Received: 2 April 2020 | Revised: 9 September 2020 | Accepted: 19 November 2020

DOI: 10.1111/anu.13201

ORIGINAL ARTICLE

Aquaculture Nutrition WILEY

Potential use of black soldier fly (*Hermetia illucens*) and mealworm (*Tenebrio molitor*) insectmeals in diets for rainbow trout (*Oncorhynchus mykiss*)

F. Melenchón[1] | A.M. Larrán[1] | E. de Mercado[1] | M.C. Hidalgo[2] | G. Cardenete[2] | F.G. Barroso[3] | D. Fabrikov[3] | H.M. Lourenço[4] | M.F. Pessoa[5] | C. Tomás-Almenar[1]

[1]Research Aquaculture Centre, Agro-Technological Institute of Castilla y León, Segovia, Spain

[2]Departmentt of Zoology, Facultad de Ciencias, University of Granada, Granada, Spain

[3]Department of Biology and Geology, University of Almería, Almería, Spain

[4]Division of Aquaculture, Valorisation and Bioprospecting, Portuguese Institute for Sea and Atmosphere (IPMA, IP), Lisboa, Portugal

[5]GeoBioTec, Departamento de Ciências da Terra, Faculdade de Ciências e Tecnologia, Universida de Nova de Lisboa, Caparica, Portugal

Correspondence
C. Tomás-Almenar, Research Aquaculture Centre, Agro-Technological Institute of Castilla y León, Ctra. Arévalo s/n, 40196, Segovia, Spain
Email: critoal@gmail.com

Funding information
Agencia Estatal de Investigación, Grant/Award Number: BES2017-080567; Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Grant/Award Number: RTA 2015-00021-C03; European Social Fund, Grant/Award Number: BES2017-080567; European Regional Development Fund, Grant/Award Number: RTA 2015-00021-C03; Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Grant/Award Number: BES2017-080567

Abstract

The aquaculture industry is diminishing the amount of fishmeal needed to maintain its protein demand. Alternatives are tested in this way, being insects one of the most promising. In this study, two different insectmeals were tested at different fishmeal replacement proportions (150 and 300 g·kg^{-1}) in diets for *Oncorhynchus mykiss*. This study covers diverse aspects related to growth, protein utilization, physiological status of the fish, and quality of the final product. The inclusion of insectmeals had no negative effects on growth, protein utilization and on the physiological status of the fish. At the highest fishmeal replacement level with *Tenebrio molitor*, fish showed a higher digestibility of the protein, a reduction in viscerosomatic index and a higher aerobic catabolism, generating a pro-oxidative environment that was compensated by an increase in antioxidant enzymes, revealing the importance of choosing the appropriate insectmeal. A significant reduction in omega-3 fatty acids in the fillet was observed with an increasing insectmeal inclusion. The study proves that insectmeal can be a viable alternative for the partial replacement of fishmeal in rainbow trout, but further studies are needed to determine the most appropriate insectmeal, and to deal with the reduction of omega-3 fatty acids.

KEYWORDS

Black soldier fly, fatty acids, fishmeal replacement, insectmeal, mealworm, rainbow trout

1 | INTRODUCTION

Global population is in constant growth, and so is its global protein demand. Aquaculture has been highlighted as one of the best possibilities to satisfy animal protein demand due to the remarkable efficiency of fish growth in comparison with terrestrial animals (Fry, 2018), and to its fast development as an industrial food sector. The most commonly farmed fish require aquafeeds with a high content in protein, whose demand has been traditionally satisfied with a significant inclusion of fishmeal (FM) in fish feeding formulations. In order to favour this continuous development in a sustainable way, the scientific and industrial communities have invested big efforts on

reducing the inclusion of FM by searching for alternative ingredients (Gasco, 2020; Mousavi, 2020; Tran, 2015). Nonetheless, and even though it has been proven that it is possible to drastically reduce the amount of FM in fish feedings (Motte, 2019; Stenberg, 2019) the increase of FM overall absolute consumption is expected to keep increasing until 2030 together with the growth of aquaculture (FAO, 2020). With this increase come two main problems: the ecological impact of using high quantities of harvested wild sea fish for this objective, and the consequential increase of FM economic value.

The search for viable alternatives to FM continues to be a main challenge. In this sense, the use of insectmeals (IM) as sources of protein is one of the most promising options (Hua, 2019; Tran, 2015). First, they have shown a big potential as a sustainable animal protein source, due to the facts that they grow and reproduce easily, their growth ratios are very efficient, and their production requires low resources regarding to space and energy investment (van Huis, 2013). Also, there is a wide bibliography that proves its promising results in different fish species. In *Sparus aurata*, Piccolo (2017) achieved the highest growth performance at 330 g·kg^{-1} FM replacement with *Tenebrio molitor* (TM) in feed formulation. Magalhães (2017) highlighted that *Hermetia illucens* (HI) meal may successfully replace up to 195 g·kg^{-1} FM in diets for juvenile *Dicentrarchus labrax* without adverse effects on growth performance. Ji (2015) also expanded this line of work by demonstrating that even at high levels of FM replacement (500–600 g·kg^{-1}) with silkworm meal (*Bombyx mori*), a species such as Jian carp (*Cyprinus carpio* var. Jian) can maintain growth ratios similar to those which could be seen with no replacement.

More specifically for salmonids, different levels of FM replacement with different IMs have been tried. As examples of not impairing growth, Terova (2019) replaced up to 300 g·kg^{-1} FM with HI in rainbow trout. Belghit (2019) described the possibility of completely replacing FM for Atlantic salmon (*Salmo salar*), using HI as one of the chosen protein sources. With a slightly different approach, the work of Mikołajczak (2020) tested a 400 g·kg^{-1} replacement of FM with two hydrolysed IMs (TM, and superworm, *Zophobas morio*) on sea trout fingerlings (*Salmo trutta*), and only noticed a change in protein efficiency ratio. Similarly, a recent trial of Chemello (2020) noted no changes in growth for rainbow trout with a complete substitution of FM, in this case, using TM as a main source of protein. According to these and other published data, it is quite easy to assume that, with little differences among species, but in general terms, a partial replacement of FM with IM has no negative effects on the growth of most fish species, especially when there is more than one main protein source involved.

The different composition of the diets, and therefore different sources of protein, may affect the physiological status of fish. The use of IM has been related with differences in digestive enzyme activities (Rapatsa & Moyo, 2017, 2019), changes in liver metabolic enzymes (Chaklader, 2019), and an increase in the antioxidant capacity (Li, 2017; Taufek, 2016). Following this line of knowledge, this work had the objective of helping clarify the impact of the partial replacement of FM by IM in the feeding formulation of rainbow trout (*Oncorhynchus mykiss*). Two different insect species, black soldier fly (HI) and mealworm (TM), at two different FM replacement levels (150 and 300 g·kg^{-1}) were used, and the effect on different parameters related to growth efficiency, physiological status and final quality of the fish were evaluated, to try to elucidate the nutritional feasibility of both IM in feed for rainbow trout.

2 | MATERIAL AND METHODS

2.1 | Experimental diets

Commercial insectmeal (IM) from two different species, *Hermetia illucens* (HI) and *Tenebrio molitor* (TM; Mealfood Europe S.L., Spain) in larvae stage were used in this study. Before feed formulation, IM were analysed (Table 1). In this study, a total of five isoproteic (460 g·kg^{-1}) and isolipidic (170 g·kg^{-1}) diets were formulated (Table 2), a control diet with no IM, and four diets with two replacement levels and two sources of IM: H1 and T1 had 150 g·kg^{-1} FM replacement, and H2 and T2 had 300 g·kg^{-1} FM replacement. Ingredients were provided by 'Lorca Nutrición Animal S.A.' (Murcia, Spain). Diets were supplemented with methionine and phenylalanine to address the nutritional requirements of rainbow trout (Blanco Cachafeiro, 1995; National Research Council, 1993), manufactured by LifeBIOENCAPSULATION S.L. (Almería, Spain), and extruded in 2 mm pellets. The dough was passed through a single screw laboratory extruder (Miltenz 51SP, JSConwell Ltd, New Zealand). The extruder barrel consisted of four sections and the temperature profile in each section (from inlet to outlet) was 100 °C, 95 °C, 90 °C and 85 °C, respectively. Finally, pellets were dried in a drying chamber at 30 °C for 24 hr (Airfrio, Spain) and kept in sealed plastic bags at −20°C until use.

2.2 | Experimental animals and rearing conditions

600 female rainbow trouts with an initial weight of 55 ± 0.7 g were acquired from a commercial farm (Piscifactoría Fuente del Campillo, Guadalajara, Spain), and transported to the experimental facilities of the Aquaculture Research Centre of "Instituto Tecnológico Agrario de Castilla y León" (ITACyL). Fish were held without treatment for acclimation during 15 days before growth trial, and then, they were randomly allocated in groups of 30 animals into 20 cylindrical fiberglass tanks (500 L) in a recirculating system. Diets were assayed in quadruplicate. Fish were hand-fed to apparent satiation once a day (9 a.m., up to a maximum of 3.100 g fish^{-1}·day^{-1} feed intake) for 46 days. During the growth trial, water temperature was maintained at 15 ± 1°C, dissolved oxygen at 7.6 ± 1 mg·L^{-1}, and the photoperiod was 12 hr light:12 hr dark. The concentration of ammonia and nitrite in water were monitored daily to make sure that they were at optimal levels (ammonia < 0.1 mg/L and nitrite < 0.1 mg/L).

All procedures for the care and handling of rainbow trout were conducted in accordance with specific regulations, the Directive of the European Union Council (2010/63/EU) and the Spanish

TABLE 1 Composition of insectmeals from *Tenebrio molitor* and *Hermetia illucens* used in the experiment

Proximate composition (g·kg^{-1} insectmeal)	*Hermetia illucens*	*Tenebrio molitor*
Crude protein	300.0	420.2
Crude fat	339.2	283.4
Crude ash	105.9	36.8
Moisture	32.2	53.4
Phosphorus	6	7.1
Calcium	48	1.2
Chitin	165.7	58.7
Fatty acid composition (g·kg^{-1} fatty acids)		
C10:0 (*capric acid*)	11.89	0
C12:0 (*lauric acid*)	429.1	0
C14:0 (*myristic acid*)	83.3	23.9
C16:0 (*palmitic acid*)	147.4	178.1
C16:1n7 (*palmitoleic acid*)	24.4	14.1
C18:0 (*stearic acid*)	26.9	34.8
C18:1n9 (*oleic acid*)	153.5	345.7
C18:2n6 (*linoleic acid*)	88.7	374.7
C18:3n3 (*α-linolenic acid*)	5.7	17
C18:4n3 (*stearidonic acid*)	9.79	0
Amino acid composition (g·kg^{-1} insectmeal)		
Asp (aspartate)	26.8	33.4
Thr (threonine)	7.7	12.4
Ser (serine)	12.1	20.8
Glu (glutamate)	28.3	48.2
Pro (proline)	13.9	29.6
Gly (glycine)	15.8	23.2
Ala (alanine)	22.7	34.0
Val (valine)	12.2	18.7
Met (methionine)	4.0	5.1
Ile (isoleucine)	8.0	12.2
Leu (leucine)	16.6	25.3
Tyr (tyrosine)	14.1	23.9
Phe (phenylalanine)	10.8	15.3
His (histidine)	6.6	13.2
Lys (lysine)	13.8	21.3
Arg (arginine)	10.5	19.3

Government (Real Decreto 53/2013), and previously approved by the Bioethical Committee of the "ITACyL" (Authorization number: 2017/19/CEEA).

