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RESEARCH ARTICLE
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Nutritional Quality, Feed Efficiency and Survival of Barramundi (*Lates Calcarifer*) Fed With Varying Substitution Levels of Black Soldier Fly Larvae Flour

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ABSTRACT

Barramundi (*Lates calcarifer*) is a fish species known for its fast growth, euryhalinity, and adaptability to cultural environments. The quality of feed provided to Barramundi (*L. calcarifer*) can influence the growth rate of the fish and aquaculture production. However, the price of fishmeal is increasing, and its availability is decreasing, mostly due to the increased demand for its supply. The maggot flour derived from Black Soldier Fly (BSF) larvae is known to be an important alternative to fishmeal. This study aimed to investigate the impact of replacing fishmeal in artificial feed with different levels of BSF flour on the survival rate and growth performane of Barramundi fingerlings. The treatments were based on the level of maggot flour per 100g of feed and were: A (0%), B (5%), C (10%), D (15%), and E (20%) maggot flour per 100g of feed. The test subjects were Barramundi fingerlings (n=120 fish), with a mean body weight of 3.69±0.27g and a body length of 6.62±0.21cm. Experimental Treatments were fed to fingerlings of the respective group for 49 days. The findings indicated that substituting fish meal with varying levels of maggot flour significantly influenced total feed consumption (TFC), feed conversion ratio (FCR), protein efficiency ratio (PER), efficiency of feed utilization (EFU), specific growth rate (SGR), and absolute body weight and body length ($P<0.05$). However, treatments had no effect on the survival rate of the Barramundi fish. Treatment E (15% maggot flour/100g feed) exhibited superior overall performance, characterized by optimal values for TFC (113.36±3.66g), FCR (1.40±0.15), PER (1.49±0.07), FUE (66.61±2.92%), SGR (2.67±0.14%/day), absolute weight (8.46±1.30g), absolute length (5.09±1.19cm), and survival rate (93.33±11.55%). Notably, Treatment D (20% maggot flour/100g feed) stood out for its exceptional nutritional profile, boasting the highest protein content (63.75±0.05%), fat content (8.24±0.02%), total essential amino acids (57g/100g protein), and eicosapentaenoic acid content (8.35±0.06g/100g fat). These findings suggest that Treatment D is effective in enhancing growth performance, while Treatment E excels in improving the nutritional quality of the feed.

Keywords: Nutrition, Growth, Feed, BSF larvae.

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INTRODUCTION

Barramundi (*Lates calcarifer*) aquaculture mainly relies on feed, which accounts for 50–60% of production costs (Hassan et al., 2021). Precise management of both the quality and quantity of the feed ingredients is imperative for optimizing growth performance in fish. Fishmeal constitutes an essential component of aquafeed, and is known for its high protein content, balanced amino acid composition, and superior digestibility in fish (Gupta et al., 2020). However, the increasing cost and limited availability of fishmeal have become serious issues (Siddik et al., 2018). Consequently, it is essential to identify alternative protein sources with similar nutritional benefits that meet the

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dietary requirements of farmed fish, are economically viable, are not in competition with the food resources of humans, and are readily accessible.

Black soldier fly (BSF) larvae meal serves as a promising alternative to fishmeal. It has protein contents between 30 and 45%, making it a more economical option compared to the fishmeal (Indriawati et al., 2021). Moreover, the essential amino acid profile of BSF larvae meal closely resembles that of fishmeal (Henry et al., 2015). A comparative analysis of amino acid profiles between fishmeal and BSF larvae meal, based on amino acid composition relative to lysine, indicates that the levels of isoleucine, leucine, threonine, valine, phenylalanine, and arginine are relatively higher in BSF larvae meal than in fishmeal (Magalhães et al., 2017). However, a notable difference lies in the histidine content, which varies significantly between fishmeal and BSF larvae meal. Additionally, the methionine content in BSF larvae meal is relatively lower than in fishmeal. Nonetheless, substituting fishmeal with BSF larvae meal in formulated diets is quite feasible and has been successfully implemented in aquafeed formulations (Arifin et al., 2020).

Numerous studies indicate that BSF larval meal can substitute for fish meal in animal feeds (Weththasinghe et al., 2021; Anggraeni et al., 2024). However, information regarding the application of BSF larvae meal in formulated feeds for marine fish species, particularly Barramundi (*L.calcarifer*), remains limited. Therefore, the present study was carried out to investigate the possibe beneficial impact of substituting BSF larvae meal in fish feed on the growth performane and survival rate of Barramundi fingerlings.

MATERIALS & METHODS

Experimental Animals

Barramundi fingerlings (n=120 fish), with an average body weight of 3.69±0.27g, body length of 6.62±0.21cm, and a stocking intensity of 1 individual/2 L were used in this study. The fish were obtained directly from the Center for Brackishwater Aquaculture (Balai Besar Perikanan Budidaya Laut, BBPBL), Lampung, Indonesia.

Experimental Containers

The experimental units consisted of 15 plastic containers, each measuring 50x30x20cm, and equipped with aeration systems. Each container was filled with 20L of seawater, equivalent to approximately 70% of the total container volume. All containers were thoroughly cleaned before use to remove any soap residues or other contaminants.

Culture Medium

The culture medium used in this study was seawater (>28ppt) obtained from the Center for Brackishwater Aquaculture (BBPBL) in Pesawaran, Lampung, Indonesia. Before use, the seawater was stored, filtered, and ensured to be free from pollutants and contaminants. This treatment was given to 120 fish, each group for 49 days.

Feed Ingredients and Preparation

The experimental diets were meticulously formulated using a diverse array of ingredients, including BSF larvae meal at varying substitution levels, fishmeal, shrimp head meal, rice bran meal, soybean meal, corn oil, fish oil, a vitamin-mineral premix and carboxymethyl cellulose (CMC) as a binder. The feed formulation was based on principle of Haryono et al. (2015). Table 1 and 2 present detailed information on the proximate composition of the feed ingredients and formulation of the experimental diets.

