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Solid Fermentation of Pelletized Fish Feeds Containing Black Soldier Fly (*Hermetia illucens*) Larvae Meal to Enhance Growth Performance of Catfish (*Clarias* sp.)

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Abstract

The unsustainable use of fish meals as protein and lipid ingredients in aquafeed has driven the search for an alternative. Black soldier fly (BSF) (*Hermetia illucens*) larvae have been widely studied for substitution purposes. This study aims at investigating the effect of solid fermentation of pelleted diets containing BSF larvae meal (BSFLM) on the growth performance of catfish *Clarias* sp. Four feed pellets were formulated: F1 (non-fermented, 60% BSFLM), F2 (fermented, 60% BSFLM), F3 (non-fermented, 50% BSFLM), and F4 (fermented, 50% BSFLM). These formulated diets and commercial feed pellets (positive control) were separately fed to the catfishes in 5 aquaria (10 fishes per aquaria in triplicate). Results showed that the fermentation increased the protein level by 10–11%, but kept the crude lipid concentrations unchanged. Specific growth rate (SGR) and weight gain (WG) profiles for fermented feeds F2 and F4 were higher than the non-fermented feeds F1 and F3, respectively. F4 gave the best feed conversion ratio (FCR) of 1.78, which was 15–25% more efficient than F1, F2, and F3. Compared to the commercial feed, the 4 formulated feeds underperformed by around 50% in all of the growth parameters except survival rates (SR) which were not significantly different (93–100%).

INTRODUCTION

In the manufacture of aquaculture feed, fish meal and fish oil are amongst the most important components due to their high nutritional quality and digestibility, being the main source of favorable amino acids and fatty acids (De Silva and Turchini, 2009), notably docosahexaenoic acid and omega-3 fatty acids (Vannuccini *et al*., 2020). However, since fish meal and fish oil are obtained from captured forage fish in the wild, its increasing use as ingredients in aquafeed can no longer be sustained in the future, thus necessitating alternative protein sources (Hua *et al*., 2019). Amongst feed ingredients already studied to replace fish meal, whose price continues to raise, are insects as well as the by-products of fisheries and terrestrial animals (Luthada-Raswiswi *et al*., 2021).

Amongst the insects widely studied for their potential to replace fish meal as a protein source with lower production cost is black soldier fly (BSF) (*Hermetia illucens*) larvae (Gougbedji *et al*., 2021).

This insect species can grow in diverse environmental conditions by feeding on decaying organic materials (Raksasat *et al*., 2020). Nutritional compositions of BSF larvae vary, depending on the feeding substrate (Fuso *et al*., 2021) and the life cycle stages (Liu *et al*., 2017), and could contain 37.3% and 61.1% protein in the form of flour and protein extract, respectively (Queiroz *et al*., 2021). Another study found BSF prepupae containing between 35% and 49% protein on a dry weight basis (Fuso *et al*., 2021). Experimentally, dried BSF larvae's amino acid score could meet the requirement of FAO/WHO for indispensable amino acids, with the ratio of essential amino acids to total amino acids being > 40% (Huang *et al*., 2018). In vivo digestibility study of oven-dried BSF larvae protein gave the values of 85% with the digestible indispensable amino acid score of 73% (Traksele *et al*., 2021). In addition, BSF larvae contain 25–34% fatty acids (Oonincx *et al*., 2015).

Feeding tests of BSF larvae-containing feeds have been conducted on a number of aquatic species. For example, formulated feeds with partial or total replacement of fish meal or other protein sources with BSF larvae have been tried on yellow catfish (*Pelteobagrus fulvidraco*) (Xiao *et al*., 2018), mirror carp (*Cyprinus carpio* var. *specularis*) (Xu *et al*., 2020), Japanese seabass (*Lateolabrax japonicus*), Thai climbing perch (*Anabas testudineus*) (Mapanao *et al*., 2021), Siberian sturgeon (Rawski *et al*., 2020, 2021), Nile tilapia (*Oreochromis niloticus*) (Tippayadara *et al*., 2021), gilthead seabream (*Sparus aurata*) (Randazzo *et al*., 2021), African catfish (*Clarias gariepinus*) (Fawole *et al*., 2020), and meagre (*Argyrosomus regius*) (Guerreiro *et al*., 2021). For most of those experiments, partial replacement of fish meal by BSF larvae as a protein-rich ingredient in the formulated feeds was possible in order not to negatively impact the growth performance of the fishes. Exceptionally though, dietary inclusion of BSF larvae in the formulated feeds completely devoid of fish meal was demonstrated in the case of feeding trials on Nile tilapia (Tippayadara *et al*., 2021) and gilthead seabream (Randazzo *et al*., 2021) without deleterious effects.