2.3 | Growth trial and sampling collection

During the experiment, feed intake and mortality were monitored, and all fish were weighed each 21 days to monitor their growth. In order to achieve that, after fasting for one day, fish were anesthetized with tricaine methanesulfonate (MS-222;180 mg·mL^{-1}), and body length and weight were measured using a graduated ictiometer (± 0.1 mm) and scale (± 0.1 g), respectively. To analyse apparent digestibility, faeces were collected during the last two weeks of the growth trial by a modified Guelph method (Cho, 1982), gathering the faeces produced throughout 24 hr in a settling column. The faeces samples were frozen at −80°C until their analysis.

At the end of the experiment, two fish were randomly sampled from each tank (8 fish per diet), and sacrificed by an overdose of MS-222 (300 mg·mL^{-1}). Blood, liver, stomach, intestine with pyloric ceca, and fillet samples were collected and individually analysed.

Blood samples were collected with heparinized syringes and its plasma separated by centrifugation at 3,500 x g for 15 min at 4 °C. Individual plasma samples were frozen at −80 °C until their analysis.

For enzyme determinations the tissue samples were immediately frozen in liquid nitrogen and maintained at −80 °C until their analysis. For chemical analysis and fatty acid (FA) determinations, the samples were directly frozen at −80 °C until their analysis.

2.4 | Analytical determinations

2.4.1 | Chemical analysis

Insect meal, diets and fish fillets were analysed for fat content, moisture according to AOAC methods (2005), and crude protein content was analysed according to Dumas method (Saint-Denis & Goupy, 2004) in a nitrogen analyser (FP 528, LECO, St. Joseph, USA). The conversion factor for protein content determination was 6.25 for feeds and faeces, and 4.67 for HI and 4.75 for TM (Janssen, 2017).

The apparent digestibility of the protein was determined using acid-insoluble ash as marker in feeds and faeces (Atkinson et al., 1984).

In the case of insect meal, phosphorus (P) was determined by molecular absorption spectrophotometry according to ISO standard (1996) using a spectrophotometer (UV/Vis UV2, UNICAM, Cambridge, UK). For the determination of calcium (Ca) X-ray fluorescence method of Dispersive Energy (ED-XRF) was used, based on the methodology described by Pessoa (2016). Chitin was isolated from insect meal using a methodology described by Gamage and Shahidi (2007). The obtained chitin residue was washed with acetone, dried and weighed.

For the amino acids, 1.6 and 1.5 mg of IM samples (*T. molitor* and *H. illucens*, respectively), were hydrolysed with 200 µl of 6 N HCl for 22 hr at 110°C, and the determination was performed by ion-exchange liquid chromatography and postcolumn continuous reaction with ninhydrin (Biochrom 30; United Kingdom) to provide qualitative and quantitative compositional analysis.

2.4.2 | Fatty acids determination

FA analyses in IMs and fish fillets were carried out as in previous studies (Lepage & Roy, 1984; Rodríguez Ruiz et al., 1998). FA were

TABLE 2 Ingredients, proximate and fatty acids composition of experimental diets

Ingredients (g·kg^{-1} on wet basis)	C	H1	H2	T1	T2
Fishmeal	368	315.4	259.1	313.3	258.9
HI meal	0.0	56.3	109.1	0.0	0.0
TM meal	0.0	0.0	0.0	51.3	102.7
Wheat gluten	105.3	127.3	148.3	120.9	126.1
Soy protein concentrate	150.9	158.6	170.2	150.7	165.3
Wheat meal	162	139.5	119.2	156	146.3
Soy lecithin	12.7	9.7	9.7	9.7	7.8
Fish oil	116.8	108	98.3	112.8	106.9
Vitamins and minerals	19.5	19.5	19.5	19.5	19.4
Goma guar	19.5	19.5	19.5	19.5	19.4
Hemoglobin	38.9	38.9	38.9	38.9	38.9
Methionine	0.8	1.5	2.2	1.5	2.2
Lysine	5.6	5.8	6.0	5.9	6.1
Total	**1,000**	**1,000**	**1,000**	**1,000**	**1,000**
Proximate composition (g·kg^{-1})					
Crude protein	458.1	466.2	454.3	465.4	463.1
Crude fat	164.6	174.1	173.2	174.2	167.7
Crude fiber	15.2	18.9	14.7	13.1	19.0
Ash	81.0	79.2	77.8	74.3	81.8
Fatty acid composition (g·kg^{-1} fatty acids)					
C12:0 (*lauric*)	0	52.2	96	0	0
C14:0 (*myristic*)	49.5	52	54.4	46.4	44.6
C16:0 (*palmitic*)	193.1	179.2	178	187.1	190.2
C16:1n7 (*palmitoleic*)	50	46.9	44.2	47.4	44.1
C17:0 (*margaric*)	7.76	6.90	6.08	7.13	6.46
C18:0 (*stearic*)	40.8	37.1	35.6	38.9	39.1
C18:1n9 (*oleic*)	143.8	145.6	145.9	163.3	181.5
C18:1n7 (*vaccenic*)	27.7	24.6	22.6	25.6	23
C18:2n6 (*linoleic*)	93.9	96.4	106.8	120.6	149.7
C18:3n3 (*linolenic*)	16.36	15.2	14.9	16.4	16.7
C18:4n3 (*stearidonic*)	15.1	13.4	11.6	13.9	12.5
C20:1n9 (*eicosenoic*)	30.3	28.2	22.4	26.6	23.7
C20:4n6 (*arachidonic*)	10.2	9.18	8.12	9.42	8.45
C20:4n3 (*eicosatetraenoic*)	5.45	4.87	4.31	5.04	4.56
C20:5n3 (*eicosapentaenoic; EPA*)	88.9	79.5	69.7	82.4	74.2
C22:1n11 (*cetoleic*)	37.1	33.4	29.3	34.7	30.9
C22:5n3 (*docosapentaenoic; DPA*)	13.4	11.6	9.87	12	10.8
C22:6n3 (*docosahexaenoic; DHA*)	118.4	106.1	90	109.1	96.8
C24:1n9 (*nervonic*)	4.56	3.89	3.29	4.04	3.57
SFA (*saturated fatty acids*)	291.2	327.4	370.1	279.5	280.4
MUFA (*monounsaturated fatty acids*)	293.4	282.5	267.7	301.5	306.9
PUFA (*polyunsaturated fatty acids*)	361.7	336.2	315.4	368.9	373.6
n−3	257.6	230.6	200.5	238.8	215.5
n−6	104	105.6	114.9	130.1	158.1
n−6/n−3	0.40	0.46	0.57	0.54	0.73

(Continues)

TABLE 2 (Continued)

Note: Vitamin and mineral premix (g·kg^{-1} unless otherwise specified): vitamin A 2,000,000 UI; vitamin D3: 200,000 UI; vitamin E: 12; vitamin K3: 2.6; vitamin B1: 3; vitamin B2: 3; vitamin B6: 2; vitamin B9:1.5; vitamin B12: 0.01; vitamin H: 0.3; inositol: 50; betaine: 50; calcium pantothenate: 10; nicotic acid: 20; Co: 0.06; Cu: 0.9; Fe: 0.6; I: 0.05; Mn: 0.95; Se: 0.001; Zn: 0.75; Ca: 190; K: 24; Na: 41.C: control diet (no insectmeal inclusion); H1 and H2: 150 and 300 g·kg^{-1} fishmeal replacement with Hermetia illucens (HI), respectively; T1 and T2: 150 and 300 g·kg^{-1} fishmeal replacement with Tenebrio molitor (TM) respectively. n−6/n−3 is a non-dimensional ratio.

measured after direct derivatization to FA methyl esters (FAME). FAME were analysed in a Focus GC (Thermo Electron. Cambridge. UK) equipped with a flame ionisation detector (FID) and an Omegawax capillary column (30 m x 0.25 mm i.d. x 0.25 μm film thickness; Supelco. Bellefonte. USA), as previously described (Guil-Guerrero, 2014). Peaks were identified by retention times obtained for known FAME standards (PUFAs No. 1, 47,033 from Sigma, St. Louis, MO, USA), and FA contents were estimated by using methyl pentadecanoate (15:0; 995 g·kg^{-1} purity; 76,560 Fluka) from Sigma as internal standard. The relative retention factors for each FA were estimated as reported by Cladis (2014) were considered for quantification.

2.4.3 | Digestive enzymes determination

For digestive enzymes, stomach, and intestine with pyloric ceca were separately processed. Individual digestive samples were first homogenized with distilled water (250 mg·mL^{-1}) at 4°C. Stomach extracts were used to measure acid protease activity, and extracts of intestine with pyloric ceca were used to measure amylase and alkaline protease activities. The concentration of soluble protein in samples was determined using Pierce™ BCA Protein Assay Kit (Thermo Scientific TM, Rockford, IL) using bovine serum albumin as a standard. Amylase activity was determined according to the Somogy-Nelson method (Somogyi, 1952) using soluble starch 20 g·kg^{-1} as substrate. One unit of activity was defined as the amount of enzyme able to produce 1 μg of maltose per minute and mg of protein. Alkaline protease activity was measured by Walter method (1984) using casein 10 g·kg^{-1} as substrate. Acid protease activity was measured by Anson method (1938), using hemoglobin 5 g·kg^{-1} as substrate. One unit of activity for both proteases was defined as 1 μg of tyrosine released per minute and mg of protein. All digestive enzyme activity analysis were performed at 37 °C.

