DIETARY INCLUSION OF *TENEbrio MOLITOR* MEAL IN SEA TROUT LARVAE REARING: EFFECTS ON FISH GROWTH PERFORMANCE, SURVIVAL, CONDITION, AND GIT AND LIVER ENZYMATIC ACTIVITY*

Lilianna Hoffmann¹*, Mateusz Rawski¹, Silvia Nogales-Merida², Jan Mazurkiewicz^{1,2}

¹Division of Inland Fisheries and Aquaculture, Institute of Zoology,
Poznan University of Life Sciences, Wojska Polskiego 71C, 60-625 Poznań, Poland

²HiProMine S.A., Poznańska 8, 62-023 Robakowo, Poland

*Corresponding author: lilianna.graczyk@up.poznan.pl

Abstract

Sea trout (*Salmo trutta m. trutta*) is a species for which effective methods of rearing are still being developed. They need high-quality protein in their diet, but, considering the ecological consequences of fishmeal production, new sources of protein are needed. Presently, insect meal is one of the most promising alternative sources of protein in the diets of farm animals. Insect production does not result in excessive gas emissions, waste generation or the risk of obtaining low-quality dietary protein. The study on sea trout larvae was carried out for 60 days. Four diets were given to the fish: one control (C), without the inclusion of insect meal, and three experimental, with one unhydrolysed *Tenebrio molitor* meal (TM) and two hydrolysed *Tenebrio molitor* meal treatments. The effects of the diets were evaluated on the basis of growth performance (weight and length of the fish, SR, SGR, RGR, FCR and PER), somatic indices (HSI and VSI) and fish condition (CF). We observed that the highest body mass and weight gain were obtained in the control group. However, the lowest mortality and the highest values of RGR were observed only in groups fed diets containing mealworm meal. The results for the hepato- and viscerosomatic indices showed a lack of statistically significant differences between the control group and the unhydrolysed *Tenebrio molitor* meal group in terms of the enzymatic activities of amylase, lipase and trypsin in the fish intestine and both aminotransferases in the liver. Our study demonstrated that the inclusion of 20% mealworm meal in practical diets for sea trout did not negatively affect growth performance or gastrointestinal tract (GIT) enzyme activity. The hydrolysed mealworm meal and the non-processed mealworm meal had similar effects.

Key words: insect meal, sea trout, larvae rearing, growth performance, hydrolysed protein

*This research was supported by several sources, i.e., grant no. 507.511.59 of the Young Researcher Program of the Faculty of Veterinary Medicine and Animal Science, Poznan University of Life Sciences, Poland, financed by the Polish Ministry of Science and Higher Education; TEAM TECH/2016-2/11-0026, project entitled Insects as novel protein sources for fish and poultry, financed by the Foundation of Polish Science (POIR 4.4); funds of the National Centre for Research and Development, no. POIR.01.01.01-00-0828/15, entitled InnSecta: innovative technology of feedstuffs production based on insect biomass; and by the funds of Poznań University of Life Sciences.

Aquaculture is a constantly growing area of animal farming worldwide; in the last decade (2008–2018), it increased by 5.8% (FAO, 2018). Currently, 598 species of animals are bred in aquaculture, of which almost 62% are fish. Some of these fish constitute an alternative to well-known and widely cultured species. Sea trout (*Salmo trutta* m. *trutta*) is one of these species; despite its high economic value, it is still harvested mainly from the wild.

For that reason, sea trout, as well as other salmonid fish species, require captive breeding programmes to conserve their biodiversity for the future (Fraser, 2008). Although its Red List status indicates that it is a species of least concern (CITES, 2017), in Poland, the sea trout is treated as a conservation-dependent (CD) species (Witkowski et al., 2009) despite numerous breeding programmes (Domagała et al., 2014). This is undoubtedly due to the biology of the species; migration causes significant losses in the population, and high environmental requirements. Decreasing water quality and fragmentation of habitats also leads to the disappearance of natural spawning grounds (Freeman et al., 2003).

In order to effectively rear sea trout, there is a strong emphasis on the first stages of development of this species, namely, hatching and larvae-rearing (Hansen, 1985; Was and Wenne, 2002; Alp et al., 2010). The survival of the larvae depends on biotic and abiotic conditions, which are correlated with proper growth and development (Hamre et al., 2013). Another commonly studied aspect of breeding sea trout is developing balanced, practical diets for the fry stage. The quality of the diet is crucial, especially the amino acid (AA) profile (Salze and Davis, 2015), as well as the origin of the protein source. Based on the well-known protein requirements of other morphs of the genus *Salmo trutta*, the most highly absorbable source of protein is fishmeal (Arzel et al., 1995). Fishmeal is still one of the most absorbable sources of protein, and thus, the majority of marine fish stocks have been overfished, which has resulted in reduced abundance and biodiversity (FAO, 2016). Fishmeal content in diets for fish is decreasing, and the use of soybean meal is constantly increasing (Stamer, 2015). To prevent the deforestation of rainforests for soybean crops, other available sources of protein should be used (Fearnside, 2001). Insects have major potential for animal nutrition. Although insects have modest fatty acid compositions in comparison to fishmeal, their utility as a component of aquaculture diets has been proven (Rumpold et al., 2017).

Currently, the mealworm (*Tenebrio molitor*) is an innovative and one of the most popular components of human diets and animal feeds (Van Huis et al., 2013). Mealworms are commonly used in breeding juvenile forms of fish as well as in the pre-growth stages in salmonids (Antonopoulou et al., 2019; Le Féon et al., 2019). Insects in animal feeds can be used for any livestock feed (Sánchez-Muros et al., 2014) because of their lower greenhouse gas emissions (Oonincx et al., 2010); insects can also be used for the bioconversion of manure into biodiesel (Li et al., 2011), the production of antimicrobial peptides (Hull et al., 2012) or the provision of chitin for medical purposes (Muzzarelli, 2010). The inclusion of mealworms in aquaculture diets varies, from about a few percent even up to 50%, but in most studies, satisfying rearing results occurred with inclusion at a level above 25% (Henry et al., 2015). The key to effective and feasible fish farming using insect meals may be the hydrolysis

of their protein, which improves the retention of the protein in the digestive system (Gilbert et al., 2008). Most hydrolysed proteins originate from marine resources and they are widely used in feeds for livestock (Pasupuleti and Braun, 2010; Chalamaiyah et al., 2012). However, there are limited data on the hydrolysis of insect material, despite the fact that insects are rich in bioactive peptides (Vercruysse et al., 2009).

Considering the facts mentioned above, the aim of this research was to examine hydrolysed and unhydrolysed mealworm protein in diets for sea trout (*Salmo trutta* m. *trutta*) fry. The effects were evaluated in terms of the growth performance and visceral and condition indices as well as laboratory analyses of changes in the enzymatic activities in the liver and intestine.

Material and methods

Ethics statement

The study was carried out in strict accordance with the recommendations of the National Ethics Commission (Warsaw, Poland). All procedures and experiments complied with the guidelines of the Local Ethics Commission of the Poznań University of Life Sciences (Poznań, Poland) with respect to animal experimentation and the care of the animals in the study, and all efforts were made to minimize animal suffering, strictly following the guidelines of the Polish law and the EU directive (no 2010/63/EU).

