfish fed with 35% maggot flour substitution (C) had the highest yield of methionine (7.10%). The production of nucleic acids and tissues, protein, other amino acids (cysteine), and vitamins all need methionine as an essential component (choline). Methionine, along with vitamin B12 and folic acid, works to aid the body in controlling an excessive amount of protein. Milkfish need 3.6% of their food to be composed of methionine to grow.

Methionine is an essential amino acid; one of its functions is to help maintain a healthy equilibrium between the other amino acids in the body. Methionine is an essential component in producing proteins and other physiological functions. According to **Boonyoung *et al.* (2013)**, methionine and cysteine are mammals' primary sources of amino sulfate. However, cysteine is unnecessary since it may be generated from methionine. According to **Rolland *et al.* (2015)**, ribosome synthesis relies on the necessary amino acids carried into the tissue via DNA. The efficiency and quantity of protein synthesis in tissue cells are strongly influenced by the completeness and balance of amino acids circulating in the tissue. The primary determinants of tissue protein synthesis are the completeness and concentration of amino acids entering or being transported into tissue cells. In addition, to kick off the process of protein synthesis, fish need methionine, which may affect the growth of their muscles **(Belghit *et al.*, 2014)**. It has been shown that adding methionine to feed affects both the growth rate and the immune response **(Kuang *et al.*, 2012; Boonyoung *et al.*, 2013; Rolland *et al.*, 2015)**.

The fatty acid profile of feed that was substituted with 35% maggot flour (C) resulted in the highest E.P.A. yield (5.07%), while the yield that was found in the fish meal that was substituted with 0% maggot flour (A) was the lowest (1.95%). The research conducted by **Ebeneezar *et al.* (2021)** showed that the essential fatty acid known as eicosapentaenoic acid (E.P.A.; 20: 5n-3) plays a crucial role in the maintenance of life, especially in growth. Phospholipids, which may be found in cell membranes and brain tissue need the presence of the EPA. When larvae first eat food, they have a neurosomatic index that is exceptionally high; as a result, they need a high (n-3 H.U.F.A.) level to avoid abnormal neuron growth. According to **Henry *et al.* (2015)**, fatty acids are an essential component that cannot be ignored during the feeding of shrimp during the maturation process of their gonads.

Survival rate

Survival rate (S.R.) is the ratio of fish alive after an experiment to the number of fish living at the beginning. The S.R. calculation variable demonstrates that the maggot flour added to artificial feed has a substantial impact ($P > 0.05$), as shown by the S.R. variable. Several internal and environmental variables are believed to influence the survival value of fish over the 30-day research period. Age, size and adaptability to the cultural environment are internal elements that are intrinsic to fish. The external factors might impact the water quality of the medium used for maintenance. If the water conditions do not match the fish being maintained, they will grow agitated and lose their appetite, ultimately leading to their demise. **Pradika *et al.* (2021)** stated that, internal and external variables affect the survival rate—internal variables include parasites, population density and the adaptability of an animal. The physical and chemical qualities of water are external elements that have an impact. The survival rate in the research is deemed satisfactory since it remains within the ideal range. **Islam *et al.* (2020)** stated that, the fish survival value is considered excellent if it exceeds 50%, whereas a value below 30% is deemed poor.

The results showed that the water temperature during the 35- day study was between 24.1- 26.5^{o}C. The water temperature in the research media is still in the optimal range for milkfish culture. According to **Kuang *et al.* (2012)**, the optimum temperature for the growth and survival of milkfish is 24- 31^{o}C, while the optimal temperature for rearing milkfish ranges from 23- 32^{o}C. Water temperature is critical to note; this is because the temperature can affect the metabolic processes of fish and fish growth. If the milk is not suitable, then the metabolic process of the fish will be hampered, and the growth of the fish will be slow as well. This is reinforced in the study of **Rakhfid *et al.* (2020)** who reported that, temperature affects metabolic activity and aquatic biota's life and growth. Water temperature can indirectly affect aquatic biota's life through its influence on the solubility of oxygen in the water. Dissolved oxygen (D.O.) in the culture media during the study ranged from 3.53- 5.98mg/ L. The dissolved oxygen in this study corresponds to the optimal range for milkfish culture.

In comparison, the salinity of the water during maintenance is in the range of 24- 27 ppt. **Herawati *et al.* (2020a)** explained that, milkfish have good growth at 15- 35 ppt salinity and the availability of dissolved oxygen in waters of more than three mg/ l. If dissolved oxygen in aquaculture waters does not meet the needs of milkfish, it will cause inhibition of fish activity, slow growth, and may even cause fish death. **Wicaksono *et al.* (2018)** stated that, oxygen is one of the limiting factors, thus if its availability is insufficient for the needs of aquaculture biota, all biota activities will be hampered. Fish need oxygen for respiration and burning their fuel (food) to produce activities such as swimming, growth and reproduction. Therefore, a lack of oxygen in the water (D.O.) can interfere with fish life, its growth rate and even death. Based on observations during the study, the pH value in milkfish culture ranged from 7.9-8.2. The pH value during the study was still within the optimal threshold for the cultivation and growth of the reared bandage fish. According to **Adriansyah *et al.* (2022)**, milkfish live in pH conditions ranging from 8- 9 because they are suitable for the growth and reproduction of organisms. Water quality is very influential on the growth and survival of farmed fish. pH that is not following the optimal range in milkfish will inhibit the metabolic processes carried out by fish.

CONCLUSION

Fish meal substitution using maggot flour in artificial feed has a significant effect on total feed consumption (TFC), feed utilization efficiency (FUE), feed conversion ratio (FCR), protein efficiency ratio (PER), specific growth rate (SGR), total weight length, absolute length and survival rate (SR). The best dose in this study, which was 35% maggot flour, was able to produce TFC, FCR, FUE, whole length, absolute weight, and SGR: 90.40±1.93 grams, 1.53±0.21, 57.32±0.75%, 5.33±0.35 cm, 51.82±1.7 grams 2.64±0.05%/ day. The best protein retention results were found in treatment B with the addition of 30% maggot flour (76.57%). Based on the nutritional quality analysis, milkfish fed with maggot flour substitution of 35% (C) gave the highest yields of 49.95% protein and 18.54% fat; methionine was 7.10% and 5.07% of EPA.

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