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Insect Larval Meal as A Possible Alternative to Fish Meal in Rainbow Trout (*Oncorhynchus mykiss*) Diets: Black Soldier Fly (*Hermetia illucens*), Mealworm (*Tenebrio molitor*)

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ABSTRACT

This study was conducted to determine the effect on growth performance and intestinal histomorphology of the rainbow trout (*Oncorhynchus mykiss*) fed diets including black soldier fly (*Hermetia illucens*) prepupae meal (HI) and mealworm (*Tenebrio molitor*) larvae meal (TM) used instead of fish meal. Six diets with HI and TM at three inclusion levels (10%, 20% and 30%) and a control diet based on fish meal were prepared. Test diets were encoded as control, HI10, HI20, HI30, TM10, TM20 and TM30. Fish (average initial weight of 34.17±0.88 g) were randomly placed (30 for each) in 500 L fibreglass tanks. Each of the seven diet treatments was tested in triplicated tanks. Fish were fed by hand at a level of 2.5% of body weight three times a day for 90 days. Results demonstrated that the growth performance and intestinal histomorphology were significantly affected by black soldier fly (HI) prepupae meal substitution ($p<0.05$). HI prepupae meal used instead of the fish meal had a negative effect on the growth performance, but not on the intestinal villi length. In addition, intestinal villi width decreased in fish on diets containing 10% or 20% HI prepupae meal. The growth performance and intestinal histomorphology were significantly affected by diets including TM. The diets containing 20% and 30% TM meal significantly decreased growth performance variables, but intestinal villi length increased. The results suggest that mealworm meal (10%) can be included in diets of rainbow trout at a level of 10% instead of fish meal without adversely affecting growth performance. Future studies should be expanded using a highly defatted TM and HI larvae meals.

Keywords: Aquaculture, trout, nutrition, growth, villus

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Gökkuşağı alabalığı (*Oncorhynchus mykiss*) yemlerinde balık ununa alternatif olarak böcek unu: Siyah asker sineği (*Hermetia illucens*), Un kurdu (*Tenebrio molitor*)

Öz: Bu çalışma balık unu yerine siyah asker sineği (*Hermetia illucens*) prepupa unu (HI) ve un kurdu (*Tenebrio molitor*) larva unu (TM) ile beslenen Gökkuşağı alabalığı (*Oncorhynchus mykiss*)'nın büyüme performansı ve bağırsak histomorfolojisi üzerine etkisini belirlemek için yapılmıştır. Bu amaçla, üç farklı seviyede (%10, %20 ve %30) HI ve TM içeren altı diyet ve balık unu esaslı bir kontrol diyeti test edilmiştir. Test diyetleri sırasıyla Kontrol, HI10, HI20, HI30, TM10, TM20 ve TM30 olarak kodlanmıştır. Balıklar (ortalama başlangıç ağırlığı 34,17±0,88 g) 500 L'lik fiberglas tanklarda (112x112 cm kare, 40 cm derinlikte) rastgele (her biri için 30) dağıtılmıştır. Yedi muamele 3 tekerrürlü olarak denenmiştir. Balıklar 90 gün boyunca günde üç kez vücut ağırlığının %2,5'i oranında elle beslenmiştir. Büyüme performansı ve bağırsak histomorfolojisi ile ilgili veriler siyah asker sineği (HI) prepupae unu ikamesinden önemli ölçüde etkilendiğini göstermiştir ($p<0,05$). Balık unu yerine kullanılan BSF prepupa unu büyüme performansı üzerinde olumsuz bir etki göstermişt ancak bağırsak villus uzunluğu üzerinde olumlu etki göstermiştir. Ek olarak, %10 veya %20 HI prepupa unu içeren diyetle beslenmede bağırsak villus genişliği azalmıştır. Büyüme performansı ve bağırsak histomorfolojisi, diyetsel un kurdu unu düzeylerinden önemli ölçüde etkilenmiştir. Zira, %20 ve %30 un kurdu unu içeren diyetlerle beslemede balıkların büyüme performansı azalırken, bağırsak villus uzunluğu artış göstermiştir. Sonuçlar, büyüme performansı üzerinde herhangi bir olumsuz etki göstermeksizin balık unu yerine un kurdu ununun (%10) kullanılabileceğini göstermiştir. Bununla birlikte, ilerideki çalışmaların yağı yüksek oranda alınmış un kurdu ve siyah asker sineği larva unu kullanımı üzerine genişletilmesi önerilerilebilir.