Table 1: Composition of 100g feed (% dry weight) offered to fish of five experimental groups

Material Type	Feed composition (%/100g Feed)				
Feed Compiler	A	B	C	D	E
Fish meal	45.00	42.75	40.50	38.25	36.00
Black Soldier Fly (BSF) larvae meal	0.00	2.25	4.50	6.75	9.00
Shrimp head flour	3.50	4.00	4.50	3.90	4.40
Soybean meal flour	39.50	39.00	38.50	38.60	38.10
Bran flour	3.00	3.00	3.00	3.50	3.50
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
Carboxymethyl Cellulose (CMC)	2.00	2.00	2.00	2.00	2.00
TOTAL (%)	100.00	100.00	100.00	100.00	100.0
Protein (%)	45.88	45.89	45.89	45.88	45.89
Nitrogen-Free Extract (NFE) (%)	19.70	19.53	19.36	19.49	19.32
Fat (%)	7.79	7.73	7.67	7.59	7.53
Energy (kcal/g)	410.94	409.71	408.48	408.25	407.02
Energy-to-Protein (E/P) Ratio	8.96	8.93	8.90	8.90	8.87

Calculated based on Digestible Energy according to Furuichi (1988) for 1g of protein is 3.0kcal/g, 1g of carbohydrate is 2.5 kcal/g and 1g of fat is 8.1kcal/g According to De Silva (1987), the E/P value for optimal fish growth ranges between 8-9kcal/g

Table 2: Proximate Analysis of Feed Ingredients (% dry weight) used in the study

MaterialType	Component (%)						Total
	Water	Protein	NFE	Fat	Fiber	Ash	(%)
Fish meal*	8.65	45.70	12.27	6.18	3.26	23.94	100.00
Black Soldier Fly (BSF) larvae meal**	4.70	52.34	11.17	3.52	9.04	19.23	100.00
Shrimp head flour ***	11.98	30.45	4.69	2.87	5.26	44.75	100.00
Soybean meal flour ****	9.32	50.37	28.58	1.33	2.29	8.11	100.00
Bran flour *	11.48	6.36	30.13	1.59	34.77	15.67	100.00

*: Hernowo et al. (2020); **: Proximate Test Results from the Center for Brackish Water Aquaculture (BBPBAP) Jepara; ***: Sudrajat & Effendi (2002); ****: Wibowo et al. (2018)

Proximate Analysis

Proximate analysis of the experimental diets (Table 2) and fish (Table 5) of different groups was carried out following the methodologies outlined by AOAC (2005). The estimation of crude protein contents in diets was accomplished through multiplying the total nitrogen factor by the crude protein content while accounting for the carbohydrate content.

Amino Acid Profile

For the determination of amino acid profiles in experimental diets (Table 3) and fish (Table 6) from different groups, the mobile phase consisted of 60% Acetonitrile and AccqTag Eluent. A fluorescence detector was used to maintain a flow rate of 1.0mL/min. High-performance liquid chromatography (HPLC) was used to analyze amino acid composition (Waters Corporation, USA).

Fatty Acids Profile

Gas chromatography (GC) was used to determine fatty acids profile in the experimental diets (Table 4) and fish (Table 7) of different groups. A Shimadzu GC-14B machine from Japan was used for this study. This machine had a

Table 3: Results of amino acid analysis of feed offered to Barramundi fish of different treatment groups during the 49-day study

Amino acid (%)	A (0%)	B (5%)	C (10%)	D (15%)	E (20%)
Arginine	1.54±0.04^{a}	1.88±0.09^{a}	2.05±0.09^{a}	2.15±0.06^{b}	2.40±0.07^{b}
Histidine	0.70±0.05^{a}	1.17± 0.03^{a}	1.35±0.08^{a}	1.22±0.08^{a}	1.39±0.09^{b}
Isoleucine	1.58±0.15^{a}	1.95±0.05^{a}	1.70±0.07^{a}	2.20±0.01^{b}	2.41±0.02^{b}
Lysine	2.42±0.09^{a}	2.77±0.07^{a}	3.04±0.09^{a}	3.35±0.09^{b}	3.56±0.05^{b}
Leucine	1.79±0.05^{a}	2.28±0.09^{a}	2.30±0.04^{a}	2.55±0.07^{a}	2.75±0.08^{b}
Methionine	1.12±0.15^{a}	1.16±0.04^{a}	2.25±0.09^{a}	3.24±0.09^{b}	3.40±0.03^{b}
Phenylalanine	1.65±0.09^{a}	1.19±0.07^{a}	2.05±0.06^{a}	2.25±0.07^{a}	2.38±0.01^{b}
Threonine	1.53±0.02^{a}	1.53±0.03^{a}	1.45±0.08^{a}	1.79±0.05^{a}	2.19±0.09^{b}
Tryptophan	0.68±0.09^{a}	1.17±0.02^{a}	2.25±0.09^{a}	1.20±0.08^{a}	1.69±0.05^{b}
Valine	1.55±0.08^{a}	1.84±0.09^{a}	1.35±0.02^{a}	2.10±0.07^{b}	2.25±0.08^{b}

Values with different superscripts in the same row differ significantly ($P<0.05$).

Table 4: Fatty acid profile of feed offered to Barramundi fish of different groups during the 49-day study

Saturated fatty acid	Samples					
	Req	A (0%)	B (5%)	C (10%)	D (15%)	E (20)
Methyl Butyrate	< 0.1	3.82±0.09^{a}	2.65±0.09^{a}	4.78±0.02^{b}	3.82±0.04^{a}	4.75±0.02^{b}
Methyl Hexanoate	< 0.1	3.46±0.04^{a}	3.75±0.08^{a}	4.99±0.09^{b}	3.86±0.08^{a}	4.83±0.08^{b}
Methyl Undecanoate	< 0.1	1.09±0.03^{a}	2.25±0.04^{a}	3.47±0.08^{b}	3.09±0.02^{a}	3.70±0.03^{b}
Methyl Laurate	0.23	1.83±0.08^{a}	3.70±0.07^{a}	3.82±0.05^{b}	4.83±0.08^{a}	6.80±0.06^{b}
Methyl Tridecanoate	0.89	0.63±0.05^{a}	0.88±0.02^{a}	2.66±0.05^{b}	0.88±0.05^{a}	2.76±0.02^{b}
Methyl Pentadecanoate	2.27	1.59±0.06^{a}	1.93±0.08^{a}	3.52±0.02^{b}	1.89±0.04^{a}	3.62±0.06^{b}
Methyl Palmitate	0.73	3.85±0.03^{a}	3.93±0.04^{a}	4.09±0.02^{b}	3.85±0.02^{a}	4.99±0.05^{b}
Methyl Heptadecanoate	0.97	1.28±0.09^{a}	2.80±0.03^{a}	3.15±0.09^{b}	3.28±0.09^{a}	4.40±0.04^{b}
Methyl Arachidate	4.15	3.73±0.08^{a}	3.89±0.03^{a}	4.65±0.05^{b}	4.37±0.05^{a}	4.90±0.07^{b}
Methyl Tricosanoate	1.26	1.35±0.06^{a}	1.93±0.05^{a}	2.09±0.07^{b}	1.85±0.02^{a}	2.39±0.02^{b}
Unsaturated Fatty Acid						
Linoleic	< 0.1	1.08±0.01^{a}	2.07±0.06^{b}	2.67±0.09^{b}	4.98±0.06^{a}	5.87±0.02^{b}
Linolenic	< 0.1	0.72±0.09^{a}	2.15±0.09^{a}	2.59±0.05^{a}	3.22±0.02^{a}	4.98±0.04^{b}
Erucate	2.93	1.63±0.09^{a}	2.62±0.02^{a}	3.05±0.04^{b}	2.83±0.07^{a}	3.17±0.08^{b}
Eicosapentaenoic	0.93	2.06±0.03^{a}	2.97±0.03^{a}	4.55±0.06^{a}	4.17±0.05^{a}	4.10±0.06^{b}
Docosahexaenoic	< 0.1	2.74±0.07^{a}	3.08±0.09^{a}	4.96±0.09^{b}	3.74±0.02^{a}	4.94±0.09^{b}

Values with different superscripts in the same row differ significantly ($P<0.05$).