High dietary inclusion of BSF larvae intended for complete fish meal substitution in fish feed is hardly achieved in most cases (Gasco *et al*., 2018). This has been suggested to be due to the high chitin content of BSF prepupae, which could reach 9% on the dry weight basis, which is the fourth most abundant macromolecule after lipids (37%), proteins (32%), and minerals (19%) (Caligiani *et al*., 2018). This chitin component is indicated as a non-digestible fiber that reduces the digestibility of proteins and lipids (Soetemans *et al*., 2020). Thus, the method that could enhance the fishes' utilization of BSF larvae-supplemented feeds is interesting to explore, and such method as fermentation has so far not yet been studied in this context. Therefore, this study aims at finding out the effect of BSF larvae-supplemented feeds fermented using tempeh starter on some growth parameters of the tested catfish *Clarias* sp. Tempeh starter is an inoculum used to ferment cooked soybeans as the solid substrate to produce the cake-like Indonesian traditional food called Tempeh (Ahnan-Winarno *et al*., 2021). The edible fungi that grow predominantly on the soybean tempeh are from the genus *Rhizopus*, whose mycelial biomass has been studied for their potential as a protein alternative for aquafeed (Asadollahzadeh *et al*., 2018) and for providing water buoyancy and stability to the feed in water without mechanical extrusion (Hariyono *et al*., 2021; Sriherwanto *et al*., 2021).

METHODOLOGY

Place and Time

The studies were conducted at the Teaching Factory and Fish Breeding Laboratory of Marine and Fishery Polytechnic, Sidoarjo, East Java, Indonesia. The research was carried out

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during the period of December 2019 - June 2020.

Research Materials

All materials used were purchased in Sidoarjo District, East Java province, in powder form, except Black Soldier Fly (BSF) larvae. The materials used in the feed formulation were BSF larvae, okara, rice bran, tapioca flour (Gunung Agung, PT Budi Starch & Sweetener Tbk., Lampung, Indonesia), and complete vitamin-mineral premix (Aquavita, PT Indosco Dwijayasakti, Surabaya, Indonesia). Soybean residue (okara) and rice bran were obtained from the tofu factory in di Pandean Neighborhood, Banjarkemantren Village and Pager Neighborhood, Sawotratap Village, respectively. Life BSF larvae were purchased from Puspa Agro Central Market and treated as described previously (Maulana *et al*., 2020): washing to remove dirt, steaming for 15 minutes, and oven-drying at 100 °C (Cosmos CO-9919 R) for 45 minutes. The dried larvae were then pulverized using a grinder (Fomac FCT Z300). Catfish (*Clarias* sp.) were obtained from Fish Boster Centre Farm, Sidoarjo.

Research Design

A feeding test was carried out on catfish (*Clarias* sp.) obtained from Fish Boster Centre Farm, Sidoarjo, East. As many as 225 2-month-old, 9-cm-length catfishes were acclimatized one week before the feeding experiment in 15 aquaria (60 × 40 × 40 cm), each containing 10 L water and 15 catfishes. Ten healthiest fishes with normal color and no sign of stress were selected from each aquarium for feeding test using 4 formulated diets (Table 1) and 1 commercial floating fish feed (Hi-Pro-Vite 781-3, PT. Central Proteina Prima, Tbk) as a positive control, with each feeding treatment triplicated in three aquaria. Prior to the study, the initial weight and length of each fish were measured. External environmental factors such as light intensity and temperature (28 ± 1 °C) were maintained the same for all of the aquaria.

Work Procedure

Feed formulation (Table 1) was determined through linear programming using the SOLVER add-ins in the Excel program to achieve approximately iso-nitrogenous and iso-energetic experimental diets. All of the feed ingredients were mixed with water (200 mL tap water per 1 kg feed mix) homogenously to produce dough which was then pelletized using a hand pelletizer with a rotating knife (Maksindo MKS-PLT10). The molded feed was then dried in an oven at 50 °C for 2 hours, resulting in pellets with approximately 3 mm diameter and 5 mm length.

Table 1. The feed formulation used in the study.