Insect meal preparation

The mealworm (*Tenebrio molitor*) larvae were fed with organic vegetable by-products at Hipromine S.A. (Robakowo, Poland). All insects were hatched for experimental purposes, reared at 28°C and fed vegetable by-products. The obtained insect biomass was frozen at -20°C and air-dried at 50°C until it reached a constant weight (24–48 h). The dried insects were finely ground with no additional conditioning. The chemical composition of full-fat insect meal and fishmeal were analysed by the independent and certified laboratory (JSH Hamilton Poland) and the results are shown in the table (Table 1) together with the amino acid composition of both protein components.

Table 1. Chemical composition of fish meal (FM) and *Tenebrio molitor* (TM) meals used in the experimental diets

Nutrients	FM	TM
1	2	3
Dry matter	90.5	92.3
Crude protein (% DM)	71.4	47.0
Alanine	4.28	3.99
Arginine	3.72	2.40

Table 1 – contd.

1	2	3
Aspartic acid	6.11	3.65
Glutamic acid	9.18	5.76
Glycine	4.13	2.53
Histidine	1.34	1.35
Isoleucine	3.02	1.99
Leucine	5.03	3.61
Lysine	5.03	2.53
Methionine	1.96	0.70
Phenylalanine	2.51	1.84
Proline	2.71	2.99
Serine	2.80	2.18
Threonine	2.77	1.87
Tyrosine	1.95	3.00
Valine	3.63	3.18
Crude fat (% DM)	12.1	29.6
Crude ash (% DM)	14.0	4.51
Phosphorus	0.49	0.69
Calcium	0.31	0.047

Explanations: % DM – percentage share of dry matter.

Hydrolysis procedure

The mealworm meal used in the study was hydrolysed using two endopeptidases: Colorase 7090 (AB Enzymes, Germany) and Flavourzyme (Novozymes, USA). The selection of hydrolysis parameters and of the assortment of enzymes used were made according to the literature (Ovissipour et al., 2010; Zheng et al., 2012; Wang et al., 2013; Minh, 2015).

Both enzymes were added to the insect meal in liquid form. Each enzyme was diluted with 500 ml of deionized water at 30°C. The enzymes were added to the insect meal at 0.5% (0.75 g/kg⁻¹ of protein) in the TMH_{0.5} diet and at 1.0% (1.5 g/kg⁻¹ of protein) in the TMH_{1.0} diet. The pH of the insect meal was measured three times: before adding enzymes and after adding each enzyme. The mean pH of the insect meal was 7.0, which is the optimum value for the activity of Colorase 7090® and Flavourzyme®. The insect meal with enzymes was placed in an incubator (Pol-Eko-Aparatura type ST 3 COMF), and the temperature was set at 50°C. The insect meal with Colorase 7090® spent 4 h in the incubator, and that with Flavourzyme® spent 3 h in the incubator. The order of enzyme addition was crucial because Flavourzyme® is an endopeptidase that removes a bitter taste from animal feed after enzymatic hydrolysis (based on the manufacturer's data). Therefore, Flavourzyme® was added as the second enzyme after the hydrolysis of the insect meal with Colorase 7090®.

Diets

Four diets were designed for sea trout fry: control (labelled as C; without insect meal) and three experimental diets with mealworm meal (TM) from the larval stage of the mealworm (*Tenebrio molitor* Linnaeus 1758), labelled TM, TMH_{0.5} and TMH_{1.0}. The TM diet included the unprocessed mealworm meal, while the TMH_{0.5} and TMH_{1.0} diets included the insect meal hydrolysed using endopeptidases at 0.5 and 1.0%. The diets for the sea trout fry were formulated using commercial ingredients, and the inclusion levels of full-fat insect meal in the experimental diets were 200 g · kg⁻¹ (Table 2).

Table 2. Experimental diet composition

Ingredients (g · kg ⁻¹)	C	TM	TMH _{0.5}	TMH _{1.0}
Fish meal ¹	306	276	276	276
Mealworm meal	–	200	–	–
Hydrolysed mealworm meal	–	–	200	200
Red blood cells (dried) ²	80	80	80	80
Casein	70	70	70	70
Squid meal	44	44	44	44
Krill meal	57	57	57	57
Wheat gluten	200	200	200	200
Whey meal	210	40	40	40
Fish oil	5	5	5	5
Lecithin	10	10	10	10
Premix ³	15	15	15	15
Vitazol AD ₃ E ⁴	1	1	1	1
Choline chloride ⁵	2	2	2	2
TOTAL	1000	1000	1000	1000

¹Danish fishmeal, type F, 720 g kg⁻¹ protein, 120 g kg⁻¹ fat, FFSkagen, Denmark. ²Spray dried, 900 g kg⁻¹ protein, APC Europe, Spain. ³Polfamix W, BASF Polska Ltd. Kutno, Poland – containing per 1 kg: vitamin A 1 000 000 IU, vitamin D₃ 200 000 IU, vitamin E 1.5 g, vitamin K 0.2 g, vitamin B₁ 0.05 g, vitamin B₂ 0.4 g, vitamin B₁₂ 0.001 g, nicotinic acid 2.5 g, D-calcium pantothenate 1.0 g, choline chloride 7.5 g, folic acid 0.1 g, methionine 150.0 g, lysine 150.0 g, Fe 2.5 g, Mn 6.5 g, Cu 0.8 g, Co 0.04 g, Zn 4.0 g, I 0.008 g. ⁴Vitamin premix AD3EC, BLOWET Drwalew, Poland – contains in 1 kg: vitamin A 50 000 IU, vitamin D₃ 5000 IU, vitamin E 30.0 mg, vitamin C 100.0 mg. ⁵Shandong Aoceter Chemical Co. Ltd, Shandong, China, 60% corn cob.

All the experimental diets were formulated to reach levels of protein and energy advised in the literature and practical diets: 55–60% of protein and 20–23 MJ/kg of gross energy (Arzel et al., 1995; Storebakken, 2002). The chosen nutrient requirements were also taken into consideration with other scientific literature (Cowey and Sargent, 1972; Hasan, 2001; National Research Council, 1981; National Research Council, 1993).

Table 3. Nutritional value of the experimental diets

Nutrient	Diet			
	C	TM	TMH _{0.5}	TMH _{1.0}
Dry matter (%)	93.50	93.90	93.69	93.73
Crude protein (% DM)	60.75	64.20	62.21	60.18
Crude fat (% DM)	6.09	4.56	6.51	6.67
Crude fibre (% DM)	1.61	2.85	3.60	3.57
Crude ash (% DM)	6.04	4.33	5.10	5.09
Gross energy (MJ kg ⁻¹)	19.95	19.97	19.98	19.91

Explanations: % DM – percentage share of dry matter.

The diets were prepared by extrusion processing with a single-screw warm extruder (Metalchem S-60 Gliwice, Poland). The processing conditions were as follows: 90°C cylinder temperature in the increasing pressure zone, 100°C cylinder temperature in the high-pressure zone, 110°C head temperature, 52 rpm speed screw and 6 mm nozzle diameter. After that, each feed was ground separately using a mechanical grinder and sieved with a sieve shaker (LPzE-3e, Multiserv, Brzeźnica, Poland) to obtain the correct granule sizes with final diameters of 0.5, 1.0 and 1.5 mm.