Table 5: Results of proximate analysis of Barramundi fish of different treatment groups

Treatment	Protein (%)	Carbohydrate (%)	Crude fat (%)	Ash (%)	Crude fiber (%)
A (0%)	50.26±0.05	18.55 ± 0.04	5.04±0.04	20.52±0.02	5.63±0.06
B (5%)	53.67±0.05	14.25±0.08	6.40±0.01	20.08±0.04	5.60±0.04
C (10%)	54.74±0.04	14.87±0.07	6.89±0.03	18.31±0.05	5.19±0.02
D (15%)	58.65±0.03	13.07±0.03	6.57±0.05	15.93±0.01	5.78±0.04
E (20%)	63.75±0.05	12.28±0.08	8.24±0.02	11.28±0.05	4.45±0.01

Table 6: Amino acid profile of Barramundi fish of different treatment groups

Amino Acid (%)	A (0%)	B (5%)	C (10%)	D (15%)	E (20%)
Arginine	4.03±0.08	4.10±0.03	4.17±0.06	4.23±0.01	4.53±0.04
Histidine	0.99±0.05	1.89±0.03	0.69±0.05	2.06±0.08	9.04±0.02
Isoleucine	3.66±0.07	3.88±0.02	3.48±0.03	3.52±0.03	3.69±0.06
Leucine	4.78±0.026	4.99±0.04	4.67±0.0	5.07±0.06	5.25±0.02
Lysine	10.23±0.08	10.99±0.07	11.25±0.04	12.65±0.05	14.57±0.08
Methionine	7.50±0.09	7.98±0.09	7.77±0.03	8.05±0.01	1.52±0.03
Phenylalanine	2.45±0.04	2.16±0.07	1.36±0.05	1.36±0.02	1.96±0.09
Threonine	8.85±0.07	8.77±0.09	8.61±0.07	8.96±0.05	8.07±0.04
Cystine	2.85±0.08	2.05±0.02	2.75±0.08	2.80±0.03	2.87±0.05
Valine	2.19±0.05	2.13±0.05	2.23±0.04	2.30±0.05	2.43±0.03
Alanine	10.12±0.02	10.57±0.08	10.52±0.02	10.60±0.02	11.06±0.09
Aspartic acid	6.52±0.09	6.89±0.03	7.22±0.06	6.99±0.03	6.52±0.01
Glutamic acid	10.13±0.07	9.85±0.08	10.25±0.02	10.57±0.06	11.49±0.09
Glycine	6.95±0.04	6.90±0.05	6.75±0.09	6.96±0.03	7.60±0.05
Serine	4.23±0.05	4.35±0.09	4.65±0.04	4.88±0.05	4.23±0.09
Tyrosine	5.97±0.09	5.17±0.05	5.88±0.07	6.27±0.03	5.07±0.02
Total EAA	50.45	51.74	53.00	53.73	57.00
Total NEAA	41.00	41.13	41.44	43.54	42.90
Total AA	91.45	92.67	94.68	97.27	99.90

flame ionization detector and a capillary column. This was followed by the AOAC 2005 standards. Table 4 shows the fatty acids in Barramundi fish diets fed over 49 days.

Feed Utilization Efficiency (FUE)

The calculation of feed utilization efficiency of fish of different groups was based on the following formula suggested by Zonneveld et al. (1991):

$$\mathrm{FUE} = \frac{\mathrm{W_t} - \mathrm{W_0}}{\mathrm{F}} \text{ x } 100\%$$

Where:

FUE: Feed Utilization Efficiency; Wt: Final fish weight (g); W0: Initial fish weight (g); F: Total amount of feed consumed.

Table 7: Fatty acid profile of Barramundi fish of different treatment groups

Fatty Acids (%)	A	B	C	D	E
SAFA					
10:0	0.70±0.07	0.78±0.04	0.88±0.02	0.52±0.03	0.78±0.01
11:0	2.39±0.01	2.97±0.02	2.87±0.04	3.10±0.01	2.17±0.05
12:0	0.41± 0.02	0.36±0.02	0.46± 0.03	0.37±0.03	0.31± 0.03
13:0	0.41±0.06	0.91±0.03	0.81±0.06	0.56±0.06	0.40±0.02
14:0	6.16±0.06	5.92±0.03	6.92±0.05	0.69±0.01	0.55±0.02
15:0	9.76±0.05	11.50± 0.07	11.57±0.06	8.12±0.06	21.08±0.08
16:0	20.48±0.02	20.45±0.05	20.78±0.01	24.83±0.02	30.04±0.06
17:0	14.15±0.05	15.80±0.08	17.80±0.05	16.10±0.05	14.51±0.02
18:0	6.99±0.04	4.60±0.03	2.60±0.02	11.80±0.06	0.91±0.05
20:4	2.43±0.02	2.72±0.03	2.65±0.03	2.22±0.04	0.83±0.04
MUFA					
14:1	5.44±0.03	4.19±0.03	4.19±0.01	2.33±0.02	1.89±0.01
15:1	0.24±0.02	1.39±0.09	1.45±0.08	1.78±0.01	3.32±0.04
16:1	8.58±0.01	9.05±0.02	9.05±0.02	8.02±0.02	6.22±0.03
18:1	4.16±0.06	4.56±0.04	4.32±0.03	4.37±0.06	0.77±0.02
18:2c	6.46±0.01	2.77±0.02	2.77±0.03	2.12±0.03	1.04±0.02
PUFA					
18:3ω3	4.21±0.02	4.63±0.04	4.48±0.02	4.91±0.07	6.83±0.02
20:5ω3	7.04±0.03	7.41±0.03	7.41±0.01	8.15±0.02	8.35±0.06
Σ SFA	63.87	66.00	66.33	68.32	71.58
Σ MUFA	34.68	30.98	30.74	27.99	22.27
Σ PUFA	1.45	3.02	2.93	3.69	6.15

Protein Efficiency Ratio (PER)

The calculation of the protein efficiency ratio (PER) was carried out by the following formula from Tacon (1987):

$$PER = \frac{\mathrm{W_t} - \mathrm{W_0}}{Pi} \times 100\%$$

Where:

PER: Protein Efficiency Ratio (%); Wt: Final total weight of sample (g); W_0: Initial total weight of sample (g); Pi: Weight of feed consumed x % of feed protein (g).