Feed Code	Ingredients (%)				
	BSF Larvae Meal	Okara	Rice Bran	Tapioca Flour	Vitamin Premix
F1	60	24	0.6	10	2
F2	60	24	0.6	10	2
F3	50	10	20	10	2
F4	50	10	20	10	2

Note: Water was added to make the total sum of individual ingredients up to 100%. F2 and F4 were fermented using tempeh starter, whereas F1 and F3 were not fermented. According to the manufacturer's information, the vitamin-mineral premix contained (per 1 g premix): vitamin A (3.000 IU), D_3 (1.000 IU), K_3 (1.2 mg), E (7.5 mg), B_1 (3.0 mg), B_2 (4.5 mg), B_6 (3.0 mg), B_{12} (3.0 mg), C (8.0 mg), calcium pantothenate (4.5 mg), folic acid (1.5 mg), biotin (1.0 mg), inositol (12.5 mg), nicotinamide (20.0 mg), choline chloride (15.0 mg), L-lysine (20.0 mg), DL-methionine (5.0 mg), and trace amounts of Co, Cu, I, Mn, Se, and Zn.

The feed fermentation was carried out using a 3% tempeh starter (Raprima), whereby 200 g feed was mixed with the starter and sprayed with 20 mL tap water to provide a moist substrate while preventing waterlogging. The mixture was then transferred into a tray (25 × 18 × 3.5 cm) and leveled. The tray was then covered with a perforated, clear plastic sheet, and incubated at ± 27 °C for 50 hours (Hariyono *et al*., 2021). Regular stirring using a comb-like tool (Doriya and Kumar, 2018a, 2018b) was carried out manually every 6 hours to prevent the formation of lumps due to the hyphae interweaving the feed pellets (Hariyono *et al*., 2021). Afterward, the fermented feeds were oven-dried at 50 °C for 3 hours before being sent to the Feed Laboratory, Livestock and Fisheries Department of Blitar District, Blitar City, East Java, Indonesia, for proximate analysis of the nutritional content. Percentage increase or decrease in nutritional components due to *Rhizopus*-fermentation was calculated as described previously (Kawee-ai and Seesuriyachan, 2019).

The formulated feeds as well as the control feed was tested on catfishes with the dosage of 3% of their total body weight with the frequency of two times a day at 7.00 and 16.00 hours, every day for up to 35 days. Catfishes were subjected to the weekly measurement of weight (digital kitchen scale SF-400) and length. Meanwhile, the aquarium water was also measured for its temperature (Benetech GM320 infrared thermometer) and pH (Universal Indicator pH strips 0-14 9535, Merck Germany). Feed utilization and growth performance were evaluated with reference to weight gain (WG), specific growth rate (SGR), protein efficiency ratio (PER), survival rate (SR), feed conversion ratio (FCR), and feed efficiency ratio (FER). These values were obtained by calculations using the previously described formulae (Gabriel *et al*., 2019; Musthafa *et al*., 2017).

Data Analysis

Data analysis was performed using statistical analysis of variance (ANOVA) at a 5% significant difference level.

RESULTS AND DISCUSSION

To our knowledge, this is the first published work on the growth performance of catfishes fed with BSF-supplemented feed pellets fermented with tempeh mold. The use of tempeh mold in this study was based on previous reports in which the biomass of the fungus *Rhizopus*, the dominant edible fungus genus in the fermentation of the Indonesian traditional food soybean tempeh, showed its potential as a feed ingredient alternative due to its high protein content with high-quality amino acids and lipids (Karimi *et al*., 2021). The fungal colonization of the feed pellets in this study increased the protein content by 10–11% of their original values, from the non-fermented values of 25.72 ± 0.11% (F1) and 26.88 ± 0.16% (F3) into the fermented values of 28.32 ± 0.07% (F2) and 29.76 ± 0.06 (F4), respectively (Table 2). Similar results were reported in the previous solid fermentation studies using *Rhizopus* as the fermenting microorganism, in which the protein content also underwent an increase by 28% (Gmoser *et al*., 2020) and 267% of the original values (Lima *et al*., 2021). The much higher increased protein content achieved by Lima *et al*. (2021) was likely due to the comprehensive preliminary optimization of fermentation conditions.

The whole pellet fermentation method in this study is different from those commonly reported in previous studies where only a certain ingredient was fermented, and this resulting fermented ingredient being subsequently mixed at certain inclusion levels with other non-fermented ingredients to produce the fish diets to be tested. Such microbiologically-treated ingredient examples are fermented rapeseed meal (Dossou *et al*., 2019), fermented *Jatropha curcas* kernel (Okomoda *et al*., 2020), and fermented de-oiled rice bran (Ranjan *et al.*, 2018).