Technical conditions of the growth trial

The study was carried out in the internal fish rearing hall in open flow tanks, and the growth trial lasted for 60 days. The applied water temperature was $15^{\circ} \pm 1^{\circ}\text{C}$, controlled by a thermostat (temperature regulator type R-3001, KS, Luboń, Poland). The content of dissolved oxygen in the water, above 70%, was established by a HIBLOW HP-40 membrane blower (Hiblow, Japan).

All of the tanks for the fish were made of fibreglass with a capacity of 60 dm³. The construction of all the tanks allowed the maintenance of the maximum water level with a constant water flow, while the individual water inlet and outflow systems allowed cyclic control catches for all fish. Every day at 8 a.m., all residues from the bottom of the tanks were removed. During the growth trial, artificial light in the rearing hall was applied using a Bemko type TSGE2 digital programmed timer (Bemko, Warsaw, Poland). The applied photoperiod was 16 h of light (from 7 a.m. to 9 p.m.) and 8 h of darkness.

At the beginning and end of the growth trial and at the end of every 10-day period, the fish were measured and weighed with a solution of MS-222. The total body length (*longitudo totalis*) was measured from the mouth to the end of the caudal fin using a S_Cal PRO IP67 digital calliper (Sylvac, Switzerland) for a sample of 20 fish per tank. The body weight was recorded on the basis of the total number of fish and their entire biomass in each tank.

Fish

The study was carried out on sea trout fry (*Salmo trutta* m. *trutta*, Linnaeus 1758). A total of 2400 sea trout larvae of 0.14 g indiv.⁻¹ mean body weight were randomly allocated per experimental tank. All animals were divided into four groups in three

replicates for each diet (200 fish per tank). The experimental design of the study is shown in Figure 1.

Figure 1. Experimental design of sea trout feeding experiment

Explanations: Diet C – control diet with fishmeal instead of insect meal as protein source; TM – diet with unhydrolysed full-fat insect meal, consisting of mealworm beetle (*Tenebrio molitor*); TMH_{0.5} – diet with hydrolysed above-mentioned full-fat insect meal using 0.5% mixture of enzymes; TMH_{1.0} – diet with hydrolysed mealworm beetle, using 1.0% mixture of enzymes

Tissue sampling

At the end of the experiment, fish were euthanized using an overdose of MS-222 (Leary et al., 2013) and decapitated for dissection and sampling for further analysis. Three samples of liver and intestine per tank were taken and immediately frozen at -80°C .

Growth performance and visceral and condition indices

The effects of the diets were evaluated on the basis of stock biomass, diet utilization and dissection of the digestive system. The equations recommended by Hardy and Barrows (2002) were used to calculate factors such as SR, SGR, RGR, FCR and PER:

1. Survival rate (SR, %):

$$SR = (N_t \times N_o^{-1}) \times 100$$

where N_t is the final number of fish (pc) and N_o is the initial number of fish (pc).

2. Specific growth rate (SGR, % day⁻¹):

$$SGR = (100 \times (\ln w_t - \ln w_o) \times t^{-1})$$

where w_o is the initial mean fish weight (g), w_t is the final mean fish weight (g), and t is the number of study days.

3. Relative growth rate (RGR, %):

$$RGR = [100 \times (w_t - w_o)^{-1}]$$

where w_o is the initial mean fish weight (g) and w_t is the final mean fish weight (g).

4. Feed Conversion Ratio (FCR):

$$FCR = f_d \cdot (w_f - w_o)^{-1}$$

where f_d is the weight of the diet (g), w_f is the final mean fish weight (g), and w_o is the initial mean fish weight (g).

5. Protein Efficiency Ratio (PER):

$$PER = (w_f - w_o) \times P^{-1}$$

where w_o is the initial mean fish weight (g), w_f is the final mean fish weight (g), and P is the weight of the protein in the fish diet (g).

The indices for the evaluation of the effect of diet on the liver and viscera and on fish condition were calculated as described by Piccolo et al. (2017) with some modifications as follows:

1. Hepatosomatic Index (HSI, %):

$$HSI = (l_f \times w_f^{-1}) \times 100$$

where l_f is the final liver weight (g) and w_f is the final mean fish weight (g).

2. Viscerosomatic Index (VSI, %):

$$VSI = (v_f \times w_f^{-1}) \times 100$$

where v_f is the final viscera weight (g) and w_f is the final mean fish weight (g).

3. Fulton's Condition Factor (CF):

$$CF = [w_f / (l_g)^3] \times 100$$

where w_f is the final mean fish weight (g) and l_g is the total length of the body (cm).

Aminotransferase activity in liver

The determination of aminotransferase activity in the liver was performed as previously described by González et al. (2012) with some modifications. In brief, liver samples were homogenized with cell lysis buffer (50 mM Tris-HCl, pH 7.5, 4 mM EDTA, 50 mM NaF, 0.5 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM dithiothreitol and 250 mM sucrose) and centrifuged at $12000 \times g$ for 30 minutes at 4°C. Moreover, the activity of both transaminases (alanine aminotransferase, ALT and aspartate aminotransferase, AST) were analysed using commercial Pointe Scientific kits (Canton, United States) and were expressed in IU per g of tissue.

GIT enzymatic activity

For the enzymatic activity in the intestine, the inner part of the intestinal sample was frozen, homogenized using PBS buffer and centrifuged at $9000 \times g$ for 20 minutes at 4°C. Next, the supernatant was transferred into new tubes, divided into parts and stored for analysis.

For amylase, the supernatant was diluted 1 000 times using a commercially supplied buffer. Amylase activity was measured with an amylase activity colorimetric assay kit from Alpha Diagnostic (Warsaw, Poland). For lipase activity, the supernatant was diluted 100 times in PBS buffer, and its activity was measured with a lipase activity colorimetric assay kit from Pointe Scientific (Warsaw, Poland). The trypsin activity was measured using a trypsin activity colorimetric assay kit from BioVision (Milpitas, USA). The supernatant was diluted 10 times using PBS and incubated for

30 minutes at 37°C with 1% enterokinase from Sigma–Aldrich (St. Louis, United States) and diluted in 0.1 M Tris-HCl with 0.1 M CaCl₂, pH 7.2 to convert the trypsinogen. All procedures were performed according to the manufacturer's instructions. The results are shown as % of the control.

Statistical analysis

All obtained data were tested for normality of distribution using the Kolmogorov-Smirnov test. Analysis of variance was conducted using Bartlett's test. The significance of differences among groups was determined by Duncan's multiple range test (for growth performance and diet utilization) at the significance level of $P \leq 0.05$. The calculations were tested using SAS® Studio 3.5 software (SAS Institute Inc., 2016). The following general model was used:

$$Y_i = \mu + \alpha_i + \delta_{ij}$$

where Y_i was the observed dependent variable, μ was the overall mean, α_i was the effect of the diet, and δ_{ij} was the random error.

For the GIT enzymatic activity, the calculations and graphs were performed in GraphPad Prism, version 5 (GraphPad Software, San Diego, USA) as well as the above-mentioned parameters.

Results

Growth performance and diet utilization

There were no statistically significant differences in the average body length (for the whole period of the experiment), weight or increase in body mass at the first phase of the experiment (Table 4). During the second month of the growth trial, the statistically significant differences occurred only in the increase in body mass between the control and the experimental treatments ($P=0.0046$). At the end of the experiment, statistically significant differences were noted for average body mass and its increase with P -values of 0.0104 and 0.0134, respectively. Despite the differences between the control group and the experimental treatments, there were no statistically significant differences among the experimental groups.