2.4.4 | Intermediary metabolism and antioxidant status

Liver samples were individually homogenized in nine volumes of ice-cold 100 mM Tris-HCl buffer containing 0.1 mM EDTA and 1 g·kg^{-1} (v/v) Triton X-100, pH 7.8. All procedures were performed on ice. Homogenates were centrifuged at 30,000 x g for 30 min at 4°C and the resultant supernatants were kept in aliquots and stored at −80°C for further enzyme assays.

Fructose 1,6-bisphosphatase (FBPase; EC 3.1.3.11), glycerol kinase (GyK; EC 2.7.1.30), pyruvate kinase (PK, EC 2.7.1.40), lactate dehydrogenase (LDH, EC 1.1.1.27), citrate synthase (CS; EC 4.1.3.7), β-hydroxyacyl CoA dehydrogenase (HOAD; EC 1.1.1.35), glutamate pyruvate transaminase (GPT; EC 2.6.1.2), glutamate oxaloacetate transaminase (GOT; EC 2.6.1.1), and glutamate dehydrogenase (GDH; EC 1.4.1.2) were determined as previously described by Furné (2012).

Superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), glutathione peroxidase (GPX, EC 1.11.1.9), glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49), and glutathione reductase (GR, EC 1.6.4.2) were determined according to Pérez-Jiménez (2009).

All enzyme assays were carried out at 25°C and changes in absorbance were monitored to determine the enzyme activity using a PowerWaveX microplate scanning spectrophotometer (Bio-Tek Instruments, USA). The optimal substrate and protein concentrations for the measurement of maximal activity for each enzyme were established by preliminary assays. The millimolar extinction coefficients used for NADH/NADPH, DTNB, and H_2O_2 were 6.22, 13.6, and 0.039·mM^{-1}·cm^{-1}, respectively. One unit of SOD activity was defined as the amount of enzyme necessary to produce a 50% inhibition of the ferricytochrome C reduction rate. For the rest of enzymes, one unit of enzyme activity was defined as the amount of enzyme required to transform 1 μmol of substrate per min. Lipid peroxidation was determined based on malondialdehyde (MDA) levels. In the presence of thiobarbituric acid, MDA reacts producing coloured thiobarbituric acid reacting substances (TBARS) that were measured as previously described by Pérez-Jiménez (2009).

Soluble protein concentration in tissue homogenates was analysed using the method of Bradford (1976), with bovine serum albumin used as a standard.

2.4.5 | Non-specific immune status

Plasma lysozyme activity was assayed according to Swain (2007), using a turbidometric method with *Micrococcus lysodeikticus* (Sigma, St. Louis, USA). For the standard curve hen egg white lysozyme was used. The reaction run for 20 min at 35°C and measured at 450 nm.

Carbonic anhydrase has esterase activity. This plasma activity was assayed according to Mashiter and Morgan (1975). For this, the total esterase activity was measured at 25 °C, using 0.8 mM p-nitrophenyl acetate as a substrate, and subtracting the activity of the rest of the esterases except the carbonic anhydrase that is inhibited, using 1.6 mM acetazolamide as an inhibitor. After an incubation period of 10 min for each of the reactions, the absorbance increase at 405 nm was measured for 5 min.

A trypsin assay in absence of plasma was used as control to measure antiprotease activity according to the method of Thompson (1995). The production of 4-nitroaniline was determined by the variation of the OD (optical density) at 410 nm for 30 min. Trypsin activity was used as a control (CAS 90002–07–7, Acofarma, Spain).

Phosphatase activity was measured using p-nitrophenil phosphate (Sigma) as substrate. For this, a buffer at pH 10 ($NaHCO_3$/NaOH 0.05 M, $MgCl_2$ 1 mM) was used to measure alkaline phosphatase activity and a buffer at pH 5 (CH_3COOH/CH_3COONa 0.1 M, $MgCl_2$ 1 mM) for acid phosphatase activity. The measurement was performed for 30 min, at 37°C and at 405 nm (Huang, 2011).

Peroxidase activity in plasma was assayed according to Mohanty and Sahoo (2010), using a solution 20 mM TMB (3, 3', 5, 5'-Tetramethylbenzidine) as substrate. The colour change reaction was stopped after 2 min; then samples were read at 450 nm. Plasma-free standard samples were measured as controls. The activity was expressed in OD (optical density).

Total immunoglobulin in plasma was determined as described by Panigrahi (2005). Immunoglobulins were separated from the total proteins by precipitation with polyethylene glycol. Supernatant total protein was determined by Bradford method (Bradford, 1976). Total immunoglobulin content was determined by subtracting the protein content resulting from the total protein content in the untreated plasma.

2.5 | Statistical analysis

SAS system version 9.0 (SAS Institute Inc., Cary, North Carolina, USA) was used for the statistical analysis. Since all the variables fit normal distribution, the data were analysed using a general linear model (PROC GLM) analyses of variance (ANOVA). The values are showed as mean ± standard error of the mean.

3 | RESULTS AND DISCUSSION

The approval of the use of insects as ingredients in aquafeeds by the European regulation (EU, 2017) has increased the interest in these ingredients by both manufacturers and scientists in the last years. The study aims to highlight the potential use of insectmeal (IM) as raw material in feeds for rainbow trout and the challenges that it faces, with the novelty of testing two different insect species (*Tenebrio molitor*, TM, and *Hermetia illucens*, HI) comparing different inclusion levels.

For the time being, the works carried out in rainbow trout involving the study of IMs have used animals between 5–545 g, with a replacement of 0–1000 g·kg^{-1} fishmeal (FM) by IM, during 56–152 feeding days. In particular, studies which included HI meal observed the tendency that this ingredient causes little to none changes in the normal physiology of rainbow trout (Dumas, 2018; Elia, 2018; Rimoldi, 2019; St-Hilaire, 2007). The most common change tends to be the reduction on growth parameters as the amount of replaced FM reaches 200–300 g·kg^{-1}, as well as the reduction of omega-3 FA (*n-3*) on the fillet, which is due to the different composition of insect fat versus fish oil. Studies which used TM as replacement of FM in rainbow trout highlighted, in general, a better result in growth parameters, whilst the proportion of *n-3* in fillet tends to be even lower than the case of studies based on HI (Belforti, 2015; Borgogno, 2017; Iaconisi, 2017, 2018; Mancini, 2018; Renna, 2017; St-Hilaire, 2007). This difference between the performance of HI and TM could be due to their differences in composition, mostly aminoacids, calcium and chitin (Table 1).

The growth results displayed in this study were adequate for all diets. No mortality was recorded, and all diets were accepted correctly by the fish. The influence of the tested diets during the growth trial are shown in Table 3. There were no significant differences ($p > .05$) observed among the groups treated with different levels of IM inclusions, either for final body weight (FBW), specific growth rate (SGR) or daily feed intake (DFI) when comparing with the control diet. The FCR was adequate for all groups without differences respect to control diet, but it is worth to mention that the best FCR was showed for the fish fed with T1 diet, statistically different from H1 and H2 diets, which opens a possibility for future studies of improving the FCR through finding the optimal proportion and insect species in the feed formulation. Although most studies support the idea of a lesser proportion of FM (50–250 g·kg^{-1}) replacement with IM not being beneficial or disruptive for fish growth (Iaconisi, 2017; Magalhães, 2017; Renna, 2017; Terova, 2019), there are other which uphold the possibility of a particular proportion of IM as beneficial for some fish species (Ido, 2015; Ng, 2001; Rema, 2019; Sing, 2014). However, when it comes to evaluate the effect of high levels of FM replacement with IM (650–1000 g·kg^{-1}), the conclusions tend to diverge widely (Ido, 2019; Ji, 2015; Kroeckel, 2012; Lock, 2016; Vargas, 2018; Vargas-Abúndez, 2019). Similarly, when the protein utilization was evaluated, no differences were observed for protein efficiency ratio (PER) and productive protein ratio (PPV) among diets. As for apparent digestibility of the protein (ADC_{prot}), IM treatments showed higher levels, being the case of T2 as significantly different respect to control diet ($p < .05$; Table 3). In other words, the protein from IM included in the feed formulation showed a better availability for its utilization. Other authors found no differences in protein utilization in rainbow trout with the inclusion of HI (Dumas, 2018; Renna, 2017), and TM (Rema, 2019) up to 300 g·kg^{-1} in feed formulation. On the other hand, insects are part of the natural diet of rainbow trout (Metcalf, 1997; Rikardsen & Sandring, 2006); it is therefore not surprising that they are able to efficiently digest insect protein.

In relation to biometric indexes, non-negative effects were observed when the IM was included in the feed formulation (Table 3). However, it is remarkable that T2 had the lowest viscerosomatic index (VSI) value, significantly different to the rest of the diets. Changes in VSI can be related to changes in visceral fat deposition (Jobling, 1998, 2002), but a decreased VSI can also be related with a lower relative intestinal length. Since visceral fat deposition and relative intestinal length were not measured, these two indexes will be

TABLE 3 Effect of insectmeals in diets on growth performance, protein utilization and biometric indexes at the end of the feeding trial of rainbow trout

Growth performance	C	H1	H2	T1	T2	*SEM*
IBW (g)	55.4	54.9	56.5	53.7	56.0	0.7
FBW (g)	141.3	140.0	139.7	141.9	139.8	0.9
SGR (%·day^{-1})	2.04	2.02	2.02	2.05	2.02	0.01
DFI (g·100g fish^{-1}·day^{-1})	1.46	1.47	1.47	1.44	1.46	0.01
FCR	0.77^{ab}	0.78^{b}	0.78^{b}	0.75^{a}	0.77^{ab}	0.004
Protein utilization						
PER	2.85	2.85	2.84	2.86	2.85	0.03
PPV (%)	56.1	55.5	55.9	55	54.9	0.44
ADC_{prot} (%)	86.1^{a}	89.0^{ab}	88.7^{ab}	88.5^{ab}	90.6^{b}	0.9
Biometric indexes						
CF (g·cm^{-3})	1.13	1.15	1.14	1.15	1.12	0.01
HSI (%)	1.24	1.20	1.10	1.22	1.15	0.06
VSI (%)	9.58^{b}	10.16^{b}	9.87^{b}	9.88^{b}	8.82^{a}	0.25

Note: C: control diet (no insectmeal inclusion); H1 and H2: 150 and 300 g·kg^{-1} fishmeal replacement with Hermetia illucens, respectively; T1 and T2: 150 and 300 g·kg^{-1} fishmeal replacement with Tenebrio molitor respectively. IBW: initial body weight; FBW: final body weight; SGR (specific growth rate) = [(ln FBW-ln IBW)·days^{-1}] · 100; DFI (daily feed intake) = [daily feed consumption (g)·biomass^{-1} (g) at time] · 100; FCR (feed conversion ratio) = [total feed intake (g) · (FBW-IBW) $^{-1}$]; PER (protein efficiency ratio) = [total weight gain (g)·protein intake^{-1} (g)]; PPV (productive protein value) = [(protein gain (g) · protein intake^{-1} (g)) · 100]; ADC_{prot} (apparent digestibility coefficient of the protein) = 100-[(marker in diet (g)·marker in faeces^{-1}(g)) · (% protein in faeces·% protein in diet^{-1}) · 100]; CF (condition factor) = [weight (g)·length^{-3} (cm)] · 100; HIS (hepatosomatic index) = [wet liver weight·FBW^{-1}] · 100; VSI (viscerosomatic index) = [wet visceral weight·FBW^{-1}] · 100. $^{a, b}$ show statistically significant differences among diets ($p < .05$); Values are expressed as mean ± standard error of the mean (SEM; n = 4 tanks per diet, 2 fish per tank).

considered for further studies, as the present study reflects a promising match between a lower VSI and a higher apparent digestibility of the protein for T2.

