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The Usage of Black Soldier Fly (*Hermetia illucens*) Larvae Meal as Alternatıve Protein Source in Carp Diets (*Cyprinus carpio)*

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Research Article

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Abstract

A seventy-five-day feeding study was performed to investigate the usage of black soldier fly (*Hermetia illucens*) larvae meal as an alternative protein source in carp diets (*Cyprinus carpio)*. In this study, experimental fish were fed with four different diets (fish meal-based without Black soldier fly larvae (BSFL) meal (BSFL0), 50% (BSFL50), 65% (BSFL65), and 75% (BSFL75) of fish meal substitution with BSFL), with triplicate. The carps (1.045±0.083 g) were randomly stocked into twelve flow-through tanks with a density of 50 fish per tank and reared for 75 days. Diet feed was provided to carps as a proportion of their biomass (5% of their mass per day) with two meals a day. Carps fed with BSFL65 meal showed positive effects on weight gain (WG), specific growth rate (SGR), protein efficiency ratio (PER), apparent net protein utilization (ANPU), and feed conversion ratio (FCR). There were significant differences in moisture, crude protein, lipid, and ash content in the whole body among dietary groups (P<0.05). The protein content of the carp-fed BSFL75 diet group (20.22%) was significantly the highest among all three diet groups. Consequently, a BSFL meal can be considered as an alternative animal source of protein for the carp diet.

Key Words: Hermetia illucens, Insect meal, Fish meal replacement, Common carp.

Sazan (*Cyprinus carpio*) Diyetlerinde Alternatif Protein Kaynağı Olarak Siyah Asker Sineği (*Hermetia illucens*) Larvası Ununun Kullanımı

Özet

Yetmiş beş günlük besleme çalışmasında, Sazan (*Cyprinus carpio*) diyetlerinde alternatif protein kaynağı olarak Siyah Asker Sineği (*Hermetia illucens*) Larvası Ununun kullanım imkânları araştırılmıştır. Çalışmada, üç tekerrürlü olmak üzere, ASSL0 (Kontrol diyet grubu: sadece Balık unu), ASSL50 (Balık ununa ikame %50 ASSL), ASSL65 (Balık ununa ikame %65 ASSL) ve ASSL75 (Balık ununa ikame %75 ASSL) olacak şekilde dört farklı diyetle deneme balıkları beslenmiştir. Ortalama başlangıç ağırlıkları 1,045±0,083 g olan sazanlar (*Cyprinus carpio*) sürekli su akışının sağlandığı 12 adet tanka tesadüfi olarak ve her bir tanka stok yoğunluğu 50 adet olacak şekilde stoklanmış ve 75 gün süre ile besleme çalışması devam etmiştir. Deneme yemleri sazanlara vücut ağırlıklarının %5'i oranında sabah ve akşam olmak üzere günde iki kez verilmiştir. Deneme periyotu sonunda, ASSL65 diyet grubunda ağırlık kazancı, spesifik büyüme oranı, protein etkinlik oranı, net protein kullanım oranı ve yem değerlendirme oranı pozitif yönde etkilenmiştir. Diyet grupları arasında nem, ham protein, yağ ve kül içeriği açısından önemli farklılıklar tespit edilmiştir (P <0,05). ASSL75 diyet grubunun protein içeriği (% 20.22), diğer diyet grupları arasında önemli ölçüde yüksek seviyede bulunmuştur. Sonuç olarak, Siyah asker sineği larvası unu sazan diyeti için alternatif hayvansal protein kaynağı olarak düşünülebilir.

Anahtar Kelimeler: Hermetia illucens, Böcek unu, Balık unu ikame, Sazan

INTRODUCTION

Nutrition demand in aquaculture production is the most important factor because it comprises about 50% of the production costs. Fish meal is an important ingredient in aquatic feeds due to its highly digestible protein, amino acids, and also being palatable. However, the increasing demand for fishmeal and unbalanced fishmeal production caused the cost of aquaculture production to increase. The limited availability of natural resources and increasing prices have led both producers and researchers to alternative protein sources with less expensive and protein-rich animal or plant ingredients (Davis and Arnold, 2000; Zhou and Yue, 2012).