The highest survival of the fish during the whole experiment (days 1–60) was noted in the group fed the TM diet (71.00%), while the lowest survival was observed in the control group (54.67%) (Table 5). However, there were no statistically significant differences in the mortality in the group fed the control diet, which increased by over 16%. Furthermore, SGR and FCR showed a similar tendency: the only statistically significant differences were noted during the second month of the experiment. Despite the fact that the highest final result of SGR and the lowest value of the FCR were found in the control group, the P -value was more than 0.05 ($P=0.0730$ for SGR and $P=0.0755$ for FCR, respectively). In the case of RGR and PER, there were no statistically significant differences among the groups.

Table 4. Effect of diet on average total length of the body, body weight and its increase¹

Period of time	Diet				SEM	P-value
	C	TM	TMH _{0.5}	TMH _{1.0}		
Average total length of the body (mm)						
1	26.71±2.46	25.59±1.56	26.13±2.21	26.15±2.05	0.1801	0.2016
30	38.40±0.54	38.31±0.46	39.35±0.45	38.60±0.54	0.2515	0.4585
60	53.20±0.65	53.36±0.80	53.33±0.60	51.90±0.62	0.3383	0.3626
Average body mass (g indiv ⁻¹)						
1	0.18±0.02	0.16±0.01	0.16±0.01	0.17±0.01	0.0022	0.2353
30	0.50±0.03	0.47±0.05	0.50±0.01	0.47±0.01	0.0082	0.4463
60	1.35 a±0.09	1.17 b±0.11	1.18b±0.04	1.07 b±0.02	0.0355	0.0104
Increasing of body mass (g indiv ⁻¹)						
1–30	0.32±0.02	0.31±0.05	0.34±0.01	0.30±0.01	0.0084	0.4463
30–60	0.86 a±0.09	0.70 b±0.06	0.68b±0.04	0.60 b±0.02	0.0320	0.0046
1–60	1.18 a±0.09	1.01 b±0.11	1.02b±0.04	0.90 b±0.01	0.0350	0.0134

¹Mean values in each row with different letters are significantly different (P<0.05).

Table 5. Effect of diet on survival and nutrient retention¹

Period of time	Diet				SEM	P-value
	C	TM	TMH _{0.5}	TMH _{1.0}		
1-30	66.83±6.01	73.00±8.05	70.50±6.06	74.83±7.01	1.9093	0.5421
30-60	81.90 a±3.10	97.09 a±2.37	96.22 b±3.01	94.14 b±5.46	2.0554	0.0032
1-60	54.67±4.07	71.00±9.54	67.83±6.21	70.50±8.66	2.7156	0.0836
SGR (% day⁻¹)						
1-30	3.47±0.09	3.51±0.46	3.76±0.09	3.41±0.10	0.0714	0.3940
30-60	3.34 a±0.28	3.03 b±0.07	2.84 b±0.08	2.74 b±0.11	0.0803	0.0079
1-60	3.41±0.16	3.27±0.20	3.30±0.01	3.08±0.02	0.0476	0.0730
RGR (%)						
1-30	89.24±13.80	110.41±33.58	117.81±23.66	109.03±26.09	6.9951	0.5782
30-60	124.12±25.97	141.24±4.89	125.47±11.33	114.17±15.46	4.9691	0.3091
1-60	322.01±27.78	407.27±77.69	390.05±44.63	346.93±57.56	16.958	0.2841
FCR						
1-30	1.11±0.09	1.18±0.16	1.06±0.07	1.21±0.06	0.0308	0.3200
30-60	1.07a±0.03	1.32 b±0.06	1.37 b±0.02	1.42 b±0.09	0.0431	<0.001
1-60	0.82±0.09	1.03±0.14	1.00±0.09	1.12±0.13	0.0427	0.0755
PER						
1-30	1.33±0.13	1.51±0.31	1.60±0.16	1.51±0.23	0.0617	0.5175
31-60	2.02±0.24	2.18±0.03	2.06±0.10	1.91±0.16	0.0474	0.2610
1-60	1.77±0.12	1.94±0.10	1.90±0.07	1.76±0.08	0.0341	0.1051

¹Mean values in each row with different letters are significantly different (P<0.05). Explanations: SR – survival rate, SGR – specific growth rate, RGR – relative growth rate, PER – protein efficiency ratio.

Hepatosomatic Index (Table 6) showed statistically significant differences between groups C and TMH_{0.5} (P=0.0168) at the end of the experiment. The TM and TMH_{1.0} diets had similar results as the control. In the case of the Viscerosomatic Index, the results showed statistically significant differences between all treatments, except the groups fed diets containing hydrolysed insect meal (TMH_{0.5} and TMH_{1.0}). Fulton’s Condition Factor showed a lack of statistically significant differences between groups C, TM and TMH_{1.0} (P=0.0013).

Table 6. Results of somatic and condition indices after 60 days of feeding¹

Index	Diet				SEM	P-value
	C	TM	TMH _{0.5}	TMH _{1.0}		
HSI	1.18 a±0.06	1.42 ab±0.12	1.55 b±0.06	1.38 ab±0.05	0.0444	0.0168
VSI	8.99 a±0.15	10.41 b±0.22	11.49 c±0.28	11.65 c±0.38	0.2226	0.0032
CF	1.11 a±0.09	1.06 a±0.07	0.98 b±0.07	1.09 a±0.07	0.0134	0.0013

¹Mean values in each row with different letters are significantly different (P<0.05).
Explanations: HSI – hepatosomatic index, VSI – viscerosomatic index, CF – Fulton’s condition factor.

GIT enzymatic activity

The results for the changes in the GIT enzymatic activity in sea trout fry are shown in the figure below (Figure 2). There were no statistically significant differences among treatments in the values of intestinal amylase (P-value=0.7577), li-pase (P-value=0.8859) or trypsin (P-value=0.5254). In the case of trypsin, the lowest amount of the enzyme occurred in the TMH_{1.0} group because less trypsin is required for the digestion of smaller proteins (e.g., the feed after enzymatic hydrolysis). The values of both aminotransferases obtained similar values, and there were no statisti-cally significant differences among the treatments (P-value > 0.05).