Absolute Weight Gain

The absolute weight gain value for fish of experimental groups was calculated based on the Effendie (1997) formula given below:

$$W = W_t - W_0$$

Where:

W: Absolute weight gain (g); Wt: Final biomass weight (g); W0: Initial biomass weight (g).

Absolute Length Gain

The absolute length gain value for fish of experimental groups was calculated based on the following formula (Effendie, 1992):

$$Lm = L_t - L_0$$

Where

Lm: Absolute length growth (cm); Lt: Final length of fish (cm); L0: Initial length of fish (cm).

Specific Growth Rate (SGR)

According to Weatherley and Gill (1987), the Specific Growth Rate for fish of different groups was calculated using the following formula:

$$SGR \frac{\ln W_t - \ln W_0}{t} \text{ x } 100\ \%$$

Where:

SGR: Specific growth rate (%/day); Wt: Final total weight of sample (g/individual); W0: Initial total weight of sample (g/individual); t: Duration of the study (day).

Food Conversion Ratio (FCR)

According to Effendie (1997), the food conversion ratio for fish of experimental groups was calculated using the following formula:

$$FCR = \frac{F}{(W_t - W_0 + D)}$$

Where:

FCR: Food conversion ratio; Wt: Final biomass weight (g); W0: Initial biomass weight (g); F: Amount of feed consumed (g); D: Fish biomass weight that died during experiment (g).

Survival Rate (SR)

According to Effendie (1997), survival rate of fish of different groups was calculated in percentage by using the following formula:

$$SR = \frac{N_t}{N_0} \text{ x } 100\%$$

Where:

SR: Survival rate (%); Nt: Final count of fish at the end of the study; N0: Initial quantity of fish.

Total Feed Consumption (TFC)

According to Weatherly (1972), total feed consumed by fish of experimental groups was calculated using the following formula:

$$F = C - S$$

Where:

F: Feed consumption (g); C: Feed given (g); S: Remaining feed (g).

Water Quality Monitoring

Water quality parameters such as salinity, pH, and temperature were monitored weekly prior to feeding, either in the morning or in the afternoon.

Data Analysis

The data analysis was performed using IBM SPSS Statistics software. Mean±SD values were computed for different parameters of each experimental group. In order to see the magnitude of variation in these parameters among various groups, the data were subjected to ANOVA ($P<0.05$), followed by Duncan's multiple range test to compare different means, where necessary. The water quality was described and compared to standard references to determine suitability.

RESULTS & DISCUSSION

Fig. 1 presents the total amount of feed consumed by Barramundi fish during the study and shows significant differences among different groups ($P<0.05$). The highest TFC by Barramundi fish was recorded in Treatment E (20% BSF larvae meal per 100g of feed), amounting to 113.36±3.66g, whereas the lowest was observed in Treatment B (5% BSF larvae meal per 100g of feed), at 80.50±4.16g.

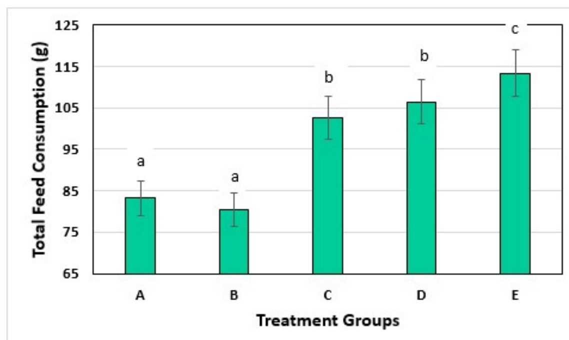

Fig. 1: Total Feed Consumption of Barramundi fish of different treatment groups. Values with different superscripts differ significantly ($P<0.05$).

Based on the feed utilization efficiency data by Barramundi fish during the study (Fig. 2), a significant difference was recorded among groups ($P<0.05$). Fish under Treatment E (20% BSF larvae meal /100g of feed) had the highest efficiency value of 66.61±2.92. In contrast, Treatment A (0% BSF larvae meal /100g of feed) had the lowest efficiency of 42.93±3.51.

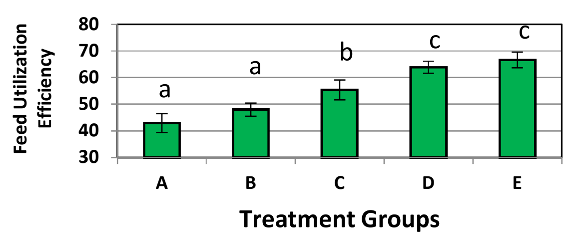

Fig. 2: Feed Utilization Efficiency of Barramundi fish of different treatment groups; Values with different superscripts differ significantly ($P<0.05$).

Fig. 3 illustrates the FCR of Barramundi fish of different groups during the study and shows that there was significant effect of treatments on FCR values (P<0.05). Further analysis revelaed that Treatment E (20% BSF larvae meal/100g of feed) showed the most favorable FCR value (1.40±0.15), while Treatment A (0% BSF larvae meal/100g of feed) had the least favorable value of FCR (1.88±0.04).

Fig. 4 depicts the SGR of Barramundi fish of five treatment groups and shows statistically significant group effects (P<0.05). It can be observed that Treatment E (20% BSF larvae meal/100g feed) exhibited the highest SGR value of 2.67±0.14%, while Treatment A (0% BSF larvae meal/100g feed) had the lowest SGR value (1.64±0.10%). As shown in Fig. 5, the PER was highest (1.49±0.07) in Treatment group E (20% BSF larvae meal/100g feed), while the lowest PER (1.08±0.09) was in Treatment group A (0% BSF larvae meal/100g feed), the effect of treatments on PER was significant (P<0.05).

When data on absolute weight was considered, statistical analysis revealed significant effects of treatments on the absolute weight of fish (Fig. 6). Further analysis indicated that absolute weight for fish of groups C, D and E was higher compared to fish of groups A and B (P<0.05). Fish under Treatment E (20% BSF larvae meal/100g of feed) showed the highest absolute weight (8.46±1.30g), while those under Treatment B (5% BSF larvae meal/100g of feed) had the lowest weight (5.06±0.42g). Similarly, analysis for the data on absolute body length (Fig. 7) revealed significant differences among treatments (P<0.05). Fish of Treatments C, D and E exhibited significantly higher body length compared to fish in Treatments A and B (P<0.05). Further analysis showed the highest absolute body length (5.09±1.19cm) for fish in Treatment E (20% BSF larvae meal/100g feed) and the lowest value (1.94±0.34cm) for fish in Treatment B (5% BSF larvae meal/100g feed).