Anahtar kelimeler: Yetiştiricilik, alabalık, besleme, büyüme, villus

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Introduction

Aquaculture is the fastest-growing food-producing sector globally, with worldwide finfish production increasing by 90% during the last decade (Rimoldi et al. 2019). The expansion of the aquaculture industry means an increase in the need for high-quality fishmeal, especially for carnivorous species (Secci el al. 2019). One of the most critical issues that threatens the sustainability and further growth of intensive aquaculture of carnivorous species is the dependence on fishmeal and fish oil in aquafeeds (Cardinaletti et al. 2019). Given that this resource is now limited with the decrease in global availability and the rising price, the aquaculture industry is forced to find more sustainable and cost-effective alternatives to fish meal (Secci el al. 2019). Protein-rich vegetable raw materials used in practical diets for carnivorous fish species have many drawbacks such as relatively low protein content, unbalanced essential amino acid profiles, low palatability, presence of antinutrients and competition with other food-feed industry sectors. Nutritional strategies are being developed to find other alternatives to fishmeal and improve the use of plant protein-based diets (Magalhaes et al. 2017). Fishmeal and soybean meal widely used as protein sources in aquaculture feeds have become unsustainable due to the progressive depletion of wild marine fish stocks and the considerable environmental cost of protein-rich plant cultivation. The most promising animal alternatives to fish meal are nonruminant by-product meals and insect meals (Rimoldi et al. 2019). Compared to conventional animal protein, insects have several advantages such as cultivability on discarded organic by-products with low water input, high feed conversion efficiency, emission of low levels of greenhouse gases and ammonia, few animal welfare issues, and low risk of transmitting zoonotic infections (Magalhaes et al. 2017). Edible insects are considered potential feed source because they contain quality protein and other nutrients (Su et al. 2017). Insects grow and reproduce quickly, have high feed efficiency, do not compete with humans and other farm animals, and are part of the natural diet of many species (Iaconisi et al. 2019). Yellow mealworm (*Tenebrio molitor*) is among authorized insect species for nutrition, and it feeds on several grains, flour, and derived products. Yellow mealworm larvae can be easily cultured with low-nutritive plant and animal waste products, and they are commercially used as pet food for birds and reptiles or fishing baits.

T. molitor larvae meal having 47–60% protein and 31–43% lipids (Henry et al. 2018) was used as a feed ingredient for several fish species such as gilthead sea bream (*Sparus aurata*) (Iaconisi et al. 2019; Piccolo et al. 2017), yellow catfish (*Pelteobagrus fulvidraco*) (Su et al. 2017), rainbow trout (*Oncorhynchus mykiss*) (Chemello et al. 2020; Henry et al. 2018), common catfish (*Ameriurus melas*) (Roncarati et al. 2015). Taking into account the fish growth performance and the diets nutrient utilization, *T. molitor* is suitable for the partial replacement of fish meal in aquafeeds for a variety of fish species such as African catfish, tilapia, yellow catfish, common catfish, rainbow trout, European sea bass, gilthead sea bream, blackspot sea bream, and red seabream (Iaconisi et al. 2019). The attention of the aquafeed industry has mainly been directed to the use of insects since compared to other animal protein sources, they, in particular, flies show several advantages (Rimoldi et al. 2019). In recent years, research and risk assessment studies have also been carried out on the larvae of the *Hermetia illucens* (Dumas et al. 2018). Its potential as a feed ingredient has been reported for poultry, pigs, Atlantic salmon, channel catfish and blue tilapia, Nile tilapia, rainbow trout and turbot (Stadtlander et al. 2017). The crude protein and crude lipid contents of *H. illucens* larvae meal can vary from 40 to 54% and 15 to 49% (dry matter basis), respectively, depending on the substrates used to grow the insect larvae and processing methods (Dumas et al. 2018). A major drawback in using *H. illucens* meal is its lack of long-chain polyunsaturated fatty acids such as EPA and DHA and the presence of chitin. When 30% full-fat *H. illucens* prepupae meal was used in channel catfish and 15% in rainbow trout, a comparable growth performance to fish fed on fish meal-based diets was observed (Cardinaletti et al. 2019).

Many monitoring indices such as growth performance, diet digestibility, liver enzyme activities (Chemello et al. 2020), gut microbiota (Rimoldi et al. 2019), fillet fatty acid profile (Secci et al. 2019), blood biochemistry, liver and intestine histology, gene expression of cytokines (Cardinaletti et al. 2019), immune response, intestinal antioxidant enzymes (Henry et al. 2018) have been carried out in studies on rainbow trout. Even a study was also conducted on the possible effect of soldier fly on villus length and width (Dumas et al. 2018). However, no study has been found on villi histomorphology of TM, and the information obtained about both insect types is quite limited. With this study, the use of alternative insect meal in fish diets will contribute to the investigation of the gut absorption capacity. More studies are required to better understand the nutritional values of HI and TM for rainbow trout. Therefore, this study was planned to determine the effects of diets containing full-fat HI and TM meals substitution of fishmeal on growth performance and intestinal histomorphology in rainbow trout (*O. mykiss*).

