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Efficacy of Black Soldier Fly (*Hermetia illucens*) Larvae Meal as Feed on Growth Performance for Juvenile Javan Mahseer (*Tor tambra*)

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ABSTRACT

Fish meal (FM) is a well known protein source for fish feed and its heavy utilization has contributed to overfishing that lead to increase price of formulated feed due to scarcity of fish resources for fish meal. Several studies were done to identify the best substitution of FM including Black Soldier Fly (*Hermetia illucens*) larvae as a potential candidate for FM replacement. This study was done to investigate the proximate composition of Black Soldier Fly Larvae Meal (BSFLM) and to determine its efficacy as feed on growth performance of Javan Mahseer (Tor tambra). Three types of treatment were used namely F0 or control (0% BSFLM and 100% FM), F50 (50% BSFLM and 50% FM) and F100 (100% BSFLM and 0% FM). Each treatment was fed twice daily for 20 days to 10 Javan Mahseer with the average initial weight of 0.1g respectively. Analyses conducted was proximate composition and growth performance like specific growth rate, feed conversion ratio, survival rate and body weight gain. Data was analysed using One-way Analysis of Variance (ANOVA) SPSS Windows 27. Results show that crude protein in F0, F50 and F100 were 37.52%, 30.36% and 27.52%; while F50 indicated best BWG (66.71%) and lowest FCR (3.16) respectively. It can be concluded that treatment 50% inclusion of BSF is the best ratio for good growth performances of Javan Mahseer.

Keywords: Fish meal substitute, Black soldier fly, growth performance, Javan Mahseer

INTRODUCTION

Fish meal (FM) is one of major ingredients used to produce feed stuff intended to feed a majority of livestock animals (Hodar et al., 2020). FM are made of processed fishes that comes from ocean. Usually, FM constitute of small pelagic fishes such as herring, anchovies, sardines, menhaden and more (Karbalaei et al., 2020; Terova et al., 2019). The fish were processed by pulverizing them to bits before remaining water and oil were pressed out and remains were cooked and crushed thoroughly until a powder texture of fish meal is formed (Karbalaei et al., 2020).

FM is an important source of ingredients to make food stuff for most domestic animals because it has high concentration of protein couple with a good balance ratio that gives a good digestibility rate (Brezas and Hardy., 2020). FM is also often used as an important ingredient in formulated diets for nursery stage fish fry as it provides good muscle growth with upregulated growth-related gene expression (Asaduzzaman, et al., 2017). FM are also excellent source of lipid, minerals and vitamin reputed as major contributors in the popularity of FM (Hodar et al., 2020). However, FM was also proven to have some disadvantages alongside its advantages especially in term of its high price and sustainability (Ljubojevic´ et al., 2015; Hodar et al., 2020; Kamarudin et al., 2021); and it does not show any sign of going down as the price of fish meal has significantly increased to over US$ 1600 per Metric tonnes (MT) (Hodar et al., 2020).

Global FM output hits approximately 7 million MT in 2010, and is expected to hit 7.6 million MT by 2030 (Karbalaei et al., 2020. This is in line with increasing unsustainable worldwide demand for aquaculture-based fish products to supply feed for aquaculture (Hodar et al., 2020; Karbalaei et al., 2020 ; Terova et al., 2019). Thus, many researches were done in search of FM substitute (Katya et al., 2017; Terova et al., 2019; Kamarudin et al., 2021) and one of the promising candidates is related to the usage of insects (Terova et al., 2019; Rimoldi et al., 2021; Kamarudin et al., 2021) which is larvae of Black Soldier Fly (*Hermetia illucens*).

In Malaysia, there are only a few studies done on research of Black Soldier Fly Larvae Meal (BSFLM) usage as a substitute for FM. The positive result of implying BSFLM as substitute for FM at a certain degree and percentage for fish feed shows its potential (Belghit et al., 2019 and further study needed to be done to conclude the usage of BSFLM as FM substitute (Tippayadara et al., 2021).