Figure 2. Enzymatic activity of sea trout GIT

Discussion

Insect meal and growth, somatic and condition indices

Belforti et al. (2015) examined a partial replacement of fishmeal (FM) with mealworm (TM) in diets for rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792). The authors found that the replacement of 25% FM with TM improved weight gain (WG), FCR, SGR and SR. The mortality in fish fed with TM meal was approximately 10–11% lower than that in fish fed with a control diet, which is in good agreement with the results of the present study. The PER index showed a similar tendency as in the above-mentioned study; it was increased by the presence of the insect meal in the fish diet, which decreased the feed intake. However, the analysis of the HSI and VSI indices showed an opposite trend. Belforti et al. (2015) showed decreased values of the HSI and VSI indices for TM diets in comparison to those for a diet composed mainly of FM. The reduced feed intake and the results of the somatic indices were probably affected by the high levels of valuable fats and the unique composition of fatty acids in mealworm meal. The same FM substitution model was used by Gasco et al. (2016) in a study on a European sea bass (*Dicentrarchus labrax* Linnaeus 1758). The lowest mortality was observed in the control group, but there were no statistically significant differences among treatments. In contrast, the results of the SGR index showed statistically significant differences between all groups, with the highest value in the group fed the FM diet. Interestingly, the FCR was similar for all treatments. On the other hand, the PER index was not affected by the presence of insect meal in the experimental diets, which corresponds to the discussed study. Likewise, Belforti et al. (2015) and Gasco et al. (2016) underlined that a diet with more than 50% mealworm meal does not cover the requirements of PUFA for some species of fish. Similar findings to those of Belforti et al. (2015) were made in a study by Ng et al. (2001), carried out on African catfish (*Clarias gariepinus* Burchell); after 20% replacement of FM by TM, the highest WG and SGR were observed, with mortality of approximately 7%. Nevertheless, when the level of FM replacement exceeded 40%, the growth performance, in particular, weight gain, SGR, FER (Feed Efficiency Ratio) and PER, was decreased. The authors suggested that the results might be caused by the increased level of chitin in the diet. In a study of black bullhead (*Ameiurus melas* Rafinesque 1820), Roncarati et al. (2015) showed statistically significant differences between treatments with fishmeal and mealworm meal. Notwithstanding, the authors noted that insect meal did not inhibit fish growth, whereas the mortality and final body weight were lower in the group fed TM. Another study, also carried out on the *Siluriformes*, was performed by Su et al. (2017) on yellowhead catfish (*Pelteobagrus fulvidraco*). The authors used three different levels of TM in the fish diets, and they observed the highest Feed Conversion Efficiency (FCE) and Feed Rate (FR) with the inclusion 9% of mealworm meal. However, as the authors emphasized, the obtained differences were not statistically significant, though their results correspond to those obtained by Belforti et al. (2015). Studies carried out by Piccolo et al. (2017) on gilthead seabream (*Sparus aurata* Linnaeus 1758) showed a higher percentage of viscera in the TM treatments and a lower biomass of the liver in the control group. SGR, PER, FCR and Fulton's Condition Factor (CF) obtained

the most satisfactory results when mealworm inclusion was 25%; this corresponds to the previous studies (despite the decreased digestibility of the proteins) and the present investigation. In the studies performed on the omnivorous blackspot seabream (*Pagellus bogaraveo* Brünnich 1768), Iaconisi et al. (2017) examined a partial substitution of FM with TM. In comparison to the previous findings, there were no statistically significant differences in the FCR, SGR or final body weight among the treatments. Interestingly, the values of HSI, VSI and CF indices showed the same tendencies as in the above-mentioned studies and the present study. Sánchez-Muros et al. (2016) examined a partial replacement of soybean meal (SBM) with mealworm meal. The results showed that inclusion at 430 g kg⁻¹ did not inhibit feed intake but significantly reduced growth performance. The highest CFs were obtained for the group with the substitution of soybean meal with mealworm meal, but there were no statistically significant differences. Nevertheless, the authors suggest that the use of insects in growth trials for fish requires a better understanding of the digestion of chitin in the gastrointestinal tract.

Hydrolysed material used in fish feeds

To obtain easily digestible proteins, enzymatic or acidic hydrolysis can be carried out. Hydrolysis of insect material was conducted by Vercruysse et al. (2009), who used endopeptidase on the cotton leafworm, *Spodoptera littoralis*. The authors suggested that insect proteins may contain biologically active peptides, which can be used as a source of natural antioxidants. Another study conducted on housefly (*Musca domestica*) larvae showed the functional properties of its hydrolysate using two endopeptidases (Wang et al., 2013). On the other hand, Fasakin et al. (2003) fed an African catfish with hydrolysed maggot meal, processed with a combination of mineral and carboxylic acids. Increased crude protein and reduced lipid levels were observed as well as an increased level of mineralization in the maggot meal. Moreover, the hydrolysed maggot meal improved the growth performance of the fish, which is in good agreement with the results of the present study. Another growth trial with hydrolysed material was carried out by Gildberg et al. (1995) on the Atlantic salmon (*Salmo salar*, Linnaeus 1758) fry. Fish were fed unhydrolysed and hydrolysed cod muscle (mixed with a concentrate of metalloprotease) and a hydrolysate culture of lactic acid bacteria (LAB) isolated from salmon intestines. The results showed that, despite low colonization of the salmon intestine by the LAB, the mortality of the fry was lower in the group fed hydrolysed and unhydrolysed cod fillets. An alternative study carried out on post-smolt salmon (Refstie et al., 2004) showed improved SGR and final weight in fish fed a fish protein hydrolysate (FPM) from pollock hydrolysed with protease. However, the authors underlined the higher feed intake (FI) and FER in the groups fed the FPM diets. Hevrøy et al. (2005) also fed post-smolts of Atlantic salmon with FPM from herring (*Clupea harengus*) processed with endopeptidase. The SGR was significantly different in the group fed with 18 and 24% FPH, but other indices (FCR, FI, HSI, CF) did not show the above-mentioned tendency. However, the authors recommended FPH as an ingredient that has no negative impacts on the health of the fish. Studies by Aksnes et al. (2006) and the results for SGR, SR and CF showed that the hydrolysed fish and plant material in feed for rainbow trout could

be used as a replacement for fishmeal in diets. Murray et al. (2003) worked on the juvenile Coho salmon (*Oncorhynchus kisutch*) and tested a hydrolysed or cooked raw material, which was a partial substitute for FM. The authors observed the highest CF in groups fed with the hydrolysed diets as well as the length and weight of the fish body.

GIT enzymatic activity

Based on the study of Torrisen et al. (1994), the weight of the fish is crucial in terms of the individual enzymatic activity in the GIT – as the fish weight increases, the enzymatic activity of the gastrointestinal tract also increases. The fish diet protein source is also important in digestion processes. Dąbrowski et al. (1989) showed the negative effects of replacing fishmeal with soybean meal; later studies showed that soybean meal could lead to histological changes in the salmonid intestine and decrease its absorption (Baeverfjord and Krogdahl, 1996; Kaushik et al., 1995; Krogdahl et al., 1994). The activity of amylase depends on the feeding behaviour, species, pH and temperature (Krogdahl et al., 1999). Herbivorous or omnivorous fish species have a lower amount of digestive protease enzymes (Hidalgo et al., 1999), which should be taken into consideration during comparisons of the GIT enzymatic activity. Sea trout, like other salmonids, is a carnivorous species, and the enzymatic activity of amylase will be lower than that in non-predator fish (Hidalgo et al., 1999). In a study evaluating dehulled lupin in diets for rainbow trout (Farhangi and Carter, 2001), the authors did not observe any statistically significant differences in the activities of amylase and trypsin (P -value > 0.05), even if the content of dehulled lupin in the diet was over 50%. Moreover, the SR for the groups fed the experimental diets was 100%, and the value of the HSI index was similar in all treatments. In a study by Furné et al. (2005), the authors observed that in the case of rainbow trout, the activity of amylase was a few times greater than the activities of lipase and proteases, which is in good agreement with the results of the present study. Moreover, in comparison to the Adriatic sturgeon (*Acipenser naccarii*), the authors emphasized that the above-mentioned species is characterized by poorer utilization of nutrients. After 60 days of starvation in both species (Furné et al., 2008), the amylase activity was affected faster than those of the other enzymes (lipases or proteases), but the recovery of the salmonid fish was faster. Frøystad et al. (2006) cloned α -amylase in Atlantic salmon and noticed its high similarity to the amylases in the other fish species, but with a sequence including a seven-amino acid deletion. The authors suggested that this could be the reason for lower activity of α -amylase in the Atlantic salmon. In another study carried out on Atlantic salmon, Refstie et al. (2006 a) showed the effects of supplementing the fish diet with inulin and oxytetracycline, which did not modify the influence of SBM on the gut morphology or enzymatic activity (amylase, trypsin). Santigosa et al. (2008) studied a replacement of FM with a mixture of plant protein sources, and Refstie et al. (2006 a) observed decreases in α -amylase and protease activity in rainbow trout and increasing levels of trypsin in gilthead seabream, which occurred, in the authors' opinion, in order to obtain a digestive balance. Azarm et al. (2013) studied the addition of two types of phospholipid to the diets of rainbow trout. They showed that the inclusion of soybean or egg lecithin did not affect the