Materials and Methods

Fish and Trial Design

This study was carried out in a freshwater research unit of Fisheries Research Institute, Elazığ, Türkiye. Rainbow trout (*O. mykiss*) used in the study were obtained from a private commercial farm located in Elazığ. After 21 days of adaptation, fish with an initial average weight of 34.17±0.88g were randomly distributed to 500 L (112x112 cm) experimental tanks with 40 cm depths. Each experimental tank was stocked with 30 fish. Seven experimental groups (Table 1), including a control diet, three *T. molitor* meal diets with TM inclusion levels of 10, 20 and 30% (coded as TM10, TM20, TM30, respectively) and three *H. illucens* prepupae meal diets with 10, 20 and 30% HI (coded as HI10, HI20 and HI30), respectively were tested in the study. All ingredients except the fish oil were mixed and extruded, and then fish oil penetrated with vacuum coating to feeds prepared. Trial feeds were prepared in a private enterprise after the rations were calculated. Feeds with 4 mm inner diameter were kept at room conditions and in a cool environment during trial period. Fish were fed by hand 2.5% of body weight three times a day (08:30, 12:30 and 16:00) for 90 days. Each experimental treatment was tested in triplicate. Water temperature (14.13±1.75ºC), pH (8.60±0.32), oxygen (8.39±0.64 mg/l) and mortality were checked daily. The water flow rate to each tank was adjusted to change the total volume 20 times per day. The tanks were daily cleaned by siphoning. 14 hours of darkness and 10 hours of light were applied. Fish were slightly anesthetised with 50 mg L^{-1} benzocaine (Oswald 1978) after by 16 hr of starvation for analyses. At the end of the experiment, fish were randomly selected from each tank and analyzed for growth performance and histomorphologic measurements.

Table 1. Formulation and proximate composition of experimenteal diets (%)

Ingredients, %	Control	HI10	HI20	HI30	TM10	TM20	TM30
Fish meal[1]	30	20	10	0	20	10	0
Soybean meal	21	21	21	21	21	21	21
Sunflower meal	9	9	9	9	9	9	9
Pea protein	6	6	6	6	6	6	6
Wheat gluten	4	6.5	8.9	11.4	5.4	6.8	8.2
Wheat flour	9	8.4	7.9	7.3	8.8	8.6	8.4
Corn gluten	9	9	9	9	9	9	9
Black soldier fly[2]	0	10	20	30	0	0	0
Mealworm[3]	0	0	0	0	10	20	30
Fish oil	11	9.1	7.2	5.3	9.8	8.6	7.4
Vitamin mix[4]	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Mineral mix[5]	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Vitamin C	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Proximate composition							
Crude protein	46.22	46.23	46.18	46.20	46.23	46.22	46.22
Crude lipid	15.35	15.36	15.38	15.39	15.34	15.35	15.36
Ash	8.77	8.81	7.71	7.50	6.60	8.62	8.54
Moisture	4.66	4.34	4.11	4.31	4.17	4.44	4.42

[1] Fish meal was a mixture of European sprat (*Sprattus sprattus)* and Atlantic herring (*Clupea harengus*) meals containing 65 % of crude protein and 8.5 % of crude lipid.
[2] Black soldier fly contained approximately 53 % protein and 24 %lipid
[3] Mealworm contained approximately 45 % protein and 31 % lipid
[4] Supplied the following (mg/kg diet): Vitamin A, 20000 IU, vitamin D 2000 IU, vitamin E 200 mg, vitamin K3 12 mg, vitamin B1 20 mg, vitamin B2 30 mg, calcium D pantothenate 50 mg, vitamin B6 20 mg, vitamin B12 0.05 mg, niacin 6 mg, folic acid 0.5 mg, biotin 200 mg, vitamin C 200 mg, inositol 300 mg
[5] Supplied the following (mg/kg diet): coper sulphate 10 mg, manganese oxide 50 mg, cobalt mono-carbonat 0.15 mg, zinc oxide 50 mg, calcium iodate 0.8 mg, sodium selenite 0.15 mg, ferric sulphate 50 mg

Diet Composisiton Analysis

Analysis of each diet was performed according to the standard methods of Helrich (1990): crude protein by Kjeldahl procedure, crude lipid by Soxhlet method, ash by drying in a muffle furnace and moisture using a forced-air oven.

Growth Indices

Growth performance was calculated using the following equations.

Spesific growth rate (SGR) % = 100 x [(In Final weight – In Initial weight) / days]

Weight gain (WG) = (In Final weight – In Initial weight)

Feed conversion ratio (FCR) = (Feed intake / Weight gain)

Survival rate (SR) = 100 x (Final number of fish / Initial number of fish)

Histomorphological Analysis

In histomorphological studies, tissues were cut as 1.0 cm pieces and placed into 10% formalin for dehydration. In the dehydration process, tissues were placed into cassettes and embedded in paraffin blocks. Then, cooled paraffin blocks were cut with a microtome equipped with the blade at 5µm. The tissue samples were transferred into slides and stained with hematoxylin and eosin solutions. Finally, stained samples were analyzed with ZEISS Primostar HD Light microscope and evaluated using an image processing system. Muscularis thickness, villi length (VL) and villi width (VW) were measured from ten fish from each group for histomorphologic analyses.