MATERIALS AND METHODS

Feed preparation

An approval Ref: UAPREC/O/3/374-3(35) was obtained from UniSZA Plant and Animal Research Ethics Committee (UAPREC) before this study started. Dried Black Soldier Larvae were obtained from a fish farm at Rawang, Selangor and transported back to Animal Feed Laboratory, Universiti Sultan Zainal Abidin to process into BSFLM. FM was purchased from a local seller from Mersing, Johor. FM was kept in cold room storage in UniSZA's animal feed storage facility prior to use. Three types of feed were prepared as shown in the Table 1 below.

Table 1. List of ingredients for each type of feed F0 (control), F50 (50% FM and 50% BSFLM) and F100 (100% BSFLM).

Ingredients (g)	F0	F50	F100
BSFLM	-	60	120
FM	120	60	-
Krill meal	30	30	30
Palm oil	21	21	21
Multivitamin mineral	54	54	54
Wheat flour	45	45	45

Sago powder	15	15	15
Cellulose	15	15	15
Total	**300g**	**300g**	**300g**

Proximate composition

Proximate analysis process was done in accordance to the standard method (AOAC, 2006) at the Animal Feed Analysis Laboratory at Universiti Sultan Zainal Abidin. There were 5 types of analysis which is for FM, BSFLM and the three types of experimental diet. Analysis were made on its crude protein, crude lipid, fibre, ash and moisture

Crude protein

For crude protein, the Kjeldahl technique was used to determine the crude protein content of the samples and percentage of crude protein in samples was calculated according to the following formula based on the standard method of AOAC (2006).

$$\text{Percentage of N} = \frac{\text{A x (T-B) x 14.007 x 100}}{\text{Weight of sample (g) x 1000}}$$

Percentage of crude protein (%) = % N x F

T = Volume of acid for sample
B = Volume of acid for blank
A = Normality of HCl
F = Protein factor, 6.25

Crude lipid

Crude lipid content was determined using Soxhlet method and percentage of crude lipid in samples was calculated according to the following formula based on standard method of AOAC (2006).

$$\text{Percentage of crude lipid (\%)} = \frac{W3 - W2}{W1} \times 100$$

W1 = weight of sample (g)
W2 = weight of extraction beaker (g)
W3 = weight of extraction beaker + fat (g)

Moisture

Moisture content was calculated according to the following formula based on standard method of AOAC (2006)

$$\text{Percentage of moisture (\%)} = \frac{W5 - W6}{W5 - W4} \times 100$$

W4 = weight of crucible (g)
W5 = weight of crucible + weight of wet sample (g)
W6 = weight of crucible + weight of dried sample (g)

Ash

Ash content was calculated according to the following formula based on standard method of AOAC (2006)

$$\text{Percentage of ash (\%)} = \frac{W9 - W7}{W8} \times 100$$

W7 = weight of crucible (g)
W8 = weight of sample (g)
W9 = weight of crucible + ash (g)

Crude fibre

Fibre content were determined by using sequential extraction method and percentage of crude protein in samples was calculated according to the following formula based on standard method of AOAC (2006).

Blank = W16 – W15

Percentage of crude fibre (%):

$$\frac{[\,(W12 - W10) - (W13 - W14)\,] \times 100}{W11}$$

W10 = weight of fibre bag (g)
W11= weight of sample (g)
W12 = weight of crucible and fibre bag after digestion (g)
W13 = weight of crucible and ash (g)
W14 = weight of blank value of empty fibre bag (g)
W15 = weight of crucible (g)
W16 = weight of crucible and ash of empty fibre bag (g)

Fish preparation and rearing

90 tails of juvenile Javan Mahseer (*Tor tambra*) with average size of 0.1g ±0.01g were purchased alive from a fish farm located at Paka, Terengganu and transported to Universiti Sultan Zainal Abidin, Besut Campus. Nine 60L-

tanks were used to rear 90 juvenile Javan Mahseer in triplicates based on three types of feed which were F0 (100% FM) (control), F50 (50% BSFLM and 50% FM) and F100 (100% BSFLM) with each tank consisted of 10 fish. The tanks used aerated static water system and water change was done once in a week. Fish in the first 3 tanks were fed with the control feed (F0) which contained 100% FM while the next 3 tanks were fed with the feed that contained 50% FM and 50% BSFLM (F50). Last 3 tanks were fed with feed that contained 100% BSFLM (F100). Fish were fed twice a day (morning and evening) for a period of 20 days based on 5% of their total body weight of fish as the amount of feed suggested by (Muchlisin et al., 2017). Data were obtained in day 10 and day 20 for their Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Survival Rate (SR) and its Body Weight Gain (BWG).