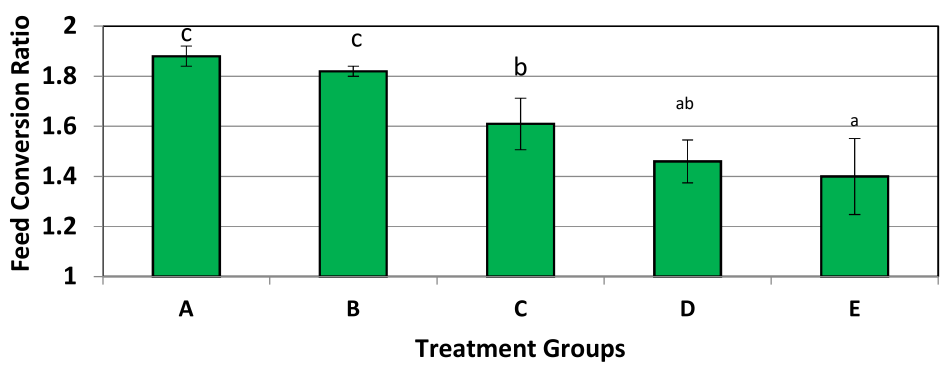

Fig. 3: Feed Conversion Ratio of Barramundi fish of different treatment groups; Values with different superscripts differ significantly (P<0.05).

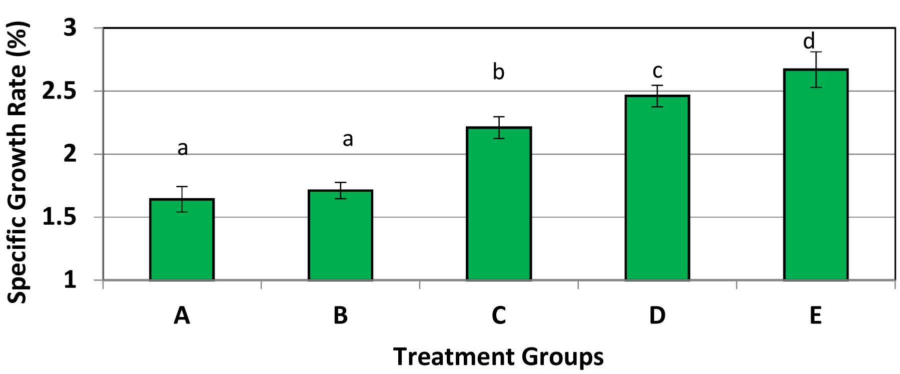

Fig. 4: Specific Growth Rate of Barramundi fish of different treatment groups; Values with different superscripts differ significantly (P<0.05).

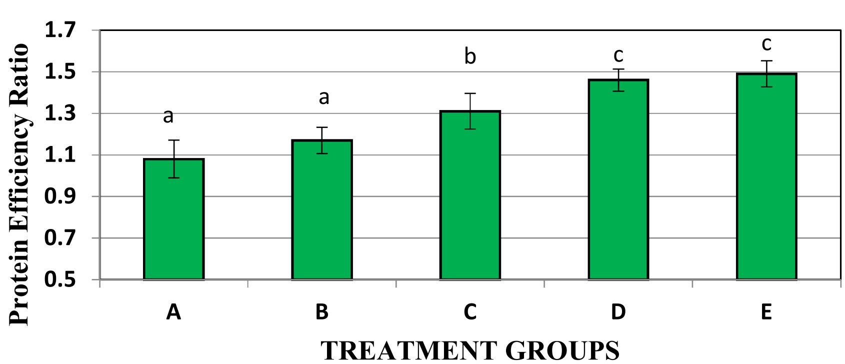

Fig. 5: Protein Efficiency Ratio of Barramundi fish of different treatment groups; Values with different superscripts differ significantly (P<0.05).

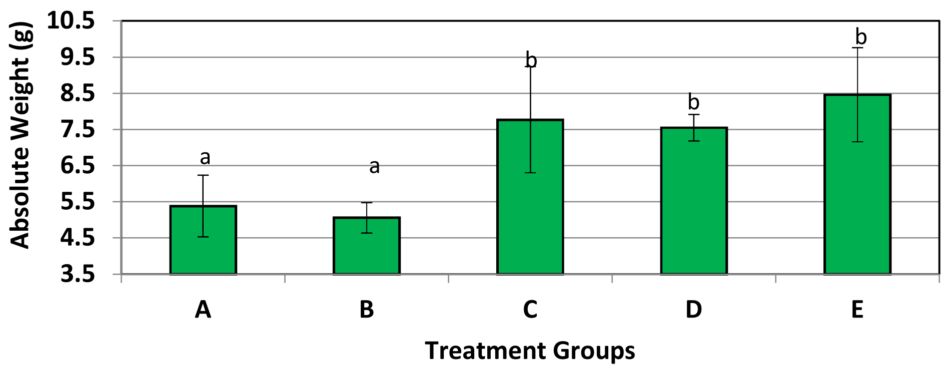

Fig. 6: Absolute Weight of Barramundi fish of different treatment groups; Values with different superscripts differ significantly (P<0.05).

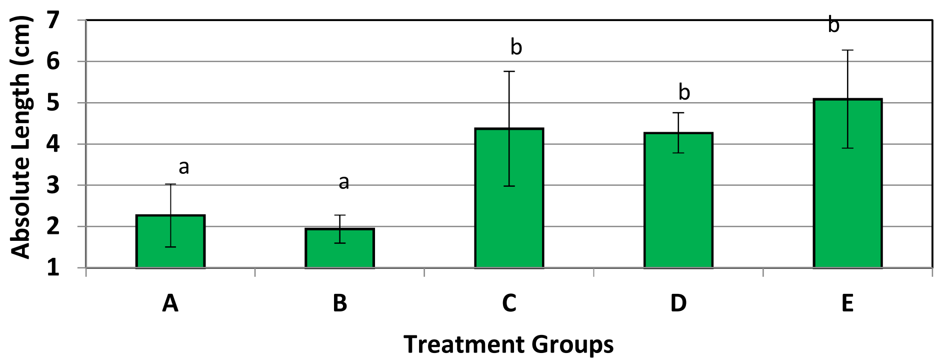

Fig. 7: Absolute Length of Barramundi fish of different treatment groups; Values with different superscripts differ significantly (P<0.05).