The inclusion of new ingredients in feed formulation could also affect the physiological status of fish, and lastly, health and welfare status. For that, different parameters related to digestive enzyme activities, key metabolic hepatic enzymes, antioxidant and immune status were analysed.

The proteolytic and amylase activity may be different between fish with different nutritional habits (Hidalgo, 1999). Different authors have described differences in the activities of proteases between *Oreochromis mossambicus* and *Clarias gariepinus*, using the same diets with increasing amounts of *Imbrasia belina* meal (Rapatsa & Moyo, 2017, 2019). For that, the analysis of these digestive enzymes was included in this study, to evaluate the acceptation by rainbow trout, and to determine possible differences between the use of one insect or another (Table 4). The higher amount of alkaline protease observed in fish fed with TM matched with the increase in ADC_{prot} observed in these fish (Table 3), suggesting that intestinal proteases could be more relevant in the proteolysis and absorption of IM protein. There were no significant differences for acid proteases (Table 4; $p > .05$). Respect to amylase, a decrease in comparison to control diet is observed in the case of H2. Contrarily, Rapatsa and Moyo (2017, 2019) highlighted that higher levels of *Imbrasia belina* meal increased the levels of amylase; however, they did not suggest a direct relation between amylase and IM itself, but between amylase activity and the remaining vegetable contents of *Imbrasia belina*. Also, German (2004) reported that amylase activity increased with the quantity of carbohydrates in the diet. In this way, due to the lack of a direct relationship between IM and amylase function (starch hydrolysis) in the bibliography, and also the fact that there was only a significant difference between C and H2, this decrease should be due to the reduction of wheat meal in the formulation of this diet (a plant-based ingredient), since it was one of the ingredients that suffered the biggest reduction to adjust protein and lipids in HI diets but not in TM ones, being the reduction of wheat meal in H2 more important.

The liver is an essential metabolic organ which governs body energy metabolism. It acts as a hub to connect metabolically various tissues, including muscle and adipose tissue (Rui, 2014). The different composition of the diets may play an important role on the regulation of the enzymes involved in catabolism and anabolism of macronutrients. In this study, different key hepatic enzymes on metabolism were analysed (Table 5) to evaluate if the inclusion of IM in the diet could affect the fate of nutrients, either as part of a metabolic pathway or for energy storage.

Concerning lipid metabolism, HOAD (β-hydroxyacyl-CoA dehydrogenase) reversibly catalyses the third step of ß-oxidation, which takes place in the mitochondria and involves the FA catabolism. The results reflect a slight increase in the oxidative lipid metabolism for

TABLE 4 Effect of insectmeals in diets on the digestive enzyme activities of rainbow trout

Digestive enzymes (U·mg protein^{-1})	C	H1	H2	T1	T2	*SEM*
Acidic proteases	140.2	133.6	163.4	123.1	115.8	23.3
Alkaline proteases	83.2^{a}	112.4^{ab}	114.8^{ab}	153.6^{b}	144.8^{b}	10.5
Amylase	53.9^{b}	36.6^{ab}	29.2^{a}	34.8^{ab}	45.5^{ab}	5.18

Note: C: control diet (no insectmeal inclusion); H1 and H2: 150 and 300 g·kg^{-1} fishmeal replacement with Hermetia illucens, respectively; T1 and T2: 150 and 300 g·kg^{-1} fishmeal replacement with Tenebrio molitor respectively. $^{a, b}$ show statistically significant differences among diets ($p < .05$); Values are expressed as mean ± standard error of the mean (SEM; n = 4 tanks per diet, 2 fish per tank).

TABLE 5 Effect of insectmeals in diets on key enzymes in hepatic metabolism of rainbow trout

Enzymes (mU·mg protein^{-1})	C	H1	H2	T1	T2	*SEM*
β-hydroxyacyl-CoA dehydrogenase (HOAD)	19.2^{abc}	22.0^{bc}	17.7^{ab}	15.8^{a}	24.1^{c}	1.89
Pyruvate kinase (PK)	15.4	17.6	18.9	24.0	23.7	2.93
Citrate synthase (CS)	17.5^{ab}	16.9^{ab}	14.5^{a}	15.6^{ab}	19.7^{b}	1.62
Lactate dehydrogenase (LDH)	764.1	911.6	797.5	809.1	828.4	89.6
Fructose 1,6-biphosphatase (FBPase)	10.5	10.4	8.70	9.93	11.1	1.08
Glycerol kinase (GyK)	15.0	19.9	15.0	18.7	19.7	1.59
Glutamate dehydrogenase (GDH)	251.9	274.2	253.2	293.6	340.7	32.4
Glutamate pyruvate transaminase (GPT)	48.9	75.6	51.1	61.8	70.6	10.2
Glutamate oxaloacetate transaminase (GOT)	459.1^{ab}	423.0^{a}	417.9^{a}	461.0^{ab}	528.0^{b}	30.4

Note: C: control diet (no insectmeal inclusion); H1 and H2: 150 and 300 g·kg^{-1} fishmeal replacement with Hermetia illucens, respectively; T1 and T2: 150 and 300 g·kg^{-1} fishmeal replacement with Tenebrio molitor respectively. $^{a, b, c}$ show statistically significant differences among diets ($p < .05$); Values are expressed as mean ± standard error of the mean (SEM; n = 4 tanks per diet, 2 fish per tank).

H1 and T2, more marked for T2 (Table 5); that could cause a higher mobilisation of the fat, which could also be linked to the low VSI showed by these fish (Table 3). However, these differences were not statistically significant when comparing with C diet.

Two groups of results were observed when talking about glucose and amino acidic metabolisms (Table 5). The first one showed differences between diets with IM (CS and GOT). For CS, T2 had the highest activity, being statistically different from H2 ($p < .05$). For GOT, T2 also had the highest activity, being statistically different from H1 and H2. The greater activity of these two enzymes in T2 would suggest an increase of the protein catabolism to obtain energy, maybe derived from a different quality of the protein. However, this disruption on the intermediary metabolism of fish fed with T2 was not in direct consonance with a disruption on growth, probably due to a compensation derived from the greater digestibility of the protein observed in T2 (Table 3). The second group of results for hepatic enzymes on intermediary metabolism (PK, GyK, GDH and GPT) did not show any statistical differences (Table 5). It is easy to notice a synchronised overall increase on their activity when comparing IM results with those of control diet. As such, the present study encourages the possibility of increasing the amount of FM replacement with IM on further studies, in order to, maybe, find significant differences on their activity. These results are partially supported by those of Chemello (2020), since neither did they describe a significant increase for GDH or GPT after treatments with TM. The differences between their GOT results and those of the present study could be due to their diets having different sources of protein. In general, fish fed with T2 showed an increase on the oxidation of lipids, glucose and amino acids, and therefore a higher aerobic catabolism.

When the antioxidant capacity in liver was evaluated, fish fed with IM showed an increase on the antioxidant parameters SOD and CAT, and a decrease in lipid oxidative damage (MDA) respect to control diet (Table 6). Greater values in GR and GPX were observed on TM diets, but without statistical significance when comparing with control diet. Possibly, the increase on intermediary metabolism resulted in an increase of reactive oxygen species (ROS) production, and therefore a pro-oxidative cellular environment. However, the decrease on the MDA (Table 6) shows the efficacy of antioxidant mechanisms and the maintenance of redox homeostasis, without

TABLE 6 Effect of insectmeals in diets on antioxidant enzyme activities and levels of lipid peroxidation in liver of rainbow trout

Antioxidant enzymes and lipid peroxidation	C	H1	H2	T1	T2	*SEM*
Superoxide dismutase (SOD)	289.6^{a}	425.5^{b}	342.7^{ab}	396.6^{ab}	425.9^{b}	34.9
Catalase (CAT)	100.1^{ab}	123.9^{bc}	96.6^{a}	129.1^{cd}	152.5^{d}	8.24
Glutathione peroxidase (GPX)	26.1^{ab}	29.9^{ab}	24.6^{a}	27.5^{ab}	31.1^{b}	1.92
Glutathione reductase (GR)	10.8^{abc}	8.14^{ab}	8.0^{a}	12.5^{c}	12.2^{bc}	1.33
Glucose−6-phosphate dehydrogenase (G6PDH)	19.5	23.8	18.6	24.4	25.0	2.65
Malondialdehyde (MDA)	36.6^{b}	28.7^{a}	32.6^{ab}	32.4^{ab}	28.1^{a}	1.93

Note: C: control diet (no insectmeal inclusion); H1 and H2: 150 and 300 g·kg^{-1} fishmeal replacement with Hermetia illucens, respectively; T1 and T2: 150 and 300 g·kg^{-1} fishmeal replacement with Tenebrio molitor respectively. SOD and CAT expressed as U·mg protein^{-1}, GPX and GR as mU·mg protein^{-1}, and MDA as nmol·g tissue^{-1}. $^{a, b, c, d}$ show statistically significant differences among diets ($p < .05$); Values are expressed as mean ± standard error of the mean (SEM; n = 4 tanks per diet, 2 fish per tank).

damage derived from oxidative stress. This matches the results of Henry (2018a) on rainbow trout fed with TM; their study described an overall increase of SOD, CAT, GPX, GR and G6PDH, and a strong decrease in MDA. However, it would be interesting to point that they studied these enzymes in different parts of fish intestine (pyloric caeca, proximal and distal intestine), and that this relation with the present study was especially strong in proximal intestine. Taufek (2016) described also an increase in CAT for African catfish (*Clarias gariepinus*) liver after feeding cricket meal (*Gryllus bimaculatus*), while SOD and GST (glutathione S-transferase, not analysed) only suffered slight non-significant increases. Li (2017) described an increase in CAT for Jian carp (*Cyprinus carpio* var. Jian) serum after feeding black soldier fly, while SOD and MDA remained still among diets. Another work on yellow catfish fed with TM (Su, 2017) highlighted similar results, with an increase in plasma SOD activity both before and after an *Edwadsiella ictaluri* challenge, and a decrease in MDA activity prior to bacterial challenge. The general tendency of the bibliography is that the use of IM for fish increases the antioxidant defences of the animals while reducing the damage generated by lipid peroxidation. It has already been described that chitin and some of its derivatives can generate different antioxidant influences, such as a direct radical species scavenging effect and the increase of intracellular glutathione (Ngo & Kim, 2014). This, together with the previously mentioned results on intermediary metabolism, support the theory that T2 could have had a pro-oxidative effect on metabolism at the same time that it gave them tools to deal with it, causing no special oxidative damage.