Statistical analysis

Data was analyzed by One-way Analysis of Variance (ANOVA) to identify the relationship between weight gain of Javan Mahseer and types of feed used. All of the required data were analyzed by using SPSS Windows 27.

RESULTS AND DISCUSSION

Proximate composition

There were 2 sets of data obtained for proximate analysis which were the comparison between each test diet which were F0, F50 and F100 and between FM and BSFLM. The result for BSFLM and FM can be seen in Table 2 below.

Table 2. Proximate analysis for BSFLM and FM.

Ingredients	Crude protein %	Crude lipid %	Ash %	Moisture %	Fibre %
BSFLM	25.11 ± 1.87^{a}	24.14 ± 0.39^{a}	8.96 ± 0.07^{b}	7.76 ± 1.30^{b}	10.37 ± 0.95^{c}
FM	33.42 ± 1.13^{a}	6.94 ± 0.14^{c}	22.09 ± 1.24^{b}	13.14 ± 0.16^{d}	6.39 ± 0.77^{c}

There are no significant differences in Table 2. Crude protein in FM of high quality should be between 57% to 77% (Karbalaei et al., 2020; Obeng et al., 2015) but results show crude protein for FM was only 33.42% ± 1.13% and this amount is lower than the amount of protein recorded in past studies. This is probably due to poor quality of FM due to degradation of quality and rancidity could have occurred during Covid19 long term storage which was approximately two years. Nevertheless, crude protein in FM used in this study was still higher than the amount of crude protein in BSFLM. Crude protein in BSFLM is estimated to be around 36% to 48% and has a balance amino acid content (Barroso et al., 2014; Rimoldi et al., 2021; Katya et al., 2017) but based on the obtained result, the crude protein of BSFLM was 25.11% ± 1.87%. Ash and moisture in FM are higher than BSFLM which are 22.09% and 8.96% in FM, while BSFLM has 13.14% FM and 7.76% moisture.

The presence of ash means the presence of minerals and it shows that FM has a superior amount of minerals which are important micronutrients that help in regulating essential metabolism in an organism (Craig et al., 2017). Ash content in fish diets typically ranges from 5.7% to 14.5% for optimal fish growth (Ibrahim et al., 2022; Xi et al., 2022).

FM has higher moisture content than BSFLM with the amount of 13.14% and 7.76% of moisture respectively. The content of moisture from both of the feed ingredients determines the best storage method and the duration of storage. FM has to be stored in a chiller to prolong its storage because of its high moisture content.

Based on the results, BSFLM has higher content of crude lipid than FM at amount of 24.94% and 6.94% respectively. Lipid is important materials for an organism as it has high energy density in comparison to protein. A sufficient amount of lipid will provide energy for an organism especially fish to sustain its energy usage without using protein as an energy source and can focus protein for growth (Muchlisin et al., 2017). Lipids provide vital fatty acid such as EPA and DHA as well as serving as a major transporter for vitamins that are fat soluble and most fish required around 7% to 15% of lipid (Craig et al. 2017).

BSFLM has the highest content of fibre with the amount of 10.37% while FM has 6.09% of fibre. Fibre is mainly indigestible in fish but play a major role of digestibility and absorption in their diet. It is beneficial especially for herbivores and omnivorous fish as fibre helps in inducing a faster movement rate in their digestibility and making them acquiring energy a lot quicker (Craig et al., 2017; Haghbayan & Mehrgan, 2015).

Table 3. Proximate analysis of F0, F50 and F100.