growth performance (CF, FCR, SR, SGR, and WG), but increased the specific activity of amylase or lipase in the experimental groups; the authors suggested that this is caused by decreased lipid droplet accumulation in the intestine. Sajjadi and Carter (2004) focused on the effects of phytic acid and phytase on i.a. trypsin activity, and there were no statistically significant differences between all treatments. The authors underlined the fact that protease activity could be blocked by phytic acid, but it is still a poorly understood issue in fish. In a study carried out on Atlantic salmon (Refstie et al., 2006 b), trypsin activity varied in different parts of the intestine, and it was also correlated with diet. Soybean meal increased the amount of trypsin in faeces and reduced the nutrient absorption mechanisms. The i.a. trypsin activity was examined by Penn et al. (2011) as well as Refstie et al. (2006 b), and the authors showed the increased trypsin activity in the distal part of the intestine and the enteropathy caused by a high inclusion level of the pea protein concentration. There are few papers about the influence of diet (especially with the inclusion of insect meal) on the levels of aminotransferases in salmonids. Hevrøy et al. (2005) showed that the addition of hydrolysed material in the diet did not negatively affect the levels of ALT and AST. In earlier findings (Martin et al., 2003), it was emphasized that the source of protein in the diet could impact amino acid metabolism, e.g., the plant protein increased the levels of some aminotransferases. According to Sánchez-Muros et al. (1998), a diet rich in protein will be reflected in high levels of ALT in salmonids, which is in good agreement with our study. The differences between the levels of ALT and AST were reported as normal in Sandnes et al. (1988), as observed in the current study.

Conclusion

This study has shown that the inclusion of 20% insect meal from *Tenebrio molitor* does not negatively affect growth performance or GIT enzymatic activity. Despite slightly reduced growth (in terms of SGR and body weight), the groups fed insect meal were characterized by the highest survival rate, which is extremely important at the first stage of fish development. The findings suggest that the GIT enzymatic activity in sea trout fry could be moderated by their diet. In the case of trypsin, it was demonstrated that some part of the dietary protein (after hydrolysis) could be digested without the participation of enzymes. Nevertheless, future work is needed to establish the proper dose of insect meal and its influence on fish health. However, mealworm meal in a proportion of 20% could be applied in practical diets for salmonids.

References

- Aksnes A., Hope B., Jönsson E., Björnsson B.T., Albrektsen S. (2006). Size-fractionated fish hydrolysate as feed ingredient for rainbow trout (*Oncorhynchus mykiss*) fed high plant protein diets. I. Growth, growth regulation and feed utilization. *Aquaculture*, 261: 305–317.
- Alp A., Erer M., Kamalak A. (2010). Eggs incubation, early development, and growth in fry of brown trout (*Salmo trutta macrostigma*) and black sea trout (*Salmo trutta labrax*). *Turkish J. Fish. Aquat. Sci.*, 10: 387–394.

- Antonopoulou E., Nikouli E., Piccolo G., Gasco L., Gai F., Chatzifotis S., Mente E., Kormas K.A. (2019). Reshaping gut bacterial communities after dietary *Tenebrio molitor* larvae meal supplementation in three fish species. *Aquaculture*, 503: 628–635.
- Arzel J., Mctaville R., Kerleguer C., Le Delliou H., Guillaume J. (1995). The protein requirement of brown trout (*Salmo trutta*) fry. *Aquaculture*, 130: 67–78.
- Azarm H.M., Kenari A.A., Hedayati M. (2013). Effect of dietary phospholipid sources and levels on growth performance, enzymes activity, cholecystokinin and lipoprotein fractions of rainbow trout (*Oncorhynchus mykiss*) fry. *Aquacult. Res.*, 44: 634–644.
- Baeverfjord G., Krogdahl A. (1996). Development and regression of soybean meal induced enteritis in Atlantic salmon, *Salmo salar* L., distal intestine: a comparison with the intestines of fasted fish. *J. Fish Dis.*, 19: 375–387.
- Belforti M., Gai F., Lussiana C., 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 filets. *Ital. J. Anim. Sci.*, 14: 670–676.
- Chalamaiah M., Kumar B.D., Hemalatha R., Jyothirmayi T. (2012). Fish protein hydrolysates: Proximate composition, amino acid composition, antioxidant activities and applications: A review. *Food Chem.*, 135: 3020–3038.
- CITES, UNEP-WCMC. (2017). The Checklist of CITES Species Website. Appendices I, II and III valid from 04 April 2017. CITES Secretariat, Geneva, Switzerland. Compiled by UNEP-WCMC, Cambridge, UK.
- Cowey C.B., Sargent J.R. (1972). Fish nutrition. *Adv. Mar. Biol.*, 10: 383–494.
- Dąbrowski K., Poczyżynski P., Köck B., Berger B. (1989). Effect of partially or totally replacing fish meal protein by soybean meal protein on growth, food utilization and proteolytic enzyme activities in rainbow trout (*Salmo gairdneri*). New in vivo test for exocrine pancreatic secretion. *Aquaculture*, 77: 29–49.
- Domagała J., Dziwulska K., Czerniawski R. (2014). Survival rate and growth in the wild of sea trout (*Salmo trutta* L.) fry obtained using frozen semen. *Arch. Pol. Fish.*, 22: 265–270.
- FAO (2016). The state of world fisheries and aquaculture. Contributing to food security and nutrition for all. Rome, Italy 200 pp.
- FAO (2018). The state of world fisheries and aquaculture, meeting the sustainable development goals. Rome, Italy 210 pp.
- Farhangi M., Carter C.G. (2001). Growth, physiological and immunological responses of rainbow trout (*Oncorhynchus mykiss*) to different dietary inclusion levels of dehulled lupin (*Lupinus angustifolius*). *Aquacult. Res.* 32 (Suppl. 1): 329–340.
- Fasakin E.A., Balogun A.M., Ajayi O.O. (2003). Evaluation of full-fat and defatted maggot meals in the feeding of clariid catfish *Clarias gariepinus* fingerlings. *Aquacult. Res.*, 34: 733–738.
- Fearnside P.M. (2001). Soybean cultivation as a threat to the environment in Brazil. *Environ. Conserv.*, 28: 23–38.
- Fraser D.J. (2008). How well can captive breeding programs conserve biodiversity? A review of salmonids. *Evol. Appl.* 1: 535–586.
- Freeman M.C., Pringle C.M., Greathouse E.A., Freeman B.J. (2003). Ecosystem-level consequences of migratory faunal depletion caused by dams. In: Biodiversity, status, and conservation of the world's shads, Limburg K.E., Waldman J.R. (eds). American Fisheries Society, Symposium Bethesda, Maryland, 35: 255–266.
- Frøystad M.K., Lilleeng E., Sundby A., Krogdahl A. (2006). Cloning and characterization of alpha-amylase from Atlantic salmon (*Salmo salar* L.). *Comp. Biochem. Physiol. A Mol. Integr. Physiol.*, 45: 479–492.
- Furné M., Hidalgo M.C., López A., García-Gallego M., Morales A.E., Domezainé J., Sanz A. (2005). Digestive enzyme activities in Adriatic sturgeon *Acipenser naccarii* and rainbow trout *Oncorhynchus mykiss*. A comparative study. *Aquaculture*, 250: 391–398.
- Furné M., García-Gallego M., Hidalgo M.C., Morales A.E., Domezainé J., Sanz A. (2008). Effect of starvation and refeeding on digestive enzyme activities in sturgeon (*Acipenser naccarii*) and trout (*Oncorhynchus mykiss*). *Comp. Biochem. Physiol. A Mol. Integr. Physiol.*, 149: 420–425.