Solid state fermentation on feeds F1 and F3 produced changes in the physical appearance of the pellets, where white cottony mycelia covered almost the entire surface of the fermented pellets (Figure 1). Feeds F2 and F4 were the fermented versions of the feeds F1 and F3, respectively (Table 2). The solid-state fermentation significantly increased the protein and ash contents by 10–11% and 12–17% of the initial values, respectively, but kept the crude lipid concentrations relatively unchanged. The crude fiber level was not significantly different between F1 and its fermented version F2, but was 30% higher in F4 than in its fermented version F3. The fermentation showed contrasting effects on the carbohydrate content of F1 and F3, namely 2% increase in F2 but 20% decrease in F4, respectively. Compared to the commercial feed (CF), however, all the fermented and non-fermented formulated feeds had 7–19% lower crude protein and 11–28% less carbohydrate, but higher concentrations of crude lipid (1.6–2.4 fold), crude fiber (2.0–3.3 fold), and ash (1.1–1.4 fold).

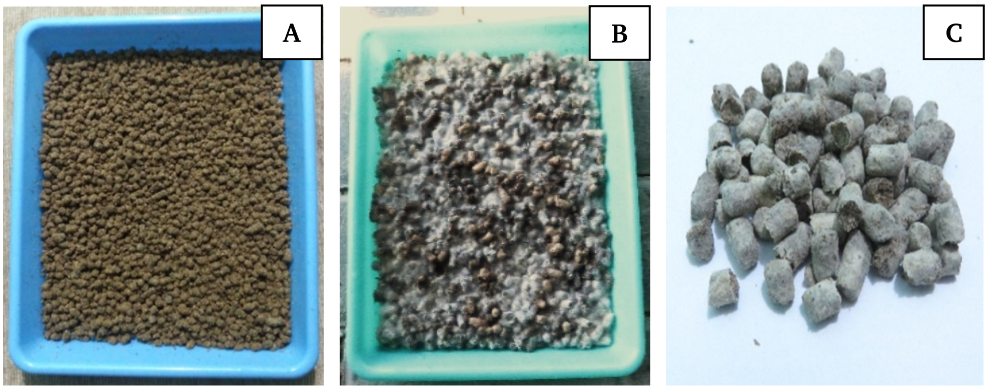

Figure 1. Formulated feed containing BSF larvae meal after being pelletized (A), then solid-fermented (B), and oven-dried (C).

Table 2. Results of proximate analysis of fermented and non-fermented feeds ($\bar{x} \pm \sigma, n = 3$).

Feed Code	Nutritional Content (%)					
	Moisture	Crude Protein	Crude Lipid	Crude Fiber	Ash	Carbohydrate (by difference)
F1	5.94 ± 0.12[c]	25.72 ± 0.11[d]	13.93 ± 0.53[a]	13.99 ± 0.62[a]	10.19 ± 0.02[b]	30.25 ± 1.12[c]
F2	7.64 ± 0.20[b]	28.32 ± 0.07[c]	12.89 ± 0.17[a]	14.89 ± 0.45[a]	11.44 ± 0.15[a]	30.83 ± 0.01[d]
F3	5.47 ± 0.19[c]	26.88 ± 0.16[e]	8.70 ± 0.01[b]	9.06 ± 0.08[c]	9.19 ± 0.11[c]	35.49 ± 0.22[a]
F4	6.26 ± 0.28[c]	29.76 ± 0.06[b]	7.79 ± 0.33[b]	11.82 ± 0.45[b]	10.73 ± 0.28[b]	28.56 ± 0.71[b]
CF	10.06 ± 0.23[a]	31.88 ± 0.15[a]	5.38 ± 0.01[c]	4.58 ± 0.18[d]	8.28 ± 0.06[d]	39.84 ± 0.16[a]

Note: Means values with the same superscript along the same column do not differ significantly ($P > 0.05$).

Overall, compared to the non-fermented feeds (F1 and F3), the fermented feeds (F2 and F4) resulted in better catfish growth as far as the average values of the growth parameters are concerned (Table 3). Upon ANOVA statistical analysis, however, most of the growth parameters were not significantly different between the feeding treatment of the non-fermented and fermented feeds. Feeding the tested catfishes either the non-fermented feed F1 or its fermented version F2 gave no significantly different values on all the growth parameters observed (Table 3).