Ingredients	Crude protein %	Crude lipid %	Ash %	Moisture %	Fibre %
F0	37.52 ± 2.34^{a}	12.55 ± 0.05^{c}	21.83 ± 0.06^{b}	4.89 ± 0.34^{d}	1.84 ± 0.17^{d}
F50	30.36 ± 2.13^{a}	16.19 ± 0.33^{b}	17.73 ± 0.31^{b}	3.30 ± 0.26^{c}	4.59 ± 0.25^{c}
F100	27.52 ± 2.03^{a}	18.21 ± 0.13^{b}	15.19 ± 0.07^{b}	5.32 ± 0.14^{c}	7.24 ± 0.80^{c}

During the feeding trial, fish were fed with 3 types of feed which were F0, F50 and F100. The only differences in the composition of F0, F50 and F100 were protein sources incorporated in the test diets which is BSFLM and FM. The differences of proximate analysis were just the same with BSFLM and FM in direct comparison with F0 and F100 with the exception of F50, the combination of both BSFLM and FM that has the intermediate result between F0 and F100 for all of the proximate analysis.

Results shown in Table 3 above shows that crude protein was the highest in F0 which is 100% FM and no BSFLM included with the amount of 37.52 ± 2.34 and the lowest crude protein is F100 which has 100% BSFLM and without FM included with protein amount of 27.52 ± 2.03 while F50 has lesser protein than F0 and more protein than F100 with the amount of 30.36 ± 2.13. Omnivorous fish especially juvenile Javan Mahseer, requires 40% to 48% protein for them to reach fast and optimum growth (Ishak et al., 2016; Bami et al., 2017; Muchlisin et al., 2017).

Lipid content recorded for the test diets were 12.55 ± 0.05, 16.19 ± 0.33 and 18.21 ± 0.13 for F0, F50 and F100 respectively. There were significant differences ($p<0.05$) between F0 with both F50 and F100 although no significant difference was detected in the later treatments. As BSFLM has a higher content of lipid in comparison with FM, the result of proximate analysis for the test diets show similar incremental patterns with incremental volume of BSFLM with F100 has the highest lipid, followed by F50 and F0. According to Bami et al. (2017), lipid requirement for Javan Mahseer is around 5% to 10% from the total of their feed diets. Mahseer species were also known to have a declining specific growth rate with inclusion of lipid content above 10% (Ishak et al., 2016). It shows that the formulated feed for F0, F50 and F100 has a higher content of lipid that the Javan Mahseer needs to achieve a good growth rate.

The results of ash were 21.83 ± 0.06, 17.73 ± 0.31, 15.19 ± 0.07 for F0, F50 and F100 respectively. There were no significant difference ($p<0.05$) for ash content among treatments. According to Craig et al. (2017), the amount of ash content in feed represents the amount of mineral composition inside the feed. Based on the result, F0 that contains 100% FM has the highest amount of ash which is 21.83 ± 0.06 and in accordance to the ash content of the FM itself while F100 which contains 100% BSFLM has the lowest ash content which is 15.19

± 0.07 and in accordance to the ash content of BSFLM itself and F50 that has the combination of both FM and BSFLM has ash content higher than F100 but lower ash content than F0. The best ash content in fish diets typically ranges from 7% to 12% for an optimal fish growth (Wei et al., 2022).

The moisture content in F0, F50 and F100 were 4.89 ± 0.34, 3.30 ± 0.26, 5.32 ± 0.14 respectively. There were significant differences ($p<0.05$) for moisture content in each feed treatment. It shows that F100 has the highest moisture content while the lowest moisture content was F50 and F0 came in second. As mentioned earlier in 4.1.1, the moisture level of feed diets dictates the appropriate storage technique and duration of storage. Moisture at less than 14% in feed coupled with proper atmosphere can limit mould formation during storage (Atter et al., 2017). The best storage method to store feed diets is to keep in a cold and dry place to prolong the shelf life of the feed.