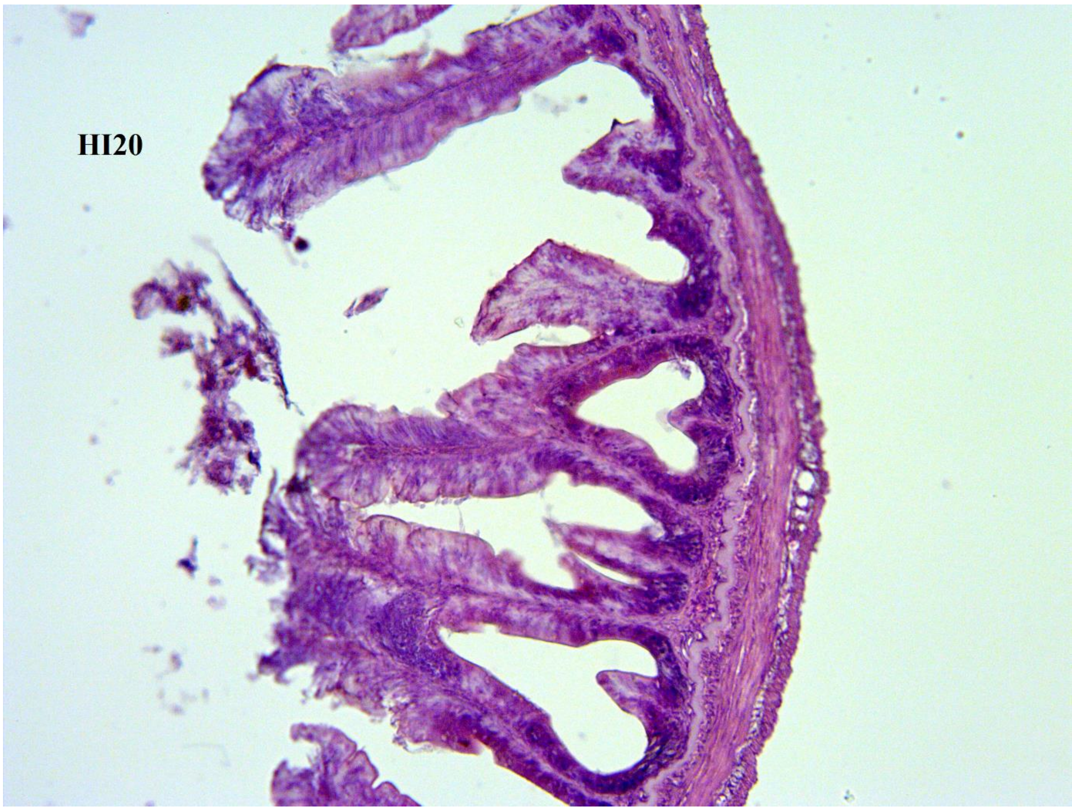
Data Analysis

Results were reported as means with standard errors. Data were submitted to one-way analysis of variance with SPSS 15. Duncan's multiple range test was performed for the significance of differences of means among the groups. The data were considered significant at the $P<0.05$ level. Two-way ANOVA analysis showed that the growth performance and intestinal histomorphology affected both insect meal and levels.