An insect is the ecosystem's largest community of organisms. In recent years, with the continual exploration and use of insect resources, the insect industry is gradually forming a healthy model of ecological development along with the agricultural industry and animal husbandry such as processing as feed protein (Sánchez-Muros et al., 2014). Besides, insects have attracted the attention of researchers due to their shorter production time and rich nutritious contents (Khan, 2018). In aquaculture, one of the most promising insect species for commercial operation is the Black soldier fly (*Hermetia illucens* L.). It has been reported that black soldier fly larvae contain 42.1% crude protein and the defatted black soldier fly larvae contain 56.9% crude protein (Makkar et al., 2014), which can be used as an alternative protein source as part of the natural diet of fish meal. Furthermore, the Larvae of the black soldier fly (*Hermetia illucens* L.) is one of the valuable feedstuffs, and has a better amino acid profile, and can replace better fish meal than soybean meal (Tran et al., 2015).

In many parts of the world, *H. illucens* is effectively used as a partial substitute of dietary fish meal in practical diets for finfish (freshwater and marine fish species) (Fasakin et al., 2003; Riddick, 2013; Magalhães et al., 2017; Belghit et al., 2019), shrimp (Cummins et al., 2017) and freshwater crustacean diets (Langer et al., 2011; Riddick, 2013). Li et al (2017) reported that at least 50% of fish meal protein could be replaced with black soldier fly meal without any adverse effects on growth performance, and digestive enzyme activities of juvenile Jian carp. Also, in the same study, it was observed that dietary stress and intestinal histopathological damage were observed when the level of soldier fly meal used exceeded 75% instead of fish meal. However, research on the optimum usage/application of black soldier fly larvae in the carp diet was scarce in the literature studies. Therefore, it has been revealed that studies should be conducted to determine the optimum usage rates of black soldier fly larvae in carp diets.

With this background, this study aims to investigate the effects of *H. illucens* larvae meal on growth performance, feed utilization, and body composition in common carp, to provide reference information for optimum usage rates of Black Soldier Fly larvae meal as a protein source.

MATERIAL and METHODS

Experimental Diets

The control diet (BSFL0) represented a commercial (Fish meal, FM100) diet where 50% of the oil was from a vegetable source (soya oil) and 50% from fish oil. The control diet (FM100) contained a 20 g 100 g^{-1} fish meal, which was gradually replaced by Black Soldier Fly (BSFL) larvae. Black soldier fly eggs were taken from Tamer Calikoglu Ankara, Turkey. The resulting larvae were grown in plastic containers (400×600×150 mm) in a climate chamber at 26–27°C and 60%– 70% humidity and were regularly fed restaurant waste once a day for 15 days. Restaurant waste was obtained from a student restaurant at Iskenderun Technical University and contained potatoes, rice, pasta, and vegetables. All larvae were harvested from a plastic container on day 15. Then, the larvae were washed in distilled water, drained, and smashed into a pulpous state with a pulverizer. So, BSFL larvae meal (40% protein, 45% lipid, 4800 Kcal kg^{-1}) were obtained for this experiment. The standard feed contained the ingredients; fishmeal (60% protein, 12% lipid, 2600 Kcal kg^{-1}), meat-bone meal, soybean meal, canola meal, cottonseed meal, sunflower meal, wheat bran, bentonite, mono-calcium, vitamin mixture. Three experimental diets were formulated, in which 50% (BSFL50), 65% (BSFL65), and 75% (BSFL) of the FM was replaced with BSFL, corresponding to dietary BSFL inclusion levels of 10 g 100 g^{-1}, 13 g 100 g^{-1} and 15 g 100 g^{-1}, respectively (Li et al., 2017) (Table 1). The same ingredients have been used for the BSFL meal diet with the only difference that 20% of the fishmeal has been substituted by BSFL, which in turn corresponded to 5% of the final diet. The feeds were prepared at the laboratory of the Faculty of Marine Science and Technology of Iskenderun Technical University, Iskenderun, Hatay, Turkey. All ingredient feed materials were blended in a kitchen robot for a homogenous mix, and sufficient water (400 g kg^{-1}) was added to the mix from soft dough. The obtained dough was passed through a mincer with a 2 mm diameter die. The diets were then dried at 40 °C and stored in plastic bags at -18 °C until feeding (Gasco et al., 2016). The feed formulation and proximate composition of the diets are presented in Table 1.

Table 1. Ingredients and proximate composition of the experimental diets.