The fibre content in F0, F50 and F100 were 1.84 ± 0.17, 4.59 ± 0.25, 7.24 ± 0.80 respectively. There were significant differences ($p<0.05$) for fibre content on each feed treatment. F100 has the highest content of fibre, followed by F50 and F0. BSFLM has highest content of fibre in comparison to FM. This pattern is similar for F100 has the highest fibre which is mostly indigestible for fish, but it plays an important role in their digestion and absorption. It is advantageous for herbivores and omnivorous fish since it causes a higher movement rate in their digestion and allows them to get energy more faster (Craig et al., 2017; Haghbayan & Mehrgan, 2015)

Analysis of growth performance

For growth performance, Table 4 summarises the growth performances of juvenile Javan Mahseer fed with three experimental diets (F0, F50, and F100) for 20 days, which is its Body Weight Gain (BWG), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), and Survival Rate (SR).

Table 4. Data recorded for Body Weight Gain (BWG), Specific Growth Rate (SGR, Feed Conversion Ratio (FCR) and Survival Rate (SR).

	Diets		
	F0	**F50**	**F100**
BWG (%)	61.20 ± 14.83^a	66.71 ± 26.98^b	59.30 ± 17.35^a
SGR (%)/day-1	3.09 ± 0.72a	3.38 ± 1.36^a	2.98 ± 0.85^a
FCR	3.27 ± 0.70^a	3.16 ± 1.12^a	3.48 ± 0.86^b
SR (%)	93.33 ± 11.55^a	96.67 ± 5.77^b	96.67 ± 5.77^b

BWG were obtained by dividing their weight gain on the data collection day with their initial weight and multiplied 100 to get a percentage of weight gained. The average initial weight of the fish was 0.1g and fish can be seen to increase in size growing steadily. During 10th day, the total weight gain were 34.52% ± 6.32%, 36.01% ± 24.56% and 34.13% ± 17.04% for juvenile Javan Mahseer fed with F0, F50 and F100 respectively (Fig.1). There are significance differences ($p<0.05$) with a high variation of weight data collected in each of the treatment. The highest recorded BWG were F50 (50%FM and 50% BSFLM), the middle BWG were the F0 (100%FM and 0%BSFLM) and the lowest BWG were the F100 (0%FM and 100%BSFLM) and there are no significant differences by comparing between each of the trial. During 20th day, the total BWG were 61.20% ± 14.83%, 66.71% ± 26.98% and 59.30% ± 17.35% for F0, F50 and F100 respectively. The significant highest total BWG were F50, followed by F0 and lastly F100. The latter treatments showed no significant differences between treatments.

Despites having lower amount protein which is 30.36%, F50 still manages to get higher BWG compared to F0 that has higher protein which is 37.52%. This could be due to the balance amount of protein, fibre and fat found in BSFLM as it has a balance amino acid, with a higher fibre and fat content that can exclude the energy usage from protein source for fat while fibre helps in digestive system in absorbing nutrients quicker and the protein inside the feed can be fully utilized for growth. (Katya et al., 2017; Craig et al., 2017; Haghbayan & Mehrgan, 2015). Fig. 1 below shows the graph for BWG.