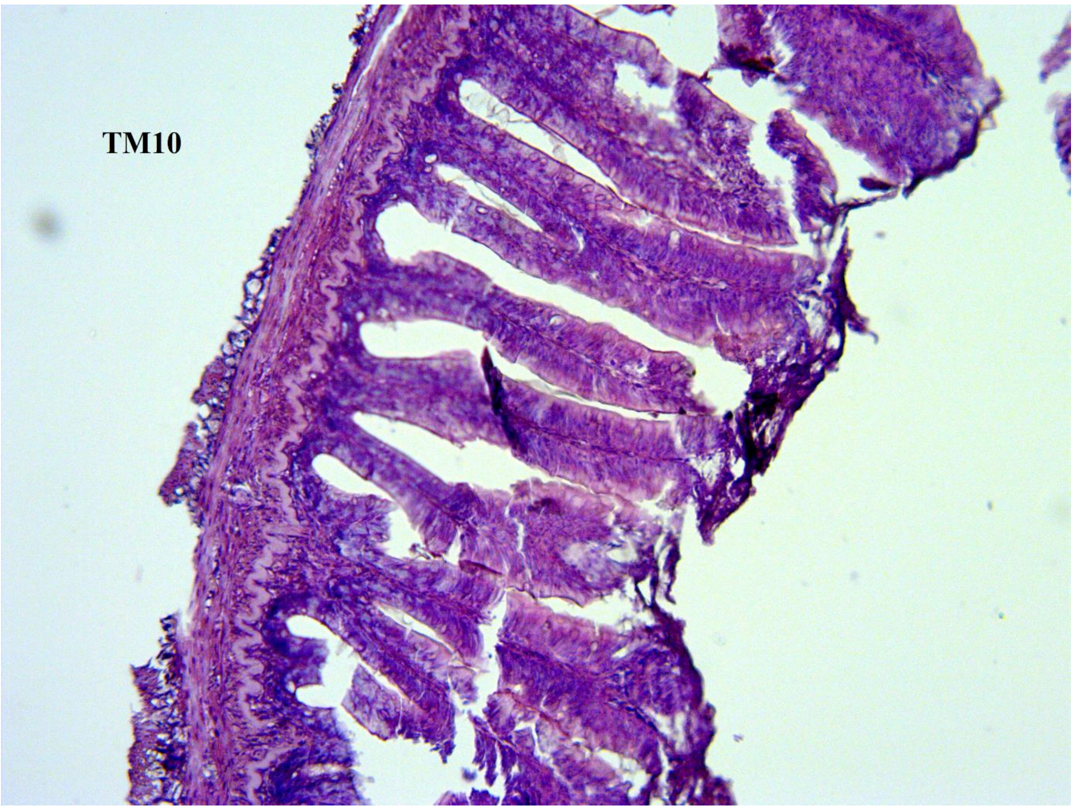
Results

Results are shown in Table 2, Table 3 and Figure 1. The growth performance and intestinal histomorphology were significantly influenced by dietary TM, and HI meal supplementation ($P<0.05$). Black soldier fly meal significantly decreased the growth performance, including FBW, FCR, WG and SGR. However, HI meal at a level of 10% did not affect feed intake. Besides, FI, FW, FCR, WG and SGR in the group fed with control diet were similar to those fed with dietary TM at a level of 10% ($p>0.05$). TM meal at a level of 30% significantly decreased the growth performance. Additionally, TM meal at a level of 20% significantly decreased the FW, WG and SGR. The growth performance decreased with the increase of dietary TM levels.


HI10


HI20


HI30


TM10

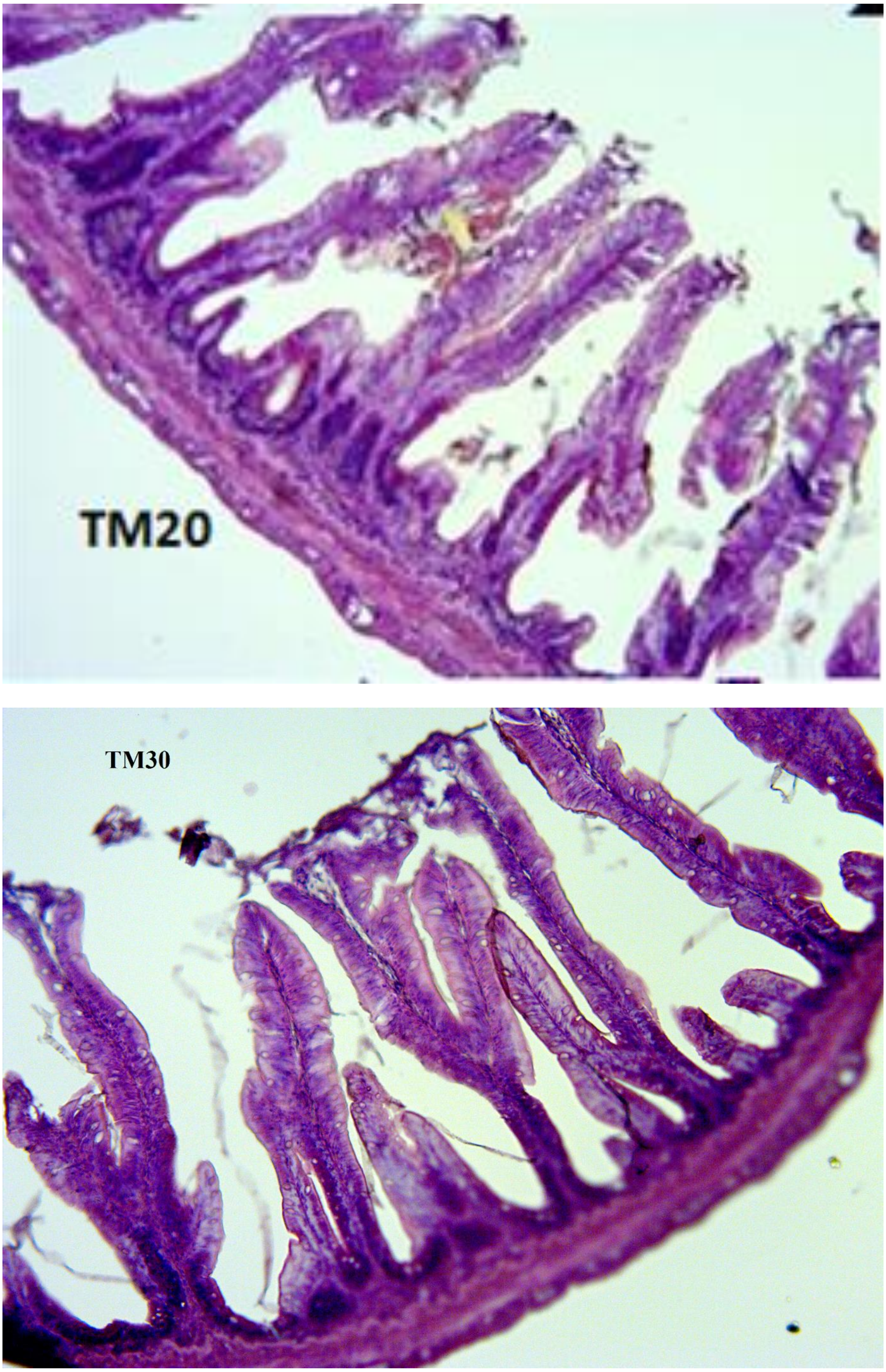


Figure 1. Intestinal histology of Rainbow trout fed with mealworm meal and Black soldier fly meal (H&E, 10X)

Table 2. Growth performance of Rainbow trout fed mealworm meal and black soldier fly meal