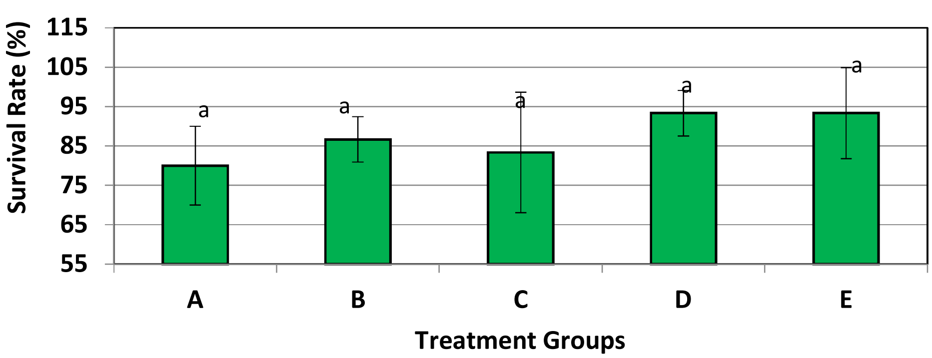

Fig. 8: Survival Rate of Barramundi fish of different treatment groups; Values with similar superscripts differ non-significantly (P>0.05).

The effects of different treatments on survival rate of fish are presented in Fig. 8, which shows statistically non-significant effects of treatments. The numerically highest survival rate (93.33±11.55%) was observed in Treatment E (20% BSF larvae meal/100g feed), while the lowest value (80±10%) was seen in fish of Treatment A (0% BSF larvae meal/100g feed), the difference was statistically non-significant.

The results of proximate composition of Barramundi fish kept of different experimental treatments over the period of 49 days are shown in Table 5. Based on the results, there was a difference in the nutritional contents, with the best protein content (63.75±0.05%) being shown by Barramundi fish in Treatment E (20% BSF larvae meal/100g feed), while the lowest value (50.26±0.05%) was seen in Treatment A (0% BSF larvae meal/100g feed).

Fat content followed a similar trend, with the highest fat in Treatment E (8.24±0.02%) and the lowest in Treatment A (5.04±0.04%). However, an opposite trend was seen for carbohydrates, ash and crude fibre contents. Furthermore, amino acid profiles for Barramundi fish of different treatments are presented in Table 6. The total essential amino acid content was highest in Treatment E (57.00g/100g protein), and lowest (50.45g/100g protein) was in Treatment A. Moreover, analysis of fatty acid profiles of Barramundi fish revealed 20% BSF larvae meal substitution treatment (Treatment E) exhibited the highest EPA value of 8.35±0.06%, while the lowest EPA value (7.04±0.03%) was recorded in fish of the control group (Table 7).

In the present study, statistical analysis of the data through the analysis of variance revealed that substituting fish meal with high concentrations (10, 15 and 20%) of black soldier fly larvae meal (BSF) significantly increased (P<0.05) total feed intake (TFI), PER, FUE, SGR, absolute weight, and absolute length and improved FCR in Barramundi fish fingerlings. However, it did not significantly affect the survival rate of Barramundi fish fingerlings.

The best performance was observed in Treatment E (with a 20% substitution), which showed the best performance across key parameters: TFC (113.36±3.66g), FCR (1.40±0.15), PER (1.49±0.07), FUE (66.61±2.92%), SGR (2.67±0.14%/day), absolute weight (8.46±1.30g), and absolute length (5.09±1.19cm). This superior performance can be attributed to the higher feed utilization efficiency (FUE), better FCR and increased TFC; all these parameters are influenced by the nutritional quality and palatability of the diet, which aligned well with the nutritional requirements of Barramundi fingerlings. The high feed intake observed in Treatment E led to enhanced growth performance, most likely due to the distinctive and more pungent aroma of the feed, which acted as an attractant and stimulated feeding behavior. According to Raharjo et al. (2014), BSF larval meal is a potential alternative to animal protein sources and functions effectively as a feed attractant. Feed intake is also influenced by feed palatability, which is determined by the protein and lipid contents of the diet.

Henry et al. (2015) emphasized that the palatability of BSF larvae meal-based feeds can vary depending on potential chemical or microbiological contamination, antinutritional factors, flavonoids, and terpenoids in the larvae. Increased feed intake reflects higher feed palatability (Putra et al., 2020). Palatability is influenced by various physical and chemical characteristics of feed, including shape, size, taste, aroma, and color. Hakim et al. (2022) further confirmed that these physical characteristics largely determine the palatability and digestibility of fish feed. Ultimately, fish growth is closely linked to the nutritional composition of the feed provided.

In the fish of Treatment E, which involved a 20% substitution of BSF larvae meal per 100g of feed, the feed utilization efficiency (FUE) was higher than in all other treatments, except Treatment D (15% substitution of BSF larvae meal). This is presumably due to the higher nutrient digestibility of the diet in this treatment, allowing the nutrients to be optimally utilized as a high-quality nutritional intake, thereby improving growth performance. According to Royani et al. (2022), increased nutrient digestibility enhances nutrient intake and utilization, supporting improved growth performance. An increase in FUE indicates that the consumed feed was of high quality and was efficiently utilized by the cultured Barramundi.

Higher dietary protein content is correlated with improved nutrient digestibility and enhanced feed efficiency. Shofura et al. (2017) also reported that feed digestibility is directly proportional to feed utilization efficiency; thus, higher feed digestibility leads to better nutrient absorption and higher feed efficiency. A high feed utilization efficiency recorded in this study indicates that the feed provided to Barramundi fingerlings was effectively converted into fish biomass. Similarly, Wulandari et al. (2021) emphasized that higher feed efficiency reflects more effective nutrient use by the fish, resulting in increased muscle mass and more efficient feed utilization. The FUE value obtained in the present study was considered satisfactory, with the highest efficiency reaching 66.61%. According to Puspasari et al. (2015), a feed utilization efficiency above 50% is considered acceptable, with values approaching 100% are taken as optimal.

The Barramundi fish subjected to treatment E (feed substituted with 20% BSF larvae meal per 100g) had a significantly lower FCR ($P < 0.05$) than those in treatments A (control), B (5% BSF larvae meal), and C (10% BSF larvae meal). This is the result of substituting fishmeal with maggot meal in formulated feeds at different inclusion levels, which results in varying FCR values. According to Raharjo et al. (2014), the optimal inclusion level of BSF larvae meal in formulated fish diets to achieve a favorable FCR is 19.01%. Similarly, in European seabass, substituting fishmeal with maggot meal at 50% level resulted in an FCR of 1.4. Abdel et al. (2020) also found that BSF larvae meal may be substituted up to 50% of fishmeal protein in diets for European seabass for delivering high-quality protein.