Chitin can also have multiple effects on the immune system due to its polymeric structure (Lee, 2008), and its immunostimulant effect in fish has previously been described (Esteban, 2001; Gopalakannan & Arul, 2006; Kumar, 2019; Mousavi, 2020). Thus, different enzyme activities in plasma with a role in the innate immune response were analysed, such as lysozyme, esterase, anti-protease, acid phosphatase, alkaline phosphatase and peroxidase. In addition, the adaptive immune system was also evaluated by measuring the presence of total immunoglobulins (Ig) in plasma.

The lysozyme activity remained invariable between the different treatments (Table 7). Sankian (2018) described a linear increase of lysozyme activity with inclusion of TM in the diet of *Siniperca scherzeri*, getting to be significant at 300 g·kg^{-1} inclusion. This difference could be due to the fact that the IM inclusion level of the present study was lower, since the diet with the highest TM inclusion reached only 102.7 g·kg^{-1}. Nevertheless, the result matched Henry (2018b), who did not describe a significant difference in lysozyme activity when feeding TM meal to *Dicentrarchus labrax* at an even higher inclusion level of 247.5 g·kg^{-1}.

In the case of IM diets, an increase in anti-protease activity was observed, being significantly remarkable on H1 respect to control diet (Table 7). In contrast, the inclusion of IM in the diets showed a reduction in the presence of acid and alkaline phosphatase respect to control diet, being statistically significant only on H2, T1 and T2 for alkaline phosphatase ($p < .05$; Table 7). The acid and alkaline phosphatases have been showed as indicatives of tissue damage in other animals (Molina, 2005; Moreno, 2003). These results would also be in accordance with the maintenance of redox homeostasis previously described for fish fed with IM diets, since an increased oxidative stress environment can be related to tissue damage (Wang, 2019; Wu & Zhou, 2013).

In the case of peroxidase activity, there were no differences between treatments (Table 7). The part concerning TM matched the analysis of Sankian (2018) on serum myeloperoxidase after feeding TM to mandarin fish (*Siniperca scherzeri*). However, the present study, as well as that of Sankian (2018), were nutritional-focused studies with no pathogen challenges. It is possible that the exposure to a real pathogen aggression could have had the capacity to increase the expression of peroxidases or other immunological parameters.

Fish fed with IM showed a higher amount of total Ig, being statistically different for T1 (Table 7). In the case of HI diets, it seems there is a linear relation with the inclusion level in the diets. This result matches partially the works of Sangma and Kamilya (2015a, 2015b), where they described an increase in serum protein with different inclusion levels of chitin for *Catla catla* diet, as well as the work of

TABLE 7 Effect of insectmeals in diets on immune parameters in plasma of rainbow trout

Immune parameters	C	H1	H2	T1	T2	*SEM*
Lysozyme activity	6.22	6.17	7.45	5.48	6.39	0.58
Esterase	378.6	365.6	310.5	330.2	371.6	19.2
Anti-protease activity	201.2^{a}	291.4^{b}	249.2^{ab}	248.5^{ab}	271.7^{ab}	16.8
Acid phosphatase	478.0	428.9	441.3	384.4	355.2	42.6
Alkaline phosphatase	1617.5^{b}	1,203.7^{ab}	1,152.9^{a}	1,088.4^{a}	1,075.6^{a}	98.0
Peroxidase	0.18	0.20	0.21	0.20	0.17	0.01
Total Ig	16.8^{a}	20.1^{ab}	26.9^{ab}	32.6^{b}	27.7^{ab}	2.93

Note: C: control diet (no insectmeal inclusion); H1 and H2: 150 and 300 g·kg^{-1} fishmeal replacement with Hermetia illucens, respectively; T1 and T2: 150 and 300 g·kg^{-1} fishmeal replacement with Tenebrio molitor respectively. Lysozyme activity expressed as μg · mL HEWL^{-1}(Hen Egg White Lysozyme), anti-protease activity as U antiprotease·mL^{-1}, acid and alkaline phosphatase as mU·mg protein^{-1}, peroxidase as optical density and total Ig as mg·mL^{-1}. $^{a, b, c}$ show statistically significant differences among diets ($p < .05$); Values are expressed as mean ± standard error of the mean (SEM; n = 4 tanks per diet, 2 fish per tank).

TABLE 8 Effect of insectmeals in diets on proximal composition and fatty acid profile in fillets of rainbow trout

Proximate composition (g·kg^{-1} fish fillet on wet matter)	C	H1	H2	T1	T2	*SEM*
Protein	186.11	191.67	190.67	188.33	185.33	2.44
Fat	12.80	16.58	12.70	17.46	13.03	3.10
Moisture	761.1	761.7	754.6	757.8	755.7	1.99
Ash	13.69	13.63	13.58	13.29	13.80	0.13
Fatty acids in fillet (g·kg^{-1} fish fillet on wet matter)						
12:0 (*lauric*)	-	0.29^{a}	0.66^{b}	-	-	0.66
14:0 (*myristic*)	0.61	0.71	0.75	0.78	0.63	0.06
16:0 (*palmitic*)	3.51	3.45	3.36	3.91	3.14	0.30
16:1*n*−7 (*palmitoleic*)	0.76	0.77	0.80	0.99	0.79	0.08
18:0 (*stearic*)	0.85	0.79	0.77	0.89	0.72	0.08
18:1*n*−9 (*oleic*)	3.90^{a}	4.40^{ab}	4.66^{ab}	6.28^{b}	5.46^{ab}	0.45
18:1*n*−7 (*vaccenic*)	0.59	0.56	0.53	0.66	0.52	0.05
18:2*n*−6 (*linoleic*)	1.94^{a}	2.12^{a}	2.36^{ab}	3.18^{c}	3.00^{bc}	0.22
18:3*n*−3 (*linolenic*)	0.37^{a}	0.38^{a}	0.38^{a}	0.51^{b}	0.44^{ab}	0.04
18:4*n*−3 (*stearidonic*)	0.15	0.17	0.17	0.22	0.17	0.03
20:1*n*−9 (*eicosenoic*)	0.45^{a}	0.43^{a}	0.47^{a}	0.72^{b}	0.58^{ab}	0.07
20:4*n*−6 (*arachidonic*)	0.22^{b}	0.19^{ab}	0.17^{ab}	0.20^{ab}	0.14^{a}	0.02
20:4*n*−3 (*eicosatetraenoic*)	0.15	0.11	0.10	0.14	0.13	0.02
20:5*n*−3 (*ecosapentaenoic*; *EPA*)	1.25^{b}	1.08^{ab}	0.88^{a}	1.11^{ab}	0.81^{a}	0.11
22:1*n*−11 (*cetoleic*)	0.29^{a}	0.26^{a}	0.25^{a}	0.42^{b}	0.33^{ab}	0.03
22:5*n*−3 (*docosapentaenoic*; *DPA*)	0.43^{b}	0.33^{ab}	0.29^{a}	0.34^{ab}	0.28^{a}	0.04
22:6*n*−3 (*docosahexaenoic*; *DHA*)	4.74^{b}	3.81^{ab}	2.99^{ab}	3.16^{ab}	2.30^{a}	0.61
SFA (*saturated fatty acids*)	4.98	5.25	5.54	5.58	4.49	0.45
MUFA (*monounsaturated fatty acids*)	5.97^{a}	6.43^{a}	6.71^{a}	9.08^{b}	7.69^{ab}	0.66
PUFA (*polyunsaturated fatty acids*)	9.26	8.17	7.35	8.87	7.28	0.80
n−3	7.09^{b}	5.86^{ab}	4.81^{ab}	5.49^{ab}	4.14^{a}	0.74
n−6	2.17^{a}	2.31^{a}	2.53^{ab}	3.38^{c}	3.14^{bc}	0.22
n−6/*n*−3	0.33^{a}	0.40^{ab}	0.53^{bc}	0.61^{c}	0.76^{d}	0.05

Note: C: control diet (no insectmeal inclusion); H1 and H2: 150 and 300 g·kg^{-1} fishmeal replacement with Hermetia illucens, respectively; T1 and T2: 150 and 300 g·kg^{-1} fishmeal replacement with Tenebrio molitor respectively. $^{a, b, c, d}$ show statistically significant differences among diets ($p < .05$); n−6/n−3 is a non-dimensional ratio. Values are expressed as mean ± standard error of the mean (SEM; n = 4 tanks per diet, 2 fish per tank).

Kumar (2019), where similar parameters were measured after a parasite challenge and similar chitin inclusions.

Some of the differences in the data presented could be related to the differences in the chitin content between IMs (Table 1). Fish fed with H1 and T2 showed similarities in some variables, such as HOAD for intermediary metabolism, and SOD and MDA for antioxidant capacity, while those differences were not as obvious on H2 and T1. This could also be due to the fact that H1 and T2 had very similar amounts of chitin on their compositions (6–9 g·kg^{-1} feed, calculated from the IM chitin composition), whilst T1 had a lower concentration, and H2 could be reaching a theoretical toxicity barrier (Shiau & Yu, 1999), having double the amount of this compound in its composition.