	Diets			
	BSFL0	**BSFL 50**	**BSFL 65**	**BSFL 75**
Ingredients (g/100g)				
Fish meal	20	10	7	5
Black soldier fly larvae (*H. illucens*) meal	0	10	13	15
Meat-bone meal	11	15	16	17
Soybean meal	25	29	29	29
Canola meal	7	5	5	5
Cottonseed meal	6	5	5	5
Sunflower meal	16	16	16	16
Wheat bran	4	2	2	2
Fish oil	4	3	2	2
Soya oil	4	2	2	1
Bentonite	1.0	1.0	1.0	1.0
Mono-calcium	1.0	1.0	1.0	1.0
Vitamin mixture	1.0	1.0	1.0	1.0
Proximate composition (%)				
Dry matter*	88.95±0.03	89.05±0.04	88.88±0.03	89.12±0.07
Crude protein*	40.35±0.42	40.46±0.23	40.31±0.13	40.36±0.45
Lipid*	12.34±0.09	12.83±0.06	12.91±0.10	12.65±0.11
Ash*	12.75±0.02	12.35±0.03	11.98±0.04	12.05±0.01
Digestible Energy** (MJ/kg)	11.5	11.8	11.8	11.7

BSFL: Black Soldier Fly Larvae (*H. illucens*). All values except for digestible energy are reported as the mean of duplicate analyses. * Calculated from analyzed. ** Digestible energy determined according to 5.0 kcal/g protein, 9.0 kcal/g lipid, 2.0 kcal/g carbohydrate (Cowey et al., 1972).

Feeding Trial

The feeding trial was conducted at Iskenderun Technical University Aquaculture Research Facility located in Iskenderun, Hatay, Turkey. Before the start of the feeding trial, carps were acclimated to the environmental conditions for two weeks. A total of 600 fish (1.045±0.083 g) were randomly distributed into 12 recirculating tanks (approximately 100 L), at a density of 50 fish per tank. The fish used in the experiment were of equivalent size and weight. Water inflow was adjusted at 6 L min^{-1}, and supplemental aeration was provided via air stone. Before the beginning of the feeding experiment, the experimental carp were fasted for 24 h and weighed.

The four experimental diets were randomly assigned to triplicate tanks and where the following: (1) a fish meal-based diet without BSFL (control diet: BSFL0), three diets with an inclusion of 50% (BSFL50), 65% (BSFL65), and 75% (BSFL75) of fish meal substitution, corresponding to BSFL dietary inclusion levels of 50% 75%, respectively. Diets were provided to carps as a proportion of their biomass (5% of their mass per day). Faces and waste feed were siphoned daily before feeding. The daily total feed ratio was separated between two equal parts and delivered at 8:00 am and 5:00 pm. The tanks were housed in a room controlled with an automatic photoperiod timing system (12 L: 12 D cycle) and each tank was supplied with continuous aeration. The water quality parameters were monitored on weekly basis, and the following parameters were recorded: the water temperature, dissolved O_2, and pH maintained at 23.0±1.5°C, 5.75 ± 1.50 mg L^{-1}, 7.65 ± 0.5, respectively. Dead fish were weighed and the mortalities were recorded during the experiments.

After 75 days, all the carps in each tank were collected, weighed, and counted for calculation of weight gain (Watanabe et al., 1990), survival rate (Watanabe et al., 1990), specific growth rate (SGR) (Clark et al., 1990). Feed use is estimated by feed conversion ratio (FCR) (Steffens, 1989), the protein efficiency ratio (PER) (Steffens, 1989), and apparent net protein utilization (ANPU) (Bender and Miller, 1953). At the beginning of the experiment, 15 fish randomly were treated with an overdose of phenoxyethanol (1.5 mg l^{-1}) solution and stored at –20 °C for the determination of body proximate composition. At the end of the feeding trial, 5 fish from each dose group (n=20 fish\per dose) were analysed for the final whole-body proximate composition (AOAC 2000).

Statistical Analysis

The data were analyzed by using the Statistical Package for the Social Sciences software (SPSS, Version 21.0). The results were subjected to Levene's test to determine homogeneity of variance and no transformation was required. One-way ANOVA was used to determine the effects of the three diets on the various responses including growth parameters for all treatments. A post hoc Duncan's multiple range test was used to determine the statistical significance of the differences in means among the treatments (Zar, 1996). Results were considered to be significant at the (P<0.05) level. The mean results were presented with ± standard error (SE).

RESULTS

The effects of experimental diets on growth performance and survival rate are presented in Table 2.