The same was observed for the non-fermented F3 and its fermented version F4, except for the parameter FCR which was 17% lower for F4 relative to F3. Compared to all the other formulated feeds' FCR (> 2.00), that of F4 (1.78) was the lowest, hence being 15–25% more efficient. Across the fermented and non-fermented formulated feeds, F4 also showed the highest WG, SGR, and PER values. When compared to the commercial feed, however, the tested formulated feeds underperformed by about 40–50% in all the growth parameters tested (WG, FCR, SGR, and PER) with the exception of SR. SR values of all catfishes fed with the formulated feeds (93.33–100%), both fermented and non-fermented, were not significantly different from those fed with the commercial feed (96.67%) (Table 3).

Table 3. Growth performance of catfish fed with fermented and non-fermented feeds ($\bar{x} \pm \sigma, n = 3$).

Treatment	Parameter				
	WG	FCR	SGR	PER	SR
F1	8.00 ± 0.75^{d}	2.37 ± 0.24^{a}	1.27 ± 0.17^{cd}	0.31 ± 0.02^{c}	93.33 ± 5.77^{a}
F2	9.03 ± 0.49^{cd}	2.10 ± 0.11^{ab}	1.41 ± 0.12^{bd}	0.32 ± 0.01^{c}	100 ± 0.00^{a}
F3	9.87 ± 0.28^{bc}	2.15 ± 0.02^{a}	1.51 ± 0.04^{bc}	0.37 ± 0.01^{bc}	96.67 ± 5.77^{a}
F4	11.67 ± 0.61^{b}	1.78 ± 0.06^{b}	1.70 ± 0.09^{b}	0.39 ± 0.02^{b}	100 ± 0.00^{a}
CF	24.93 ± 1.05^{a}	0.99 ± 0.04^{c}	3.09 ± 0.20^{a}	0.78 ± 0.03^{a}	96.67 ± 5.77^{a}

Note: Commercial feed (CF) was used as positive control. Means values with the same superscript along the same column do not differ significantly ($P > 0.05$). Abbreviation: weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER), and survival rate (SR).

Measurements of SGR and WG on weekly basis (Figure 2) showed that, in general, fermented feeds F2 and F4 gave higher weekly SGR and WG relative to the non-fermented versions of the feeds, namely F1 and F3, respectively. Feeding the catfishes with F4 gave the best weekly profiles of SGR and WG among the other treatments, except for the positive control treatment using the commercial feed. The commercial feed showed the highest SGR of 3.54 on week 2, which is almost twice higher than that of F4, which had the highest SGR of all the formulated feeds with or without fermentation. A similar situation occurred to the WG values of the commercial feed and F4. When the error bars were taken into account, however, both SGR and WG weekly profiles of all the fermented- and non-fermented-formulated feeds were not significantly different.

Although the positive control commercial feed used in this study demonstrated the best growth performance on the catfish based on the values of WG, FCR, SGR, and PER, the fermented feeds clearly showed a positive impact on the catfish growth than the non-fermented feeds did. In general, although not significantly different when the error bars are taken into account, throughout the 5-week rearing periods, catfishes fed with the fermented feed F2 or F4 demonstrated higher SGR and WG than those fed with their non-fermented versions F1 and F3, respectively (Figure 2, Table 3). The fermented feed F4 showed the lowest FCR (1.78) which was 15–25% more efficient than the other 3 formulated feeds (fermented and non-fermented) with FCR values above 2.00.

Similar results were obtained when red sea breams (*Pagrus major*) were reared for 56 days and fed an experimental diet containing *Aspergillus oryzae*-fermented rapeseed meal, in which the tested fishes showed better SGR and WG than those fishes fed with a similar diet but containing non-fermented rapeseed meal (Dossou *et al*., 2019). In another study, WG and PER values were found to be higher in *Labeo rohita* fingerlings fed with *Aspergillus niger*-fermented jatropha protein concentrate compared to those fed the non-fermented jatropha protein concentrate (Shamna *et al*., 2015). Relative to raw *Jatropha curcas* kernel, naturally fermented *J. curcas* kernel, when supplemented in diets, resulted in better WG, SGR, FCR, and SR after feeding-test on African catfish *Clarias gariepinus* (Okomoda *et al*., 2020). A different result was, however, shown when *Labeo rohita* was fed with formulated feeds containing fermented and non-fermented de-oiled rice bran, respectively. Lower growth was observed for the fishes fed the diet supplemented with the fermented de-oiled rice bran (Ranjan *et al*., 2018).