- Gasco L., Henry M., Piccolo G., Marono S., Gai F., Renna M., Lussiana C., Antonopoulou E., Mola P., Chatzifotis S. (2016). *Tenebrio molitor* meal in diets for European sea bass (*Dicentrarchus labrax* L.) juveniles: Growth performance, whole body composition and in vivo apparent digestibility. *Anim. Feed Sci. Technol.*, 220: 34–45.
- Gilbert E.R., Wong E.A., Webb K.E. (2008). Board-invited review: Peptide absorption and utilization: Implications for animal nutrition and health. *J. Anim. Sci.*, 86: 2135–2155.
- Gildberg A., Johansen A., Bøgwald J. (1995). Growth and survival of Atlantic salmon (*Salmo salar*) fry given diets supplemented with fish protein hydrolysate and lactic acid bacteria during a challenge trial with *Aeromonas salmonicida*. *Aquaculture*, 138: 23–34.
- González J.D., Caballero A., Viegas I., Metón I., Jones J.G., Barra J., Fernández F., Baanante I.V. (2012). Effects of alanine aminotransferase inhibition on the intermediary metabolism in *Sparus aurata* through dietary amino-oxyacetate supplementation. *Br. J. Nutr.*, 107: 1747–1756.
- Hamre K., Yúfera M., Rønnestad Boglione C., Conceição L.E.C., Izquierdo M. (2013). Fish larval nutrition and feed formulation: knowledge gaps and bottlenecks for advances in larval rearing. *Rev. Aquacult.*, 4: 526–558.
- Hansen T. (1985). Artificial hatching substrate: effect on yolk absorption, mortality and growth during first feeding of sea trout (*Salmo trutta*). *Aquaculture*, 46: 275–285.
- Hardy R.W., Barrows F.T. (2002). Diet formulation and manufacture. In: *Fish Nutrition*, Halver J.E., Hardy R.W. (eds). 3rd ed. Academic Press Inc., San Diego, CA, USA, pp. 506–601.
- Hasan M.R. (2001). Nutrition and feeding for sustainable aquaculture development in the third millennium. In: *Aquaculture in the Third Millennium*, Subasinghe R.P., Bueno P., Phillip M.J., Hough C., McGladdery S.E., Arthur J.R. (eds). Technical Proceedings of the Conference on Aquaculture in the Third Millennium, Bangkok, Thailand, 20–25 February 2000. NACA, Bangkok and FAO, Rome, pp. 193–219.
- Henry M., Gasco L., Piccolo G., Fountoulaki E. (2015). Review on the use of insects in the diet of farmed fish: Past and future. *Anim. Feed Sci. Technol.*, 203: 1–22.
- Hevrøy E.M., Espe M., Waagbø R., Sandnes K., Ruud M., Hemre G.-I. (2005). Nutrient utilization in Atlantic salmon (*Salmo salar* L.) fed increased levels of fish protein hydrolysate during a period of fast growth. *Aquacult. Nutr.*, 11: 301–313.
- Hidalgo M.C., Urea E., Sanz A. (1999). Comparative study of digestive enzymes in fish with different nutritional habits. Proteolytic and amylase activities. *Aquaculture*, 170: 267–283.
- Hull R., Katete R., Ntwasa M. (2012). Therapeutic potential of antimicrobial peptides from insects. *Biotechnol. Mol. Biol. Rev.*, 7: 31–47.
- Iaconisi V., Marono S., Parisi G., Gasco L., Genovese L., Maricchiolo G., Bovera F., Piccolo G. (2017). Dietary inclusion of *Tenebrio molitor* larvae meal: Effects on growth performance and final quality traits of blackspot sea bream (*Pagellus bogaraveo*). *Aquaculture*, 476: 49–58.
- Kaushik S.J., Cravedi J.P., Lalles J.P., Sumpter J., Fauconneau B., Laroche M. (1995). Partial or total replacement of fish meal by soybean protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, 133: 257–274.
- Krogdahl A., Lea T.B., Olli J.J. (1994). Soybean proteinase inhibitors affect intestinal trypsin activities and amino-acid digestibilities in rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology Part A: Physiology*, 107: 215–219.
- Krogdahl Å., Hemre G.-I., Mommsen T.P. (1999). Carbohydrates in fish nutrition: digestion and absorption in postlarval stages. *Aquacult. Nutr.*, 11: 103–122.
- Le Féon S., Thévenot A., Maillard F., Macombe C., Forteau L., Aubin J. (2019). Life cycle assessment of fish fed with insect meal: Case study of mealworm inclusion in trout feed, in France. *Aquaculture*, 500: 82–91.
- Leary S., Underwood W., Anthony R., Cartner S., Corey D., Grandin T., Greenacre C., Gwaltney-Brant S., McCrackin M.A., Meyer R., Miller D., Shearer J., Yanong R. (2013). AVMA guidelines for the euthanasia of animals: 2013 edition, pp. 32.
- Li Q., Zheng L., Qiu N., Cai H., Tomberlin J.K., Yu Z. (2011). Bioconversion of dairy manure by black soldier fly (*Diptera: Stratiomyidae*) for biodiesel and sugar production. *Waste Manage.*, 31: 1316–1320.