The feed conversion ratio (FCR) is closely related to feed utilization efficiency. Setiawati et al. (2013) emphasized that the lower the FCR value, the higher is the feed efficiency, which means that fish utilize consumed feed more effectively for growth. Furthermore, FCR is influenced by factors such as mortality rate and the ability of fish to convert feed into biomass. According to Mengistu et al. (2020), mortality rate, individual variation in feed conversion efficiency, and environmental conditions in aquaculture systems are the primary factors affecting FCR in fish.

Moreover, FCR serves as an important indicator of feed quality, reflecting the farmer's proficiency in feed management and the cost-effectiveness of feed use (Putri et al., 2019). A lower FCR corresponds to reduced feed costs, thereby increasing farmers' profitability. The FCR values obtained in this study can be considered favorable due to their low magnitude. Simamora et al. (2021) stated that a lower FCR indicates higher feed quality, while a higher FCR suggests inferior feed quality. According to Mubaraq et al. (2021), FCR values below 3.00 are considered efficient in fish culture.

Protein in fish feed plays a primary role in promoting growth. It is essential for tissue repair, body maintenance, and enhancing growth performance. For fish, protein contents in the feed also serve as the principal source of energy (Tabun et al., 2021). In the present study, the highest PER of 1.49±0.07 was recorded in treatment E. In European seabass, substituting fishmeal with BSF larvae meal at 19.5% resulted in a PER of 1.27% (Magalhães et al., 2017). The energy obtained from the feed is first digested before it can be utilized in metabolic processes. Digestion is a continuous process in fish, beginning with feed intake. According to Fitriliyani (2011) digestion entails the hydrolysis of proteins into amino acids, carbohydrates into simple sugars, and lipids into glycerol and fatty acids. These physical and chemical processes of digestion are essential for fish metabolism.

Proteins present in the feed are metabolized within the fish body through anabolic processes (synthesis of amino acids) and catabolic processes (breakdown of amino acids). The efficiency of protein metabolism, encompassing both physical and chemical processes, improves with increased feed protein digestibility, thereby enhancing fish growth. Marwan et al. (2022) also noted that when the feed composition and protein content meet the nutritional requirements of fish, feed digestibility is improved, promoting the fish growth.

Proteins are key nutrients required for fish growth. Proteins help to repair the damaged cells and support daily body functions (Wicaksono et al., 2018). Fats and carbohydrates provide energy, so fish do not use proteins for energy. This allows more proteins to be used for growth. According to Munisa et al. (2015), fats and carbohydrates help balance protein use, letting more proteins to be used for growth. In the present study, the specific growth rate (SGR) was different in each treatment, with Treatment E had the highest SGR of 2.67±0.14% per day. For freshwater fish Tengadak (*Barbonymus schwanenfeldii*), replacing fishmeal with BSF larvae meal at the level of 20% resulted in an SGR of 3.36% per day. Raharjo et al. (2014) found that the best amount of BSF larvae meal in feed for growth is 19.23%. For Atlantic salmon, using 12.5% BSF larvae meal instead of fishmeal in the diet resulted in an SGR of 2.24%/day (Weththasinghe et al., 2021).

The specific growth rate (SGR) of fish mainly depends on the nature and amount of proteins present in their food. Under optimum level of proteins in the diet, fish can efficiently use the nutrients for energy and growth (Raharjo et al., 2014). An increase in SGR is directly proportional to feed utilization efficiency (FUE); thus, higher FUE typically corresponds to a higher growth rate. Isnawati et al. (2015) also confirmed that higher growth rates are associated with higher feed efficiency. The growth of Barramundi fingerlings depends on several factors. These include the quality and amount of food they receive, the quality of water, and their health and stress levels. The factors present inside and outside the fish can also affect their growth. Internal factors originate within the fish, such as how well they can digest the food. External factors include the environment and quality of food. The most important internal factor is how well the fish can digest and use food to grow. According to Seran et al. (2020), slow growth can result because of the fish ability to digest and use food for gaining weight, and external conditions like poor food that does not have all the necessary nutrients.

The proteins present in BSF larvae meal have already been broken down into simpler compounds, amino acids, that can be more readily absorbed by the fish. The BSF larvae meal contains glutamic acid, which can support digestive metabolism. Azizah et al. (2019) have demonstrated that glutamic acid in feed can enhance metabolism in the digestive system. Furthermore, Pereira et al. (2017) showed that glutamic acid and arginine can improve fish growth. BSF larvae meal also contains amino acids that promote growth. Arifin et al. (2020) have reported that BSF larvae meal is rich in essential amino acid lysine, which optimizes the utilization of other amino acids, thereby increasing the amount of proteins available for fish growth.

The highest absolute body length (5.09±1.19cm) and weight gain (8.46±1.30g) results in Barramundi fingerlings were observed in Treatment E. Increases in body length and weight are influenced by weight gain, which is directly related to feed efficiency. As daily weight gain increases, the feed provided is utilized more efficiently for growth, thereby improving feed efficiency (Balqis, 2021). Regular feeding frequency also plays a crucial role in influencing fish growth. According to Khairul (2017), appropriate feeding frequency combined with high nutrient content in the feed can enhance the length growth of Asian seabass juveniles (*Lates calcarifer)*. Increased feeding frequency generally results in greater growth due to sufficient energy intake. Proteins are the primary energy source for fish growth, as they constitute the second-largest component of the fish body (after water), accounting for approximately 60–70% of the body composition.

Fish growth rate depends on the energy available in the feed and how efficiently the available energy is allocated for maintenance and growth processes (Karimah et al., 2018). In the present study, substitution of fishmeal with BSF larvae meal at a 20% inclusion level in formulated feed resulted in better growth performance, feed efficiency and protein utilization than other treatments. Specifically, it achieved a growth rate of 2.67±0.14% per day, feed conversion ratio of 1.40±0.15, and protein efficiency ratio of 1.49±0.07. These findings surpass those of previous studies. Weththasinghe et al. (2021) reported a growth rate of 2.12–2.24% per day for Atlantic salmon utilizing BSF larvae meal. Similarly, Abdel et al. (2020) observed a feed conversion ratio of 1.41–1.44 for European seabass with BSF larvae meal, while Magalhães et al. (2017) recorded a protein efficiency ratio of 1.27–1.38 for the same species. The survival rate, defined as the proportion of fish remaining alive at the conclusion of the study compared to the initial count, was highest in Treatment E (93.33±11.55%), whereas Treatment A exhibited the lowest survival rate (80.00±10%), though the difference was statistically non-significant. Throughout the study, the water quality of the Barramundi fingerlings was consistently maintained at an optimal level.