Different sources of proteins and lipids may affect the fillet composition, as well as the quality of the final product. In this case the inclusion of IM, as source of both proteins and lipids, did not affect to the proximate composition of the fillet (Table 8). This coincides partially with the bibliography on rainbow trout fed IM, since most authors highlight little to none differences in fillet proximate composition after IM treatments, showing in some cases a reduction in fillet lipids and a compensatory increase in fillet moisture as IM is increased (Belforti, 2015; Dumas, 2018; Mancini, 2018; Sealey, 2011). This can also apply when reviewing the literature around whole-body proximate composition (Borgogno, 2017; Rema, 2019; St-Hilaire, 2007). However, the work of Renna (2017) observed an increment in the fat content of rainbow trout fillet after substituting up to 500 g·kg^{-1} FM in the diet with HI meal which, as the work itself states, could be due to the different rearing substrate of the insects used. Nevertheless, it is possible that the present study did not reach enough IM inclusion levels to match properly those same results.

The FA composition in fish fillet generally reflects the FA composition of the feeds (Turchini, 2009). The most remarkable differences in the FA profile of the feeds were their content of MUFA with a marked increase in TM diets, a decrease in EPA, DPA and DHA in all experimental diets, and an increase in omega-6 FA (*n-6*), specifically in linoleic acid. Another pronounced change would be the higher content in oleic acid in TM diets (Table 2). These FA variations have been reflected in the FA profiles of the fillets (Table 8). The most important changes were an increase of MUFA and *n-6* FA, together with a decrease in *n-3* as FM was replaced with IM, being especially high the increases on oleic FA for T1, the increase on *n-6* for TM diets, and the decreases on EPA, DPA and DHA for almost all experimental treatments. The health benefits of incorporating fish in the human diet for their content in *n-3* FA are well known; therefore, the lower content of these *n-3* FA in the fillet could involve a decrease in the perception of the fish quality by the consumers. Similar results have been found by other authors (Belforti, 2015; de Haro, 2016; St-Hilaire, 2007), which seems to be one main concern when dealing with IM based diets for fish. Thus, the search for strategies to improve this aspect will be critical for the implementation of the use of IM in commercial aquafeeds. One of the advantages on the use of insects is that they exhibit a high easiness on changing their composition according to the substrate used for their growth. Promising results have been obtained by Barroso (2017) and Danieli (2019); they proved the possibility of changing the proportion of PUFA in HI larvae composition by dietary modification. Hence, this consideration could be a major factor to have in mind in order to improve the *n-3* FA profile of fish whose diet is based on IM.

The study has covered diverse aspects related to growth efficiency, health and welfare status, and composition of the fillet, comparing two different IM at two different levels under the same experimental conditions. In general, the inclusion up to 109.1 g·kg^{-1}g/kg of HI and 102.7 g·kg^{-1}g/kg TM (300 g·kg^{-1}g/kg FM replacement) in diets for rainbow trout does not negatively affect the growth, protein utilization and the physiological status of the fish for the parameters evaluated. The replacement of FM with TM at 300 g·kg^{-1} showed a higher digestibility of the protein, a reduction of VSI, and a higher aerobic catabolism, generating a pro-oxidative environment that was compensated. Between IM diets, fish fed with H2 showed the most remarkable differences respect to T2 in intermediary metabolism and antioxidant activity, revealing the importance of the IM composition in its use for aquafeeds.

In contrast, the 300 g·kg^{-1} FM replacement level negatively affected the content of *n-3* FA in the fillet of fish fed with IM diets, which could lastly influence the perception of the consumers as an important source of *n-3* in a healthy diet.

Further studies are required to evaluate the effect of the use of feeds with IM for a longer time, and to determine the eligibility of the most appropriate IM to target different fish species, as well as to test different strategies to compensate the weakness of the IM inclusion, in order to maximize the potential of these protein sources.

ACKNOWLEDGEMENTS

This work was funded by INIA (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria) and co-funded by FEDER funds (Ref. RTA 2015-00021-C03). Acknowledgements to AEI (Agencia Estatal de Investigación) for its funds through the financial help of reference BES2017-080567 for PhD contracts with FSE funds. Feedings manufactured by LifeBIOENCAPSULATION S.L.

DATA AVAILABILITY STATEMENT

Research data are not shared.

ORCID

F. Melenchón https://orcid.org/0000-0002-4611-4308

REFERENCES

Anson, M. L. (1938). The estimation of pepsin, trypsin, papain and cathepsin with hemoglobin. *Journal of General Physiology*, *22*, 79–89. https://doi.org/10.1085/jgp.22.1.79

AOAC. (2005). *Official methods of analysis of the association of official analytical communities international, 18th ed.*, .

Atkinson, J. L., Hilton, J. W., & Slinger, S. J. (1984). Evaluation of acid-insoluble ash as an indicator of feed digestibility in rainbow trout (*Salmo gairdneri*). *Canadian Journal of Fisheries and Aquatic Sciences*, *41*(9), 1384–1386. https://doi.org/10.1139/f84-170

Barroso, F. G., Sánchez-Muros, M. J., Segura, M., Morote, E., Torres, A., Ramos, R., & Guil, J.-L. (2017). Insects as food: Enrichment of larvae of *Hermetia illucens* with omega 3 fatty acids by means of dietary modifications. *Journal of Food Composition and Analysis*, *62*, 8–13. https://doi.org/10.1016/j.jfca.2017.04.008

Belforti, M., Gai, F., Lussiana, C., Renna, M., Malfatto, V., Rotolo, L., De Marco, M., Dabbou, S., Schiavone, A., Ivo, Z., & Gasco, L. (2015). *Tenebrio molitor* meal in rainbow trout (*Oncorhynchus mykiss*) diets: Effects on animal performance, nutrient digestibility and chemical composition of fillets. *Italian Journal of Animal Science*, *14*(4), 4170. https://doi.org/10.4081/ijas.2015.4170

Belghit, I., Liland, N. S., Gjesdal, P., Biancarosa, I., Menchetti, E., Li, Y., Waagbø, R., Krogdahl, Å., & Lock, E.-J. (2019). Black soldier fly larvae meal can replace fish meal in diets of sea-water phase Atlantic salmon (*Salmo salar*). *Aquaculture*, *503*, 609–619. https://doi.org/10.1016/j.aquaculture.2018.12.032

Blanco Cachafeiro, M. C. (1995). *La Trucha: Cría Industrial*, *2a. ed.*, Mundi-Prensa.

Borgogno, M., Dinnella, C., Iaconisi, V., Fusi, R., Scarpaleggia, C., Schiavone, A., Monteleone, E., Gasco, L., & Parisi, G. (2017). Inclusion of *Hermetia illucens* larvae meal on rainbow trout (*Oncorhynchus mykiss*) feed: Effect on sensory profile according to static and dynamic evaluations. *Journal of the Science of Food and Agriculture*, *97*, 3402–3411. https://doi.org/10.1002/jsfa.8191

Bradford, M. (1976). A rapid and sensitive method for the quatification of microgram quantities of protein utilizing the principle of protein dye-binding. *Analytical Biochemistry*, *72*, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Chaklader, M. R., Siddik, M. A. B., Fotedar, R., & Howieson, J. (2019). Insect larvae, *Hermetia illucens* in poultry by-product meal for barramundi, *Lates calcarifer* modulates histomorphology, immunity and resistance to *Vibrio harveyi*. *Scientific Reports*, *9*, 16703. https://doi.org/10.1038/s41598-019-53018-3

Chemello, G., Renna, M., Caimi, C., Guerreiro, I., Oliva-Teles, A., Enes, P., … 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), 229. https://doi.org/10.3390/ani10020229

Cho, C. Y., Slinger, S. J., & Bayley, H. S. (1982). Bioenergetics of salmonid fishes: Energy intake, expenditure and productivity. *Comparative Biochemistry and Physiology – Part A: Molecular and Integrative Physiology*, *73B*, 25–41. https://doi.org/10.1016/0305-0491(82)90198-5

Cladis, D. P., Kleiner, A. C., Freiser, H. H., & Santerre, C. R. (2014). Fatty acid profiles of commercially available finfish fillets in the United States. *Lipids*, *49*(10), 1005–1018. https://doi.org/10.1007/s11745-014-3932-5

COMMISSION REGULATION (EU)2017/893 of 24 May 2017 amending Annexes I and IV to Regulation (EC) No 999/2001 of the European Parliament and of the Council and Annexes X, XIV and XV to Commission Regulation (EU) No 142/2011 as regards the provisions on processed animal protein. http://data.europa.eu/eli/reg/2017/893/oj

Danieli, P. P., Lussiana, C., Gasco, L., Amici, A., & Ronchi, B. (2019). The effects of diet formulation on the yield, proximate composition, and fatty acid profile of the black soldier fly (*Hermetia illucens* L.) prepupae intended for animal feed. *Animals*, *9*, 178. https://doi.org/10.3390/ani9040178

De Haro, C., Ramos-Bueno, R. P., Barroso, F. G., Sánchez-Muros, M. J., Rincón-Cervera, M. A., & Guil-Guerrero, J. I. (2016). I Insect larvae as feed ingredient selectively increase arachidonic acid content in farmed gilthead seabream (*Sparus aurata* L.). *Aquaculture Research*, *47*, 2881–2887. https://doi.org/10.1111/are.12738

DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 22 September 2010 on the protection of animals used for scientific purposes. http://data.europa.eu/eli/dir/2010/63/2019-06-26

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. https://doi.org/10.1016/j.aquaculture.2018.03.038

Elia, A. C., Capucchio, M. T., Caldaroni, B., Magara, G., Dörr, A. J. M., Biasato, I., Righetti, M., Pastorino, P., Prearo, M., Gai, F., Schiavone, A., & Gasco, L. (2018). Influence of *Hermetia illucens* meal dietary inclusion on the histological traits, gut mucin composition and the oxidative stress biomarkers in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, *496*, 50–57. https://doi.org/10.1016/j.aquaculture.2018.07.009

Esteban, M. A., Cuesta, A., Ortuño, J., & Meseguer, J. (2001). Immunomodulatory effects of dietary intake of chitin on gilthead seabream (*Sparus aurata* L.) innate immune system. *Fish and Shellfish Immunology*, *11*, 303–315. https://doi.org/10.1006/fsim.2000.0315

FAO. (2020). The state of world fisheries and aquaculture, sustainability in action.

Fry, J. P., Mailloux, N. A., Love, D. C., Milli, M. C., & Cao, L. (2018). Feed conversion efficiency in aquaculture: Do we measure it correctly? *Environmental Research Letters*, *13*, 024017.