- Martin S.A.M., Vilhelmsson O., Médale F., Watt P., Kaushik S., Houlihan D.F. (2003). Proteomic sensitivity to dietary manipulations in rainbow trout. *Biochim. Biophys. Acta – Proteins and Proteomics*, 1651: 17–29.
- Minh N.P. (2015). Alcalase and protamex hydrolysis of bioactive peptides from soybean. *Bull. Environ. Pharmacol. Life Sci.*, 4: 132–143.
- Murray A.L., Pascho R.J., Alcorn S.W., Fairgrieve W.T., Shearer K.D., Roley D. (2003). Effects of various feed supplements containing fish protein hydrolysate or fish processing by-products on the innate immune functions of juvenile coho salmon (*Oncorhynchus kisutch*). *Aquaculture*, 220: 643–653.
- Muzzarelli R.A.A. (2010). Chitins and chitosans as immunoadjuvants and non-allergenic drug carriers. *Mar. Drugs*, 8: 292–312.
- Ng W.-K., Liew F.-L., Ang L.-P., Wong K.-W. (2001). Potential of mealworm (*Tenebrio molitor*) as an alternative protein source in practical diets for African catfish, *Clarias gariepinus*. *Aquacult. Res.*, 32 (Suppl. 1): 273–280.
- NRC, National Research Council (1981). Nutrient requirements of coldwater fishes (Nutrient requirements of domestic animals). National Academy Press, Washington DC.
- NRC, National Research Council (1993). Nutrient Requirements of Fish. Washington, DC: The National Academies Press.
- Oonincx D.G.A.B., van Itterbeeck J., Heetkamp M.J.W., van den Brand H., van Loon J.J.A., van Huis A. (2010). An exploration on greenhouse gas and ammonia production by insect species suitable for animal or human consumption. *PLoS One*, DOI:10.1371/journal.pone.0014445.
- Ovissipour M., Benjakul S., Safari R., Motamedzadegan A. (2010). Fish protein hydrolysates production from fin tuna *Thunnus albacares* head using Alcalase and Protamex. *Intern. Aqua. Res.*, 2: 87–95.
- Pasupuleti V.K., Braun S. (2010). State of the Art Manufacturing of Protein Hydrolysates, Chapter II. In: Protein hydrolysed in Biotechnology, Pasupuleti V.K., Demain A.L. (eds), pp. 11–32.
- Penn M.H., Bendiksen E.A., Campbell P., Krogdahl A. (2011). High level of dietary pea protein concentrate induces enteropathy in Atlantic salmon (*Salmo salar* L.). *Aquaculture*, 310: 267–273.
- Piccolo G., Iaconisi V., Marono S., Gasco L., Loponte R., Nizza S., Bovera F. (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. Technol.*, 226: 12–20.
- Refstie S., Olli J.J., Standaal H. (2004). Feed intake, growth, and protein utilisation by post-smolt Atlantic salmon (*Salmo salar*) in response to graded levels of fish protein hydrolysate in the diet. *Aquaculture*, 239: 331–349.
- Refstie S., Bakke A.M., Petcare M., Penn M.H., Sundby A., Shearer K., Krogdahl A. (2006 a). Capacity for digestive hydrolysis and amino acid absorption in Atlantic salmon (*Salmo salar*) fed diets with soybean meal or inulin or without addition of antibiotics. *Aquaculture*, 261: 392–406.
- Refstie S., Glencross B., Landsverk T., Sørensen M., Lilleeng E., Hawkins W., Krogdahl A. (2006 b). Digestive function and intestinal integrity in Atlantic salmon (*Salmo salar*) fed kernel meals and protein concentrates made from yellow or narrow-leafed lupins. *Aquaculture*, 261: 1382–1395.
- Roncarati A., Gasco L., Parisi G., Terova G. (2015). Growth performance of common catfish (*Ameiurus melas* Raf.) fingerlings fed mealworm (*Tenebrio molitor*) diet. *J. Insects as Food Feed*, 1: 233–240.
- Rumpold B.A., Klocke M., Schlüter O. (2017). Insect biodiversity: underutilized bioresource for sustainable applications in life sciences. *Reg. Environ. Change*, 17: 1445–1454.
- Sajjadi M., Carter C.G. (2004). Effect of phytic acid and phytase on feed intake, growth, digestibility and trypsin activity in Atlantic salmon (*Salmo salar*, L.). *Aquacult. Nutr.*, 10: 135–142.
- Salze G.P., Davis D.A. (2015). Taurine: a critical nutrient for future fish feeds. *Aquaculture*, 437: 215–229.

- Sandnes K., Lie Ø., Waagbø R. (1988). Normal ranges of some blood chemistry parameters in adult farmed Atlantic salmon, *Salmo salar*. J. Fish Biol., 32: 129–136.
- Santigosa E., Sánchez J., Médale F., Kaushik S., Pérez-Sánchez J., Gallardo M. A. (2008). Modifications of digestive enzymes in trout (*Oncorhynchus mykiss*) and sea bream (*Sparus aurata*) in response to dietary fish meal replacement by plant protein sources. Aquaculture, 282: 68–74.
- Sánchez-Muros M., García-Rejón L., García-Salguero L., de la Higuera M., Lupiáñez J.A. (1998). Long-term nutritional effects on the primary liver and kidney metabolism in rainbow trout. Adaptive response to starvation and a high-protein, carbohydrate-free diet on glutamate dehydrogenase and alanine aminotransferase kinetics. Int. J. Biochem. Cell Biol., 30: 55–63.
- Sánchez-Muros M.J., Barroso F.G., Manzano-Agugliaro F. (2014). Insect meal as renewable source of food for animal feeding: a review. J. Clean. Prod., 65: 16–27.
- Sánchez-Muros M.J., De Haro C., Sanz A., Trenzado C.E., Villareces S., Barroso F.G. (2016). Nutritional evaluation of *Tenebrio molitor* meal as fishmeal substitute for tilapia (*Oreochromis niloticus*) diet. Aquacult. Nutr., 22: 943–955.
- Stamer A. (2015). Insect proteins a new source for animal feed. Sci. Soc., 16: 676–680.
- Storebakken T. (2002). Atlantic salmon, *Salmo salar*. In: Nutrient Requirements and Feeding of Finfish for Aquaculture, Webster C. (ed.). Aquaculture Research Center, Kentucky State University, USA, Chhorn Lim, USDA-ARS, Fish Diseases and Parasites Research Laboratory, Auburn, Alabama, USA.
- Su J., Gong Y., Cao S., Lu F., Han D., Liu H., Jin J., Yang X., 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.
- Torrissen K.R., Lied E., Espe M. (1994). Differences in digestion and absorption of dietary protein in Atlantic salmon (*Salmo salar*) with genetically different trypsin isozymes. J. Fish Biol., 45: 1087–1104.
- Van Huis A., Van Itterbeeck J., Klunder H., Mertens E., Halloran A., Muir G., Vantomme P. (2013). Edible insects: future prospects for food and feed security. Food and Agriculture Organization of the United Nations. Forestry Paper No. 171. Rome, 2013.
- Vercruysse L., Smagghe G., Beckers T., Van Camp J. (2009). Antioxidative and ACE inhibitory activities in enzymatic hydrolysates of the cotton leafworm, *Spodoptera littoralis*. Food Chem., 114: 38–43.
- Wang Y., Dang X., Zheng X., Zhang W. (2013). Housefly larvae hydrolysate: orthogonal optimization of hydrolysis, antioxidant activity, amino acid composition and functional properties. BMC Research Notes, 6: 197.
- Was A., Wenne R. (2002). Genetic differentiation in hatchery and wild sea trout (*Salmo trutta*) in the Southern Baltic at microsatellite loci. Aquaculture, 204: 493–506.
- Witkowski A., Kotusz J., Przybylski M. (2009). The degree of threat to the freshwater ichthyofauna of Poland: Red list of fishes and lampreys – situation in 2009 (in Polish). Chrońmy Przyrodę Ojczystą, 65: 33–52.
- Zheng K., Liang M., Yao H., Wang J., Chang Q. (2012). Effect of dietary fish protein hydrolysate on growth, feed utilization and IGF-I levels in Japanese flounder (*Paralichthys olivaceus*). Aquacult. Nutr., 18: 297–303.

Received: 10 VIII 2019

Accepted: 12 XII 2019