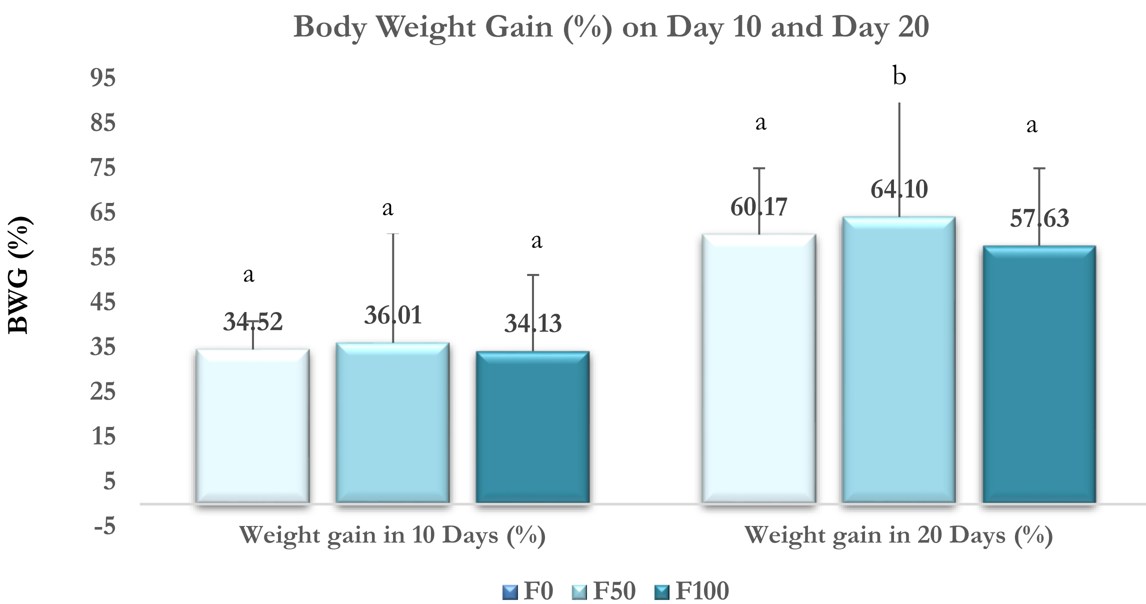

Fig. 1. *Tor tambra* fingerling Body Weight Gain (BWG) over 20 days with F0, F50, and F100 treatments.

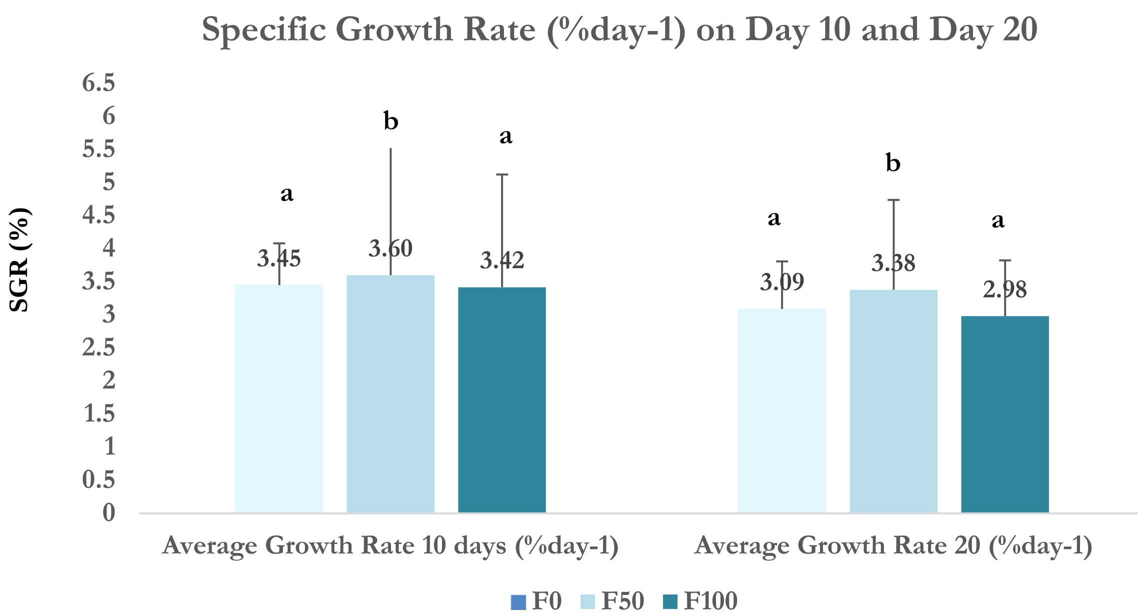

Fig. 2. *Tor tambra* fingerling Specific Growth Rate (SGR) over 20 days with F0, F50, and F100 treatments.

SGR were obtained by dividing the amount of weight gain percentage on the data collection day with the total day from the initial day to get the average percentage of fish growth on each day. During 10th day, the SGR recorded were 3.45% ± 0.63%, 3.60% ± 2.46% and 3.42% ± 1.70% for F0, F50 and F100 respectively. There were significance differences ($p<0.05$) with a high variation of SGR data collected in each treatment. The highest recorded SGR were F50 (50%FM and 50% BSFLM), the middle SGR were the F0 (100%FM and 0%BSFLM) and the lowest SGR were the F100 (0%FM and 100%BSFLM) and no significant differences were shown from comparison between each trials.