Furné, M., Morales, A. E., Trenzado, C. E., García-Gallego, M., Hidalgo, M. C., Domezain, A., & Sanz, A. (2012). The metabolic effects of prolonged starvation and refeeding in sturgeon and rainbow trout. *Journal of Comparative Physiology B*, *182*, 63–76. https://doi.org/10.1007/s00360-011-0596-9

Gamage, A., & Shahidi, F. (2007). Use of chitosan for the removal of metal ion contaminants and proteins from water. *Food Chemistry*, *104*, 989–996. https://doi.org/10.1016/j.foodchem.2007.01.004

Gasco, L., Acuti, G., Bani, P., Zotte, A. D., Danieli, P. P., De Angelis, A., Fortina, R., Marino, R., Parisi, G., Piccolo, G., Pinotti, L., Prandini, A., Schiavone, A., Terova, G., Tulli, F., & Roncarati, A. (2020). Insect and fish by-products as sustainable alternatives to conventional animal proteins in animal nutrition. *Italian Journal of Animal Science*, *19*(1), 360–372.

German, D. P., Horn, M. H., & Gawlicka, A. (2004). Digestive enzyme activities in herbivorous and carnivorous prickleback fishes (Teleostei: Stichaeidae): Ontogenetic, dietary, and phylogenetic effects. *Physiological and Biochemical Zoology*, *77*, 789–804. https://doi.org/10.1086/422228

Gopalakannan, A., & Arul, V. (2006). Immunomodulatory effects of dietary intake of chitin, chitosan and levamisole on the immune system of *Cyprinus carpio* and control of *Aeromonas hydrophila* infection in ponds. *Aquaculture*, *255*, 179–187. https://doi.org/10.1016/j.aquaculture.2006.01.012

Guil-Guerrero, J. L., Gómez-Mercado, F., Ramos-Bueno, R. P., Rincón-Cervera, M. Á., & Venegas-Venegas, E. (2014). Restricted-range boraginaceae species constitute potential sources of valuable fatty acids. *Journal of the American Oil Chemists' Society*, *91*, 301–308. https://doi.org/10.1007/s11746-013-2372-3

Henry, M. A., Gai, F., Enes, P., Pérez-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 and Shellfish Immunology*, *83*, 308–313. https://doi.org/10.1016/j.fsi.2018.09.040

Henry, M. A., Gasco, L., Chatzifotis, S., & Piccolo, G. (2018). Does dietary insect meal affect the fish immune system? The case of mealworm, *Tenebrio molitor* on European sea bass, *Dicentrarchus labrax*. *Developmental and Comparative Immunology*, *81*, 204–209. https://doi.org/10.1016/j.dci.2017.12.002

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. https://doi.org/10.1016/S0044-8486(98)00413-X

Hua, K., Cobcroft, J. M., Cole, A., Condon, K., Jerry, D. R., Mangott, A., Praeger, C., Vucko, M. J., Zeng, C., Zenger, K., & Strugnell, J. M. (2019). The future of aquatic protein: Implications for protein sources in aquaculture diets. *One Earth*, *1*(3), 316–329. https://doi.org/10.1016/j.oneear.2019.10.018

Huang, Z.-H., Ma, A.-J., & Wang, X. (2011). The immune response of turbot, *Scophthalmus maximus* (L.), skin to high water temperature. *Journal of Fish Diseases*, *34*, 619–627. https://doi.org/10.1111/j.1365-2761.2011.01275.x

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 treats of blackspot sea bream (*Pagellus bogaraveo*). *Aquaculture*, *476*, 49–58. https://doi.org/10.1016/j.aquaculture.2017.04.007

Iaconisi, V., Secci, G., Bonelli, A., Pupino, R., Gai, F., & Parisi, G. (2018). Mealworm as dietary protein source for rainbow trout: Body and fillet quality traits. *Aquaculture*, *484*, 197–204. https://doi.org/10.1016/j.aquaculture.2017.11.034

Ido, A., Hashizume, A., Ohta, T., Takahashi, T., Miura, C., & Miura, T. (2019). Replacement of fish meal by defatted yellow mealworm (*Tenebrio molitor*) larvae in diet improves growth performance and disease resistance in red seabream (*Pargus major*). *Animals*, *9*, 100. https://doi.org/10.3390/ani9030100

Ido, A., Iwai, T., Ito, K., Ohta, T., Mizushige, T., Kishida, T., Miura, C., & Miura, T. (2015). Dietary effects of housefly (*Musca domestica*) (Diptera: Muscidae) pupae on the growth performance and the resistance against bacterial pathogen in red sea bream (*Pagrus major*) (Perciformes: Sparidae). *Applied Entomology and Zoology*, *50*, 213–221. https://doi.org/10.1007/s13355-015-0325-z

ISO 13730. (1996). *Meat and meat products - determination of total phosphorus content - spectrometric method*. : ISO Technical Committee TC 34/SC 6.

Janssen, R. H., Vincken, J.-P., Van den Broek, L. A. M., Fogliano, V., & Lakemond, C. M. M. (2017). Nitrogen-to-protein conversion factors for three edible insects: *Tenebrio molitor*, *Alphitobius diaperinus*, and *Hermetia illucens*. *Journal of Agricultural and Food Chemistry*, *65*(11), 2275–2278. https://doi.org/10.1021/acs.jafc.7b00471

Ji, H., Zhang, J. L., Huang, J. Q., Cheng, X. F., & Liu, C. (2015). Effect of replacement of dietary fish meal with silkworm pupae meal on growth performance, body composition, intestinal protease activity and health status in juvenile Jian carp (*Cyprinus carpio* var. Jian). *Aquaculture Research*, *46*, 1209–1221. https://doi.org/10.1111/are.12276

Jobling, M., Koskela, J., & Savolainen, R. (1998). Influence of dietary fat level and increased adiposity on growth and fat deposition in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture Research*, *29*, 601–607. https://doi.org/10.1046/j.1365-2109.1998.00251.x

Jobling, M., Larsen, A. V., Andreassen, B., & Olsen, R. L. (2002). Adiposity and growth of post-smolt Atlantic salmon *Salmo salar* L. *Aquaculture Research*, *33*, 533–541. https://doi.org/10.1046/j.1365-2109.2002.00738.x

Kroeckel, S., Harjes, A.-G.-E., Roth, I., Katz, H., Wuertz, S., Susenbeth, A., & Schulz, C. (2012). When a turbot catches a fly: Evaluation of a pre-pupae meal of the Black Soldier Fly (*Hermetia illucens*) as fish meal substitute — Growth performance and chitin degradation in juvenile turbot (*Psetta maxima*). *Aquaculture*, *364–365*, 345–352. https://doi.org/10.1016/j.aquaculture.2012.08.041

Kumar, R., Kaur, N., & Kamilya, D. (2019). Chitin modulates immunity and resistance of *Labeo rohita* (Hamilton, 1822) against gill monogeneans. *Aquaculture*, *498*, 522–527. https://doi.org/10.1016/j.aquaculture.2018.09.013

Lee, C. G., Da Silva, C. A., Lee, J. Y., Hartl, D., & Elias, J. A. (2008). Chitin regulation of immune responses: An old molecule with new roles. *Current Opinion in Immunology*, *20*, 684–689. https://doi.org/10.1016/j.coi.2008.10.002

Lepage, G., & Roy, C. C. (1984). Improved recovery of fatty acid through direct transesterification without prior extraction or purification. *Journal of Lipid Research*, *25*, 1391–1396.

Li, S., Ji, H., Zhang, B., Zhou, J., & Yu, H. (2017). Defatted black soldier fly (*Hermetia illucens*) larvae meal in diets for juvenile Jian carp (*Cyprinus carpio* var. Jian): Growth performance, antioxidant enzyme activities, digestive enzyme activities, intestine and hepatopancreas histological structure. *Aquaculture*, *477*, 62–70. https://doi.org/10.1016/j.aquaculture.2017.04.015

Lock, E. R., Arsiwalla, T., & Waagbø, R. (2016). Insect larvae meal as an alternative source of nutrients in the diet of Atlantic salmon (*Salmo salar*) postsmolt. *Aquaculture Nutrition*, *22*, 1202–1213. https://doi.org/10.1111/anu.12343

Magalhães, R., Sánchez-Lóprez, A., Leal, R. S., Martínez, L. S., Oliva-Teles, A., & Peres, 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. https://doi.org/10.1016/j.aquaculture.2017.04.021

Mancini, S., Medina, I., Iaconisi, V., Gai, F., Basto, A., & Parisi, G. (2018). Impact of black soldier fly larvae meal on the chemical and nutritional characteristics of rainbow trout fillets. *Animal*, *12*, 1672–1681. https://doi.org/10.1017/S1751731117003421

Mashiter, K. E., & Morgan, M. R. (1975). Carbonic anhydrase levels in the tissues of flounders adapted to sea water and fresh water. *Comparative Biochemistry and Physiology Part A: Physiology*, *52*, 713–717. https://doi.org/10.1016/S0300-9629(75)80028-4

Metcalf, C., Pezold, F., & Crump, B. G. (1997). Food habits of introduced rainbow trout (*Oncorhynchus mykiss*) in the upper Little Missouri River drainage of Arkansas. *Southwestern Naturalist*, *42*, 148–154.

Mikołajczak, Z., Rawski, M., Mazurkiewicz, J., Kieronczyk, B., & Józefiak, D. (2020). The effect of hydrolyzed insect meals in sea trout fingerling (*Salmo trutta* m. *trutta*) diets on growth performance, microbiota and biochemical blood parameters. *Animals*, *10*(6), 1031. https://doi.org/10.3390/ani10061031

Mohanty, B. R., & Sahoo, P. K. (2010). Immune responses and expression profiles of some immune-related genes in Indian major carp, *Labeo rohita* to *Edwardsiella tarda* infection. *Fish and Shellfish Immunology*, *28*, 613–621. https://doi.org/10.1016/j.fsi.2009.12.025

Molina, R., Moreno, I., Pichardo, S., Jos, A., Moyano, R., Monterde, J. G., & Cameán, A. (2005). Acid and alkaline phosphatase activities and pathological changes induced in Tilapia fish (*Oreochromis sp.*) exposed subchronically to microcystins from toxic cyanobacterial blooms under laboratory conditions. *Toxicon*, *46*, 725–735. https://doi.org/10.1016/j.toxicon.2005.07.012

Moreno, I. M., Mate, A., Repetto, G., Vázquez, C. M., & Cameán, A. M. (2003). Influence of microcystin-LR on the activity of membrane enzymes in rat intestinal mucosa. *Journal of Physiology and Biochemistry*, *59*, 293–299. https://doi.org/10.1007/bf03179887

Motte, C., Rios, A., Lefebvre, T., Do, H., Henry, M., & Jintasataporn, O. (2019). Replacing fish meal with defatted insect meal (yellow mealworm *Tenebrio molitor*) improves the growth and immunity of pacific white shrimp (*Litopenaeus vannamei*). *Animals*, *9*(5), 58. https://doi.org/10.3390/ani9050258

Mousavi, S., Zahedinezhad, S., & Loh, J.-Y. (2020). A review on insect meals in aquaculture: The immunomodulatory and physiological effects. *International Aquatic Research*, *12*(2), 100–115.