Table 2. Effect of BSFLM on growth performance and survival rate of the experimental fish

	Dietary groups*			
	BSFL0	**BSFL50**	**BSFL65**	**BSFL75**
Initial weight (g)	1.024 ± 0.004^{a}	1.019 ± 0.001^{a}	1.121 ± 0.094^{a}	1.018 ± 0.003^{a}
Final Weight (g)	4.045 ± 0.109^{a}	4.901 ± 0.068^{b}	6.957 ± 0.442^{c}	4.787 ± 0.113^{ab}
Weight Gain (g)	3.021 ± 0.112^{a}	3.882 ± 0.069^{b}	5.836 ± 0.358^{c}	3.768 ± 0.112^{b}
Specific growth rate (%)	1.830 ± 0.039^{a}	2.093 ± 0.020^{b}	2.437 ± 0.051^{c}	2.062 ± 0.029^{b}
Feed conversion ratio	1.991 ± 0.074^{c}	1.546 ± 0.027^{b}	1.208 ± 0.074^{a}	1.594 ± 0.045^{b}
Protein Efficiency Ratio	1.258 ± 0.468^{a}	1.617 ± 0.029^{b}	2.084 ± 0.128^{c}	1.570 ± 0.046^{b}
Net Protein Utilization Rate	25.580 ± 0.930^{a}	30.633 ± 0.429^{a}	41.496 ± 3.307^{b}	39.891 ± 0.942^{b}
Survival rate (%)	74.66 ± 0.66^{a}	74.00 ± 1.15^{a}	74.00 ± 1.15^{a}	74.66 ± 0.66^{a}

*Values are means and standard errors with different superscripts in the same row are significantly different (P < 0.05) from each other (n=3). WG (Weight Gain) (g) = Final weight-Initial weight; Specific Growth Rate (SGR) (%) = (Ln final weight - Ln initial weight) / total number of the experimental days x 100; FCR (Feed Conversion Ratio) = Dry feed intake (g) / wet weight gain (g); PER (Protein Efficiency Ratio) = Live body weight gained (g) / protein intake (g); ANPU (Apparent Net Protein Utilization) (%)= (Protein retained / unit protein intake) x 100; Survival rate (%) = Final number of fish harvested / initial number of fish stocked × 100.

There were no significant differences in the initial weight of the experimental fish at the start of the trial (P>0.05). After 75 days of feeding, it was observed that there were significant differences in the growth performance of carps among the four diet groups (P<0.05). The mean final weight, weight gain (WG), and specific growth rate (SGR) of the carp fed with the BSFL65 diet were significantly (P<0.05) higher than those carp fed with the other three diet groups (BSFL0, BSFL50, and BSFL75) (Table2). The best weight gain (5.836±0.358 g) was found in the BSFL65 diet group and it was significantly higher than those of the other three groups (Table 2). These results indicated that the growth of carp was improved significantly by dietary Black soldier fly larvae incorporation. The highest mean specific growth rate (SGR) (2.437±0.051%) was obtained in the BSFL65 diet group whereas the lowest value (1.830±0.039%) was found in the group fed with a fish meal-based diet without BSFL (BSFL0) (Table 2).

Also, the best FCR (1.208±0.074), PER (2.084±0.128), and ANPU (41.496±3.307) were observed in the BSFL65 diet group were significantly (P<0.05) higher than that of carp fed with other three diet groups (BSFL0, BSFL50, and BSFL75) (Table 2). After 75 days of rearing, the survival rate (SR) of carp showed a range of 74.00%-74.66% (Table 2). There were no significant differences in the survival rate of the BSFL treated groups. During the study, there were no abnormal behaviors in the general activities of treated experimental carps.

Table 3. Effect of Black soldier fly larvae meal on proximate composition in tissues of fish*

	Dietary groups *			
	BSFL0	**BSFL50**	**BSFL65**	**BSFL75**
Moisture (%)	79.708±0.154[c]	79.296±0.265[bc]	78.670±0.353[b]	76.667±0.333[a]
Crude protein (%)	15.726±0.591[a]	16.059±0.244[ab]	17.509±0.549[b]	20.229±0.331[c]
Lipid (%)	4.564±0.642[ab]	4.644±0.377[b]	3.819±0.366[ab]	3.103±0.308[a]
Ash (%)	1.133±0.029[a]	1.802±0.085[c]	1.603±0.023[b]	1.767±0.048[bc]

* Values (% w.w.) are means and standard errors with different superscripts in the same row are significantly different (P < 0.05) from each other (n=5)

The statistical analysis results of the body composition of the experimental fish are shown in Table 3. There were significant differences in moisture, crude protein, lipid, and ash content in the whole body among dietary groups (*P*<0.05). The protein content of the carp-fed BSFL75 diet group (20.229%) was significantly the highest among all three diet groups (Table 3). The lipid content of BSFL treated groups was significantly lower than that of the control group (*P*<0.05).