During 20th day, the total SGR were 3.09% ± 0.72%, 3.38% ± 1.36% and 2.98% ± 0.85% for F0, F50 and F100 respectively (Fig. 2). The highest total SGR were F50, followed by F0 and lastly F100 although no significance differences ($p<0.05$) were shown among treatments. Overall SGR on 20th day was lower than SGR on 10th day that may be due to slower growth rate as they aged more. In addition, the growth impairment may also be contributed from external stress factors such as poor handling and toxic ecosystem (Lopes et al., 2022).

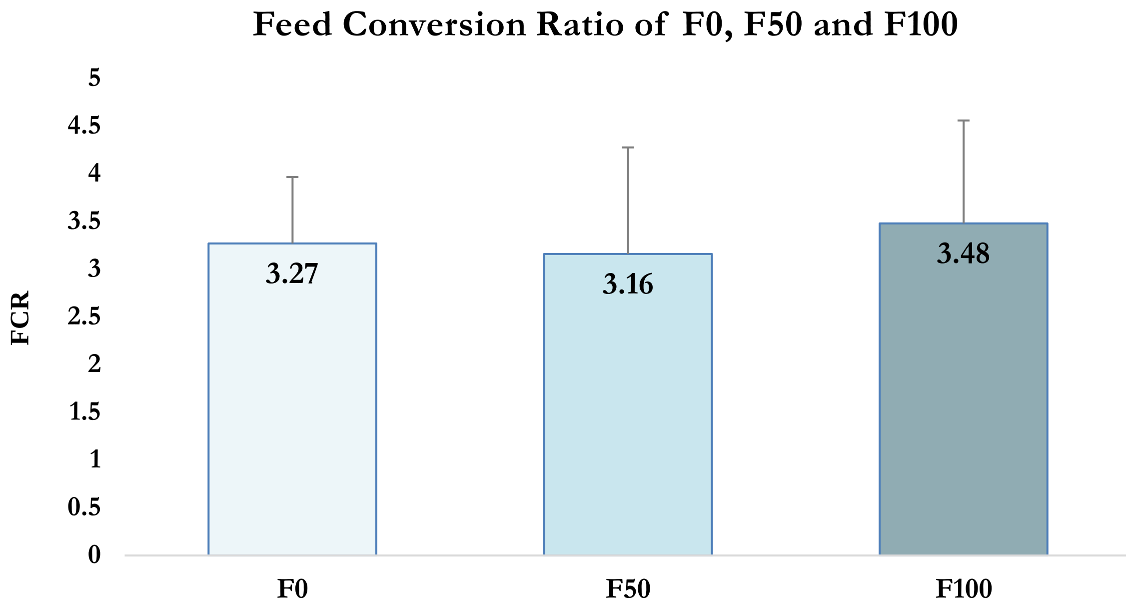

Fig. 3. *Tor tambra* fingerling Feed Conversion Ratio (FCR) over 20 days with F0, F50, and F100 treatments.

FCR means amount of feed needed for a fish to consume to increase their weight by 1kg, which means that the lower the FCR, the better the feed is. Fishermen wish to reduce the amount of feed cost by reducing the total feed given to the fish while at the same time maintaining a good growth of the fish. FCR is obtained by dividing the weight of total feed given with total weight gain of the fish. Fig. 3 shows the recorded FCR with the amount of 3.27 ± 0.70, 3.16 ± 1.12 and 3.48 ± 1.08 for F0, F50 and F100 respectively after 20 days.