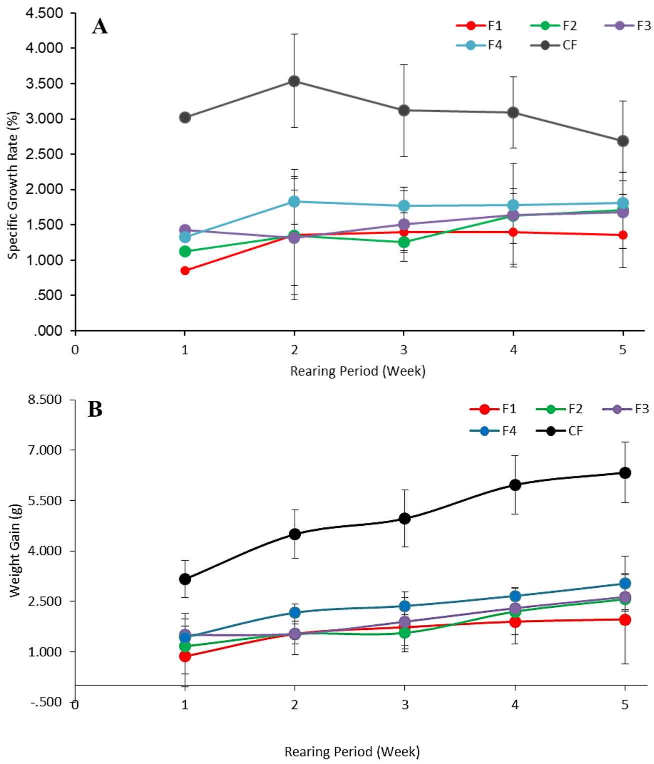

Figure 2. Weekly profiles of specific growth rate (SGR) (A) and weight gain (WG) (B) of catfishes fed with fermented and non-fermented feeds ($\bar{x} \pm \sigma, n = 3$).

Previous studies done by other authors demonstrated that better growth performance shown by fishes fed with diets containing fermented components at certain inclusion levels could be due to their simultaneous nutritional, functional, and immunological properties. For example, a study on juvenile gibel carp (*Carassius auratus* gibelio) fed with formulated feed augmented with up to 40% fermented moringa leaves as fish meal replacement showed enhancement on growth, immune response, and disease resistance (Zhang *et al*., 2020). Higher WG and SGR, protein digestibility, and health-promoting property were reported by Kari *et al*. (2022) when fermented soy pulp was used to replace 50% fish meal in the experimental diet given to African catfish (*Clarias gariepinus*). Dileep *et al*. (2021) replaced 25% fish meal with fermented guar and copra meal in a feeding test on Nile tilapia (*Oreochromis niloticus*). The yeast fermentation enhanced the protein and amino acid content, and lowered the antinutritional factors, increasing the weight gain of the Nile tilapia. Incorporating a solid-state fermentation product of *Aspergillus niger* in the test diets for carp (*Cyprinus carpio*) improved the feed utilization and growth performance (Anwar *et al*., 2020).

This study constitutes preliminary work to explore the possibility of using a solid fermentation techniques on BSF-containing fish feeds, in which tempeh mold was used as the fermenting microorganisms, to improve fish growth performance. Further works need to be carried out in order to determine whether the BSF meal component is significantly affected by the microbial bioconverting activity, and to what extent fermentation could increase the inclusion rate of the BSF component and replace the fish meal.

CONCLUSION

Solid state fermentation using tempeh starter increased the protein level of all the formulated pellets by 10–11%, but the crude lipid concentrations remained unchanged. On feeding test to *Catfish* sp., weekly-measured specific growth rate (SGR) and weight gain (WG) profiles for the fermented feeds F2 and F4 were relatively higher than the non-fermented versions F1 and F3, respectively. The fermented feed F4 showed the best feed conversion ratio (FCR) of 1.78, which was 15–25% more efficient than the other fermented and non-fermented formulated feeds (FCR > 2.00). Compared to the commercial feed as a positive control, all the tested feeds show poorer performance in all of the measured growth parameters, circa 50% lower than those of the commercial feed. The survival rate (SR) values were, however, not significantly different for all treatments (93–100%).

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