Items	Control	HI10	HI20	HI30	TM10	TM20	TM30	Two-way anova variances		
								A	B	AXB
IW	34.33±0.47	34.00±0.50	34.03±0.05	34.31±0.95	34.22±0.95	34.13±0.51	34.33±0.62	0.939	0.920	0.988
FI	126.98±6.4[a]	121.17±3.99[a]	103.92±2.28[b]	107.63±2.84[b]	124.89±3.82[a]	121.60±1.81[a]	90.66±0.94[c]	0.958	0.000	0.000
FW	158.92±4.2[a]	133.25±3.8[b]	107.15±4.04[d]	122.83±1.15[c]	152.49±1.26[a]	135.30±4.41[b]	91.16±2.27[e]	0.000	0.000	0.000
FCR	1.09±0.10[a]	1.30±0.02[a]	1.53±0.08[b]	1.23±0.06[ab]	1.12±0.04[a]	1.29±0.06[ab]	1.89±0.16[c]	0.445	0.009	0.000
WG	124.58±4.6[a]	99.25±3.39[b]	73.12±4.03[d]	88.51±2.07[d]	118.27±0.42[a]	101.17±4.37[b]	56.83±2.28[e]	0.000	0.000	0.000
SGR	1.70±0.04[a]	1.52±0.02[b]	1.27±0.04[d]	1.42±0.04[c]	1.66±0.02[a]	1.53±0.04[b]	1.09±0.03[e]	0.005	0.000	0.000
SR	82.22±2.94	82.22±4.01	84.44±1.11	90.00±1.92	83.33±1.93	84.45±2.22	81.11±2.94	0.483	0.571	0.143

Means with different superscript letters in a row are significantly different at p<0.05. IW (g): Initial body weight, FW (g): Final body weight, FI (g): Feed intake, FCR: Feed conversion ratio. A: First independent variable (insect meal), B: Second independent variable (levels), AxB: Interaction of two independent variables.

Table 3. Intestinal histomorphology of Rainbow trout fed mealworm meal and black soldier fly meal

Items	Control	HI10	HI20	HI30	TM10	TM20	TM30	Two-way anova variances		
								A	B	AXB
Villi length	443.24±12.60[b]	458.89±14.04[b]	446.30±8.31[b]	442.66±14.60[b]	454.67±13.08[b]	501.21±9.77[a]	514.22±19.10[a]	0.001	0.244	0.015
Villi width	83.80±4.03[a]	70.61±3.80[b]	69.20±3.04[b]	83.98±3.46[a]	73.51±1.85[b]	74.73±1.56[ab]	68.66±3.88[b]	0.010	0.308	0.003
Serosa	31.35±2.41[ab]	20.18±1.01[c]	26.68±2.39[b]	31.89±2.88[ab]	36.89±2.82[a]	31.31±1.57[ab]	30.63±2.15[ab]	0.002	0.839	0.371
Muscularis	45.52±4.04[bc]	33.02±1.68[d]	41.80±2.14[c]	49.55±3.52[bc]	66.29±3.29[a]	54.15±3.06[b]	48.19±2.19[bc]	0.000	0.851	0.000
Submucosa	24.40±2.58[ab]	21.48±0.67[b]	22.60±1.33[b]	22.72±1.44[b]	29.07±1.78[a]	26.45±1.28[ab]	25.96±1.91[ab]	0.001	0.432	0.001

Means with different superscript letters in a row are significantly different at p<0.05. A: First independent variable (insect meal), B: Second independent variable (levels), AxB: Interaction of two independent variables.

Intestinal villi width of fish fed with control diet were similar to those fed with dietary HI30. Similarly, muscularis thickness of fish fed with control diet was similar to those on HI20 and HI30. When intestinal histomorphological results were examined, groups fed with 20% and 30% TM had higher VL than the control group. While the highest VW was seen in the group fed with the control diet, the highest muscularis thickness was obtained in the group fed with TM10.

Discussion

Insect meal is a good candidate as a substitute for fishmeal in aquaculture feeds, and research on their use in fish feeds has revealed encouraging results (Roncarati et al. 2015). Among these potential sources, mealworm is considered a good alternative for the partial replacement of fish meal in the different fish species (Chemello et al. 2020). Besides, Terova et al. (2021) found that without causing negative effects on rainbow trout gut microbial communities, mealworm larvae meal is a valid alternative animal protein to replace fish meal in the aquafeeds. The addition of mealworm larvae meal instead of the fish meal does not have a negative effect on the growth performance of sea bream (*S. aurata*) (Piccolo et al. 2017), rainbow trout (*O. mykiss*) (Chemello et al. 2020), Yellow catfish (*P. fulvidraco*) (Su et al. 2017). African catfish fed a diet including 80% mealworm meal of fish meal showed a good growth and feed efficiency (Su et al. 2017). Although insect meal added to rainbow trout feeds had no effect on weight gain, feed conversion ratio and specific growth rate were significantly higher in TM0 since feeding rate was significantly higher in TM0 than TM50 (Belforti et al. 2015). In our study, *T. molitor* did not have a negative effect on the growth performance when included 10%. Studies on the effects of mealworm larvae meal on intestinal physiology in fish are lacking. Our results obtained with 20% and 30% mealworm were similar to those of Zadeh et al. (2019), who found that mealworm increased villi length in jejunum supplemented Japanese quails (*Coturnix japonica*). Besides, our results with mealworm at the level of 20% and 30% agree with the results of Roncarati et al. (2015), who reported that feeding with a dietary mealworm in substitution of 50% of fish meal decreased the final body weight of rainbow trout.