There are significant differences ($p<0.05$) with a high variation of FCR data collected in each of the trial. The lowest which is the best recorded FCR were F50 (50%FM and 50% BSFLM), the middle were the F0 (100%FM and 0%BSFLM) and the lowest FCR were the F100 (0%FM and 100%BSFLM). This indicates that all feeds were palatable to this fish as observed by Muchlisin et al. (2017). However, FCR results from all treatments are still considerably high than the recommended FCR as low as 1 in aquaculture for fish to reach optimal growth. FCR of more than 3 in *Tor tambra* was also reported by Muchlisin et al (2016) indicating that this species do have slow growth with total body weight gain ranged between 0.33 g to 0.54 g, from initial weight of 2.69g. In comparison, similar FCR shown in this study indicated similar growth pattern but with higher percentage of average growth rate at more than 3% per day during the first 10 days as indicated in Fig. 2. After 20 days, the growth rates seemed to decline slightly indicating that feed inefficiently may have developed at this stage. This explains importance of a lower FCR in increasing growth rate while reducing maintenance requirements per unit of growth produced towards time of slaughter as suggested by Dvergedal et al (2019).

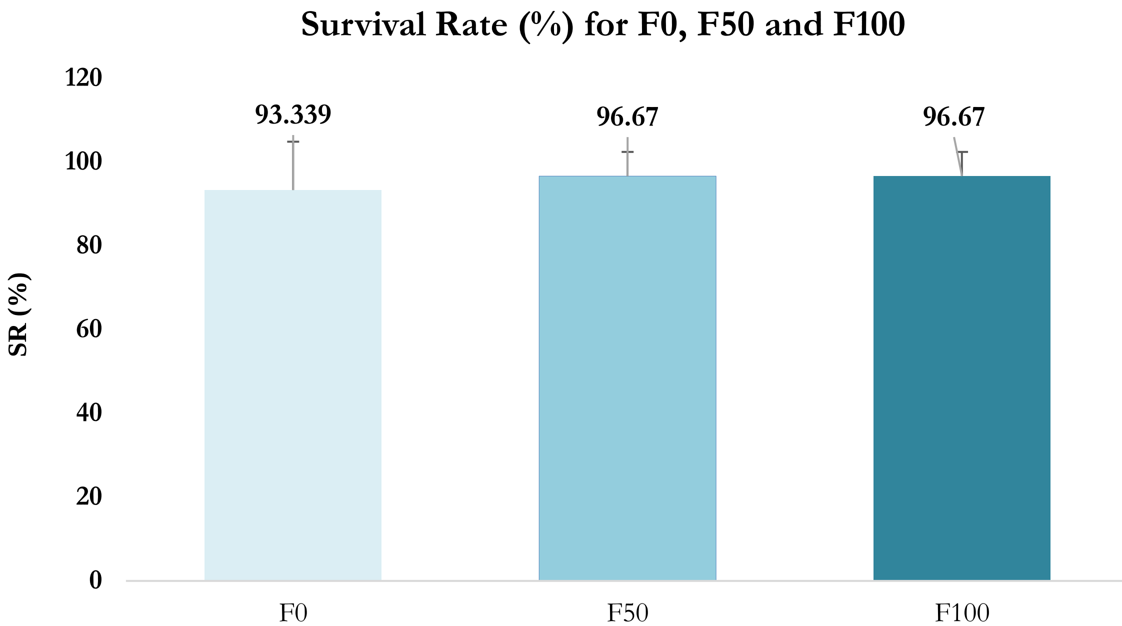

Fig. 4. *Tor tambra* fingerling Survival Rate (SR) over 20 days with F0, F50, and F100 treatments.

Survival rate is an important aspect to determine the quality of the experimental diet. The survival rate were obtained by dividing the survived fish with the initial number of fish and multiplying with 100 to obtain the percentage of alive fish. As indicated in Fig.4**,** the survival rate were 93.34% ± 11.547, 96.67% ± 5.77 and 96.67% ± 5.7774%. SR is still regarded as high with more than 90% survival rate for all treatments although SR in F0 was significantly lower than F50 and F100. Mortality occurred during electricity cut-off that had occurred for during the feeding trial.

CONCLUSION

In conclusion, the efficacy of BSFLM as a replacement of FM for protein source is proven to be true as SGR and BWG of F100 were not much different than F0 and the combination of BSFLM and FM in F50 provided the best result in the trials. It is recommended for a longer feeding trial period to determine the efficacy of BSFLM as protein source in fish feed with an optimum amount of BSFLM or mixture between BSFLM and FM to fully understand the benefits of BSFLM inclusion in animal feed especially fish.

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