DISCUSSION

The current study showed that replacement of dietary fish meal by Black soldier fly larvae had a significant effect on the growth performance and feed conversion ratio of carp, suggesting that it is possible to substitute up to 65% fishmeal by BSFL meal in diets for Common carp without negative effect on growth performance and feed utilization. Protein replacement in fish diets has been investigated using the meals obtained from both larvae and prepupae of Black soldier fly for the following fish species: turbot (*Psetta maxima*) (Kroeckel et al., 2012), hybrid tilapia (*Oreochromis niloticus X Oreochromis spilurus*) (Furrer, 2011), rainbow trout (*Oncorhynchus mykiss*) (Sealey et al., 2011), Atlantic salmon (*Salmo salar*) (Belghit et al., 2019), European seabass (*Dicentrarchus labrax*) (Magalhães et al., 2017), Jian carp (*Cyprinus carpio* var. Jian) (Li et al., 2017).

Similar results have been reported by Li et al. (2017) who indicated that adding up to 50% BSFL meal in the Jian carp diet showed good growth performance. Besides, they reported that dietary stress and intestinal histopathological damage were observed when the replacement levels exceeded 75% in some studies. For this reason, our study was conducted to determine the optimum usage rates of black soldier fly larvae meal in carp diets. Our results showed that the effect of growth performance and feed efficiency of common carp were influenced by dietary composition. The final weight, weight gain, and SGR values of carp-fed BSFL diets (65% black soldier fly larvae meal replacement) were significantly improved when compared to those of carp fed on control (BSFL0) and other diets (BSFL50, BSFL75).

Moreover, Magalhães et al. (2017) pointed out similar results in their studies that when the BSFL meal was included in the seabass diets, growth and feed conversion parameters improved. Besides, they reported that up to 19.5% of the prepupae of black soldier fly (*Hermetia illucens*) meal (corresponding to 22.5% of dietary protein) can replace 45% FM 'in diets for the European sea bass without any adverse effect on growth, and have positive effects on growth, feed use and digestive enzyme activity.

Previous studies have shown that adding insects to diets at different levels gives conflicting results in growth performance. Other studies in Nile tilapia found that replacing black soldier fly larvae with diet instead of the fish meal significantly reduced weight gain and specific growth rate, significantly improved feed intake and feed conversation rate (Webster et al., 2016). Kroeckel et al. (2012) reported that the replacement of black soldier fly larvae meal with diet instead of the fish meal significantly reduced the growth performance and feed utilization of turbot. These various results may be due to differences in fish species, fish size, insect species and substrates, and processing for the insect (Tschirner and Simon, 2015).

The survival rate was not significantly different among all groups (Table 2), suggesting that BSFL was palatable for *C. carpio*. The results of survival rate indicated that BSFL could be included in diets of carp without negative effects. Similar results have been found on juvenile mirror carp (*Cyprinus carpio*), Jian carp (*Cyprinus carpio),* yellow catfish (*Tachysurus fulvidraco*), and juvenile Jian carp (*Cyprinus carpio*) when BSF larvae were included in their diets (Li et al., 2016; Hu et al., 2017; Zhou et al., 2018; Xu et al., 2020). In the current study, it was found that the protein content of *C. carpio* body mass (whole proximate composition) was significantly influenced by adding BSFL meals (P<0.05) (Table 3). The highest protein content (20.229%) was observed in the diet feed with 75% BSFL meal (BSFL75), which had small differences from the other two groups (BSFL50 and BSFL65). The diet group (BSFL50) had the highest lipid content (4.644%) compared to the others and control group of BSFL. But, no significant differences were shown in whole-body lipid among the dietary treatments. Also, the values obtained from the whole body of carp were found to be between 76.66 and 79.70% for moisture and approximately 16-20% for protein, and these values are consistent with previous studies (Li et al., 2016; Zhou et al., 2018; Xu et al., 2020).

In conclusion, the results of this study clearly showed that the replacement of fishmeal with up to 65% BSFL meal in the diet of Common carp had positive effects on weight gain, specific growth rate, feed conversion rate, and protein efficiency ratio. This application may increase the expansion of the aquaculture industry. Therefore, we conclude that the insect meal made from BSF is a nutritionally appropriate source of protein for carp culture.

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