Black soldier fly has been recognized as a potential candidate ingredient due to its rich protein and lipid contents (Katya et al. 2017). However, the successful inclusion of black soldier fly into aquatic feeds depends on the fish species and the characteristics of insect derivatives (Dumas et al. 2018). Jia and Hing (2017) reported that black soldier fly prepupae meal alone could substitute fish meal as a protein source in the fish diet. Similarly, Magalhaes et al. (2017) found that BSF can be included in the diets of European sea bass juveniles up to 19.5%, replacing 45% of fish meal. Similar results were also obtained in juvenile mirror carp (*Cyprinus carpio* var. specularis) (Xu et al. 2020) and rainbow trout (Cardinaletti et al. 2019; Stadtlander et al. 2017). Magalhaes et al. (2017) stated that the addition of high levels (45%) of black soldier fly to the diet reduces the growth performance of channel catfish, *Ictalurus punctatus*, rainbow trout and turbot, *Scophthalmus maximus*. However, Stamer et al. (2014) reported that black soldier fly meal with 75% fish meal substitution decreased growth performance, including FW, WG, SGR and FCR. Similarly, in our study, dietary inclusion of full-fat black soldier fly meals significantly decreased growth performance. In a previous study, Dumas et al. (2018) reported that feeding with 6.6% and 13.2% black soldier fly larvae meal did not affect the growth performance of rainbow trout. In an additional study, Katya et al. (2017) reported that feeding with diets formulated to replace fish meal using processed black soldier fly larval meal at 100% decreased FCR, SGR and WG in juvenile barramundi (*Lates calcarifer*). Diets containing 6.6%, 13.2% and 26.4% Black soldier fly larva meal had no impact on villi length and villi width of the posterior intestine of rainbow trout, but villi in the anterior intestine of trout fed 26.4% black soldier fly larva meal was significantly lower compared to the control diet (Dumas et al. 2018). In our study, intestinal villi length in the middle section did not change, while villi width was significantly reduced when fish fed with diets containing10% and 20% black soldier fly larvae prepupae meal. We used full-fat insect meal to test diets in the study. Therefore, the difference in the results obtained in the studies may be due to adding partially defatted insect meal to the diet.

In conclusion, dietary 10% TM meal can be used instead of fish meal without adversely affecting growth performance. Moreover, diets containing 20 and 30% mealworm meal may have a potential to increase intestinal villi length. However, to obtain more effective results from the studies to be carried out on mealworm and black soldier fly meal, it is necessary to expand the experimental studies by highly reducing the lipid content of mealworm and black soldier fly larvae meal.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes and feed legislation.

References

Belforti M, Gai F, Lussiana G, Renna M, Malfatto V, Rotolo L, De Marco M, Dabbou S, Schiavone A, Zoccarato I, Gasco L. 2015. *Tenebrio Molitor* Meal in Rainbow Trout (*Oncorhynchus mykiss*) Diets: Effects on Animal Performance, Nutrient Digestibility and Chemical Composition of Fillets. Ital J Anim Sci. 14(4):669-676. doi: 10.4081/ijas.2015.4170

Cardinaletti G, Randazzo B, Messina M, Zarantoniello M, Giorgini E, Zimbelli A, Bruni L, Parisi G, Olivotto I, Tulli F. 2019. Effects of Graded Dietary Inclusion Level of Full-Fat *Hermetia illucens* Prepupae Meal in Practical Diets for Rainbow Trout (*Oncorhynchus mykiss*). Animals. 9(5):1-21. doi: 10.3390/ani9050251

Chemello G, Renna M, Caimi C, Guerreiro I, Oliva-Teles A, Enes P, Biasato I, Schiavone A, Gai F, Gasco L. 2020. Partially defatted *Tenebrio molitor* larva meal in diets for grow-out Rainbow trout, *Oncorhynchus mykiss* (Walbaum): Effects on growth performance, diet digestibility and metabolic responses. Animals. 10(2):1-15. doi: 10.3390/ani10020229

Dumas A, Raggi T, Barkhouse J, Lewis E, Weltzien E. 2018. The oil fraction and partially defatted meal of black soldier fly larvae (*Hermetia illucens*) affect differently growth performance, feed efficiency, nutrient deposition, blood glucose and lipid digestibility of rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 492:24-34. doi: 10.1016/j.aquaculture.2018.03.038

Helrich K. 1990. Official Methods of Analysis of the Association of Official Analytical Chemists, 15th edition. Arlington, Virginia, USA: Association of Official Analytical Chemists, Inc 1298 p.