The survival rate of fish is influenced by a variety of factors, including intrinsic factors, such as fissh age, body size, and the ability to adapt to the culture environment. In Treatment E, the feed composition included 47.55% protein and 14.23% fat, surpassing the nutritional requirements of 45% protein and 10% fat for Barramundi fingerlings. Protein and fat in the feed are essential for the structural and functional development of fish, contributing to growth, reproduction, and energy provision. Conversely, Treatment A demonstrated the lowest protein and fat content at 42.97 and 12.99%, respectively. Adequate protein availability in feed is crucial because a deficiency can lead to stunted growth (Khat et al., 2018). Conversely, an excess of protein may result in the inability of fish to effectively catabolize amino acids, thereby leading to inefficient feed utilization (Masjudi et al., 2016).

Furuichi (1988) determined that juvenile fish required 6.97% lysine. This amino acid is essential for the production of carnitine, which supports growth, reduces ammonia toxicity, and helps fish adapt to sudden temperature changes (Herawati et al., 2020). Balqis (2021) have found that good proteins are easy to digest and have the right amino acids for the farmed species. Adding lysine to fish feed can help Barramundi fingerlings to grow better and survive longer (Valverde et al., 2013). Lysine also helps to produce antibodies, strengthen the immune system, and support cell growth. It also makes easier to digest other amino acids, such as tyrosine, which is important for controlling appetite and handling stress in the fish

(Mengistu et al., 2020). According to Valverde et al. (2013), vitamin B1 helps fight viruses, aids calcium absorption, boosts appetite, and helps produce carnitine, the latter is crucial for fatty acid metabolism. Consequently, lysine indirectly aids in increasing the polyunsaturated fatty acids (PUFAs) in fish by promoting carnitine production.

Methionine is thought to serve as a chemoattractant, potentially enhancing feeding behavior in fish species. In the case of Barramundi, the highest methionine concentration was observed in Treatment E. According to Furuichi (1988), the methionine requirement for fish is 3.2%. Methionine is an important amino acid in fish production, as it helps balance and use other amino acids, supports growth, aids in protein production, and helps body functions. It is also required for the production of nucleic acids, cells, and proteins in fish (Anggraeni et al., 2024), and helps the body handle extra proteins when combined with vitamin B12 and folic acid. Diets with high methionine levels can boost growth and immune health (Juharni et al., 2022). Glutamic acid is a free amino acid found in large amounts in the plasma. This amino acid helps produce purines, glucosamine, pyrimidines, and asparagine (Valverde et al., 2013). It also improves the structure and function of the intestine, which is crucial for shrimp and fish larvae that have underdeveloped digestive systems. The addition of glutamine is expected to enhance intestinal performance and nutrient absorption (Hakim et al., 2022).

During metabolism, saturated fatty acids are broken down to create energy, which is important for fish growth. This process is facilitated by lysine, which is an essential amino acid. Lysine helps produce carnitine, a compound that improves the energy consumption of fatty acids (Weththasinghe et al., 2021). Lauric acid, a specific type of saturated fatty acids, is transformed into lauric monoglycerides, known for their antiviral, antibacterial, and antiprotozoal effects. Eicosapentaenoic acid (EPA) contributes to cellular metabolism, while arachidonic acid serves as a substrate for the production of eicosanoids. These bioactive compounds are essential for physiological functions, such as ion regulation and reproductive processes, including the maturation of eggs in the female broodstock (Furuichi , 1988). Arachidonic acid, in particular, is a precursor to eicosanoid fatty acids, such as prostaglandins, thromboxanes, and leukotrienes (Gupta et al., 2020), and is also a crucial component of phosphatidylinositol, an important phospholipid in cell membranes.

Based on the statistical analysis, substituting fishmeal with Black Soldier Fly (BSF) larvae meal in formulated feed did not significantly affect the survival rate of Barramundi (*Lates calcarifer*) fingerlings. Although differences were observed among treatments throughout the study, these differences were not statistically significant. The highest recorded survival rate was 93.33±11.55%, which is considered good and falls within the optimal range. According to Bahri et al. (2020), a survival rate above 70% is categorized as good, 50–60% as moderate, and below 50% as low.

In the present experiment, water temperature, dissolved oxygen (DO), pH, and salinity were measured. Water temperature ranged from 29°C to 30°C, dissolved oxygen was 4.80 to 5.50mg/L, salinity ranged from 30 to 31ppt, while the pH was 7.7 to 7.9. According to Jaya et al. (2013), the optimal temperature range for cultured fish in tropical regions is between 27–32°C, which supports efficient digestion and promotes growth and survival rates in species like Barramundi. Water temperature also influences oxygen solubility and chemical and biological processes in aquatic environments. Juharni et al. (2022) stated that dissolved oxygen levels of 4.5–5.7mg/L are considered optimal for fish growth and are favorable for fish. According to Shubhi et al. (2017), an ideal dissolved oxygen concentration for juvenile Barramundi is 5–7mg/L.

The salinity of the seawater used for Barramundi rearing should remain stable at 30–31ppt (Juharni et al., 2022). Barramundi is an euryhaline species capable of tolerating various salinities, including freshwater (0ppt). Rayes et al. (2013) have noted that Barramundi shows high salinity tolerance, which allows it to adapt to varying environmental conditions. The pH range during the study was 7.7–7.9, which also remained within the optimal range. Water pH is a critical factor that influences metabolic processes in fish. According to Johan et al. (2020), the optimal pH range for Barramundi culture is 7.5–7.9. All water quality parameters measured during the study were within acceptable limits and complied with standard aquaculture water quality guidelines.

Conclusion

The substitution of BSF larvae meal in formulated feed for Barramundi (*L. calcarifer*) fingerlings produced the best results in Treatment E (20% BSF larvae meal/100g of feed), with the average values for TFC, FCR, PER, PRE, SGR, absolute weight and absolute length were recorded as follows: 113.36±3.66g, 1.40±0.15, 1.49±0.07, 66.61±2.92, 2.67±0.14%/day, 8.46±1.30g, and 5.09±1.19cm, respectively. The best nutrient composition was also obtained in Treatment E (20% BSF larvae meal/100g of feed), with cultured fish exhibiting a protein content of 63.75±0.05%, lipid content of 8.24±0.02%, total essential amino acids (EAA) of 57g/100g protein, and eicosapentaenoic acid (EPA) content of 8.35±0.06g/100g fat.

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Conflict of Interest: The authors declare that they have no competing interests.

Data Availability: The additional data of this study can be obtained from the corresponding author upon a justified request.

Ethics Statement: This study did not use ethical clearance.

Author's Contribution: First Author: Conceptualization, Second and Third Author: Methodology; Fourth Author: Investigation, Fifth Author: Formal analysis, Sixth Author: writing, reviewing, and editing.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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