Henry MA, Gai F, Enes P, Peréz-Jiménez A, Gasco L. 2018. Effect of partial dietary replacement of fishmeal by yellow mealworm (*Tenebrio molitor*) larvae meal on the innate immune response and intestinal antioxidant enzymes of rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immun. 83:308-313. doi: 10.1016/j.fsi.2018.09.040

Iaconisi V, Secci G, Sabatino G, Piccolo G, Gasco L, Papini AM, Parisi G. 2019. Effect of mealworm (*Tenebrio molitor* L.) larvae meal on amino acid composition of gilthead sea bream (*Sparus aurata* L.) and rainbow trout (*Oncorhynchus mykiss* W.) fillets. Aquaculture. 513:1-8. doi: 10.1016/j.aquaculture.2019.734403

Jia FT, Hing WN. 2017. Fish feed formulation using Black soldier fly pre-pupae from fruit waste. J Eng Appl Sci. 12(Special Issue 3):6393-6397.

Katya K, Borsra MZS, Ganesan D, Kuppusamy G, Herriman M, Salter A, Ali SA. 2017. Efficacy of insect larval meal to replace fish meal in juvenile barramundi, *Lates calcarifer* reared in freshwater. Int Aquat Res. 9:303-312. doi: 10.1007/s40071-017-0178-x

Magalhaes R, Sánchez-López A, Leal RS, Martínez-Llorens S, Oliva-Telesa A, Peresa H. 2017. Black soldier fly (*Hermetia illucens*) pre-pupae meal as a fish meal replacement in diets for European seabass (*Dicentrarchus labrax*). Aquaculture 476:79-85. doi: 10.1016/j.aquaculture.2017.04.021

Oswald RL. 1978. Injection anesthesia for experimental studies in fish. Comp Biochem Phys C. 60(1):19-26. doi: 10.1016/0306-4492(78)90021-7

Piccolo G, Iaconisi V, Marono S, Gasco L, Loponte R, Nizza S, Bovera F, Parisi G. 2017. Effect of Tenebrio molitor larvae meal on growth performance, in vivo nutrients digestibility, somatic and marketable indexes of gilthead sea bream (*Sparus aurata*). Anim Feed Sci Tech. 226:12-20. doi: 10.1016/j.anifeedsci.2017.02.007

Rimoldi S, Gini E, Iannini F, Gasco L, Terova G. 2019. The Effects of Dietary Insect Meal from *Hermetia illucens* Prepupae on Autochthonous Gut Microbiota of Rainbow Trout (*Oncorhynchus mykiss*). Animals. 9(4):1-17. doi: 10.3390/ani9040143

Roncarati A, Gasco L, Parisi G, Terova G. 2015. Growth performance of common catfish (*Ameiurus melas* Raf.) fingerlings fed mealworm (*Tenebrio molitor*) diet. Journal of Insects as Food and Feed. 1(3): 233-240. doi: 10.3920/JIFF2014.0006

Secci G, Mancini S, Iaconisi V, Gasco L, Basto A, Parisi G. 2019. Can the inclusion of black soldier fly (*Hermetia illucens*) in diet affect the flesh quality/nutritional traits of rainbow trout (*Oncorhynchus mykiss*) after freezing and cooking? Int J Food Sci Nutr. 70(2):161-171. doi: 10.1080/09637486.2018.1489529

Stadtlander T, Stamer A, Buser A, Wohlfahrt J, Leiber F, Sandrock C. 2017. *Hermetia illucens* meal as fish meal replacement for rainbow trout on farm. Journal of Insects as Food and Feed. 3(3):165-175. doi: 10.3920/JIFF2016.0056

Stamer A, Wesselss S, Neidigk R, Hoerstgen-Schwarl G. 2014. Black Soldier Fly (*Hermetia illucens*) larvae-meal as an example for a new feed ingredients' class in aquaculture diets. Paper presented at: 4 th ISOFAR Scientific Conference. 'Building Organic Bridges', at the Organic World Congress; Istanbul, Türkiye.

Su J, Gong Y, Cao S, Lu F, Han D, Liu H, Jin J, Yang Y, Zhu X, Xie S. 2017. Effects of dietary Tenebrio molitor meal on the growth performance, immune response and disease resistance of yellow catfish (*Pelteobagrus fulvidraco*). Fish Shellfish Immun. 69:59-66. doi: 10.1016/j.fsi.2017.08.008

Terova G, Gini E, Gasco L, Moroni F, Antonini M, Rimoldi S. 2021. Effects of full replacement of dietary fishmeal with insect meal from Tenebrio molitor on rainbow trout gut and skin microbiota. J Anim Sci Biotechno. 12:1-14. doi: 10.1186/s40104-021-00551-9

Xu X, Ji H, Yu H, Zhou J. 2020. Influence of dietary black soldier fly (*Hermetia illucens* Linnaeus) pulp on growth performance, antioxidant capacity and intestinal health of juvenile mirror carp (*Cyprinus carpio* var. specularis). Aquacult Nutr. 26(2):432-443. doi: 10.1111/anu.13005

Zadeh ZS, Kheiri F, Faghani M. 2019. Use of yellow mealworm (*Tenebrio molitor*) as a protein source on growthperformance, carcass traits, meat quality and intestinal morphology of Japanese quails (*Coturnix japonica*). Veterinary and Animal Science. 8:1-5. doi: 10.1016/j.vas.2019.100066