National Research Council (NRC). (1993). *Nutrient requirements of fish*. National Academy Press.

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*. *Aquaculture Research*, *32*, 273–280. https://doi.org/10.1046/j.1355-557x.2001.00024.x

Ngo, D.-H., & Kim, S.-K. (2014). Chapter Two – Antioxidant effects of chitin, chitosan and their derivatives. *Advances in Food and Nutrition Research*, *73*, 15–31.

Panigrahi, A., Kiron, V., Puangkaew, J., Kobayashi, T., Satoh, S., & Sugita, H. (2005). The viability of probiotic bacteria as a factor influencing the immune response in rainbow trout *Oncorhynchus mykiss*. *Aquaculture*, *243*, 241–254. https://doi.org/10.1016/j.aquaculture.2004.09.032

Pérez-Jiménez, A., Hidalgo, M. C., Morales, A. E., Arizcun, M., Abellán, E., & Cardenete, G. (2009). Antioxidant enzymatic defenses and oxidative damage in *Dentex dentex* fed on different dietary macronutrient levels. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, *150*, 537–545. https://doi.org/10.1016/j.cbpc.2009.07.011

Pessoa, M. F. (2016). Nutritional profile of the Portuguese cabbage (*Brassica oleracea* L var. costata) and its relationship with the elemental soil analysis. *Emirates Journal of Food and Agriculture*, *28*, 381–388. https://doi.org/10.9755/ejfa.2016-04-338

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*). *Animal Feed Science and Technology*, *226*, 12–20. https://doi.org/10.1016/j.anifeedsci.2017.02.007

Rapatsa, M. M., & Moyo, N. A. G. (2017). Evaluation of *Imbrasia belina* meal as a fishmeal substitute in *Oreochromis mossambicus* diets: Growth performance, histological analysis and enzyme activity. *Aquaculture Reports*, *5*, 18–26. https://doi.org/10.1016/j.aqrep.2016.11.004

Rapatsa, M. M., & Moyo, N. A. G. (2019). Enzyme activity and histological analysis of *Clarias gariepinus* fed on *Imbrasia belina* meal used for partial replacement of fishmeal. *Fish Physiology and Biochemistry*, *45*, 1309–1320. https://doi.org/10.1007/s10695-019-00652-3

Real Decreto 53/2013, de 1 de febrero, por el que se establecen las normas básicas aplicables para la protección de los animales utilizados en experimentación y otros fines científicos, incluyendo la docencia.https://www.boe.es/eli/es/rd/2018/11/19/1386

Rema, P., Saravanan, S., Armenjon, B., Motte, C., & Dias, J. (2019). Graded incorporation of defatted yellow mealworm (*Tenebrio molitor*) in rainbow trout (*Oncorhynchus mykiss*) diet improves growth performance and nutrient retention. *Animals*, *9*, 187. https://doi.org/10.3390/ani9040187

Renna, M., Schiavone, A., Gai, F., Dabbou, S., Lussiana, C., Malfatto, V., Prearo, M., Capucchio, M. T., Biasato, I., Biasibetti, E., De Marco, M., Brugiapaglia, A., Zoccarato, I., & Gasco, L. (2017). Evaluation of the suitability of a partially defatted black soldier fly (*Hermetia illucens* L.) larvae meal as ingredient for rainbow trout (*Oncorhynchus mykiss* Walbaum) diets. *Journal of Animal Science and Biotechnology*, *8*, 57. https://doi.org/10.1186/s40104-017-0191-3

Rikardsen, A. H., & Sandring, A. (2006). Diet and size-selective feeding by escaped hatchery rainbow trout *Oncorhynchus mykiss* (Walbaum). *ICES Journal of Marine Science*, *63*, 460–465. https://doi.org/10.1016/j.icesjms.2005.07.014

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*, 143. https://doi.org/10.3390/ani9040143

Rodríguez-Ruiz, J., Belarbi, E. H., Sánchez, J. L. G., & Alonso, D. L. (1998). Rapid simultaneous lipid extraction and transesterification for fatty acid analyses. *Biotechnology Techniques*, *12*, 689–691. https://doi.org/10.1023/A:1008812904017

Rui, L. (2014). Energy metabolism in the liver. *Comprehensive Physiology*, *4*, 177–197. https://doi.org/10.1002/cphy.c130024

Saint-Denis, T., & Goupy, J. (2004). Optimization of a nitrogen analyser based on the Dumas method. *Analytica Chimica Acta*, *515*, 191–198. https://doi.org/10.1016/j.aca.2003.10.090

Sangma, T., & Kamilya, D. (2015). In vitro and dietary effects of chitin on cellular and humoral immune parameters of catla, *Catla catla* (Hamilton). *Journal of the World Aquaculture Society*, *46*, 617–623. https://doi.org/10.1111/jwas.12232

Sangma, T., & Kamilya, D. (2015). Dietary *Bacillus subtilis* FPTB13 and chitin, single or combined, modulate systemic and cutaneous mucosal immunity and resistance of catla, *Catla catla* (Hamilton) against edwardsiellosis. *Comparative Immunology, Microbiology and Infectious Diseases*, *43*, 8–15. https://doi.org/10.1016/j.cimid.2015.09.003

Sankian, Z., Khosravi, S., Kim, Y. O., & Lee, S. M. (2018). Effects of dietary inclusion of yellow mealworm (*Tenebrio molitor*) meal on growth performance, feed utilization, body composition, plasma biochemical indices, selected immune parameters and antioxidant enzyme activities of mandarin fish (*Siniperca scherzeri*) juveniles. *Aquaculture*, *496*, 79–87. https://doi.org/10.1016/j.aquaculture.2018.07.012

Sealey, W. M., Gaylord, T. G., Barrows, F. T., Tomberlin, J. K., McGuire, M. A., Ross, C., & St-Hilaire, S. (2011). Sensory analysis of rainbow trout, *Oncorhynchus mykiss*, fed enriched black soldier fly prepupae, *Hermetia illucens*. *Journal of the World Aquaculture Society*, *42*, 34–45. https://doi.org/10.1111/j.1749-7345.2010.00441.x

Shiau, S. Y., & Yu, Y. P. (1999). Dietary supplementation of chitin and chitosan depresses growth in tilapia. *Oreochromis Niloticus X O. Aureus*. *Aquaculture*, *179*, 439–446. https://doi.org/10.1016/S0044-8486(99)00177-5

Sing, K.-W., Kamarudin, M. S., Wilson, J.-J., & Sofian-Azirum, M. (2014). Evaluation of blowfly (*Chrysomya megacephala*) maggot meal as an effective, sustainable replacement for fishmeal in the diet of farmed juvenile red tilapia (*Oreochromis* sp.). *Pakistan Veterinary Journal*, *34*, 288–292. https://doi.org/10.13140/2.1.3944.0007

Somogyi, M. (1952). Notes on sugar determination. *Journal of Biological Chemistry*, *195*, 19–23.

Stenberg, O. K., Holen, E., Piemontese, L., Liland, N. S., Lock, E.-J., Espe, M., & Belghit, I. (2019). Effect of dietary replacement of fish meal with insect meal on *in vitro* bacterial and viral induced gene response in Atlantic salmon (*Salmo salar*) head kidney leukocytes. *Fish and Shellfish Immunology*, *91*, 223–232. https://doi.org/10.1016/j.fsi.2019.05.042

St-Hilaire, S., Cranfill, K., McGuire, M. A., Mosley, E. E., Tomberlin, J. K., Newton, L., Sealey, W., Sheppard, C., & Irving, S. (2007). Fish offal recycling by the black soldier fly produces a foodstuff high in omega-3 fatty acids. *Journal of the World Aquaculture Society*, *38*, 309–313. https://doi.org/10.1111/j.1749-7345.2007.00101.x

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 and Shellfish Immunology*, *69*, 59–66. https://doi.org/10.1016/j.fsi.2017.08.008

Swain, P., Dash, S., Sahoo, P. K., Routray, P., Sahoo, S. K., Gupta, S. D., Meher, P. K., & Sarangi, N. (2007). Non-specific immune parameters of brood Indian major carp Labeo rohita and their seasonal variations. *Fish and Shellfish Immunology*, *22*, 38–43. https://doi.org/10.1016/j.fsi.2006.03.010

Taufek, N. M., Aspani, F., Muin, H., Raji, A. A., Razak, S. A., & Alias, Z. (2016). The effect of dietary cricket meal (*Gryllus bimaculatus*) on growth performance, antioxidant enzyme activities, and haematological response of African catfish (*Clarias gariepinus*). *Fish Physiology and Biochemistry*, *42*, 1143–1155. https://doi.org/10.1007/s10695-016-0204-8

Terova, G., Rimoldi, S., Ascione, C., Gini, E., Ceccotti, C., & Gasco, L. (2019). Rainbow trout (*Oncorhynchus mykiss*) gut microbiota is modulated by insect meal from *Hermetia illucens* prepupae in the

diet. *Reviews in Fish Biology and Fisheries*, *29*, 465–486. https://doi.org/10.1007/s11160-019-09558-y

Thompson, I., Choubert, G., Houlihan, D. F., & Secombes, C. J. (1995). The effect of dietary vitamin A and astaxanthin on the immunocompetence of rainbow trout. *Aquaculture*, *133*, 91–102. https://doi.org/10.1016/0044-8486(95)00024-V

Tran, G., Heuzé, V., & Makkar, H. P. S. (2015). Insects in fish diets. Animal. *Frontiers*, *5*(2), 37–44. https://doi.org/10.2527/af.2015-0018

Turchini, G. M., Torstensen, B. E., & Ng, W.-K. (2009). Fish oil replacement in finfish nutrition. *Reviews in Aquaculture*, *1*, 10–57. https://doi.org/10.1111/j.1753-5131.2008.01001.x

Van Huis, A. (2013). Potential of insects as food and feed in assuring food security. *Annual Review of Entomology*, *58*, 563–583. https://doi.org/10.1146/annurev-ento-120811-153704

Vargas, A., Randazzo, B., Riolo, P., Truzzi, C., Gioacchini, G., Giorgini, E., Loreto, N., Ruschioni, S., Zarantoniello, M., Antonucci, M., Polverini, S., Cardinaletti, G., Sabbatini, S., Tulli, F., & Olivotto, I. (2018). Rearing zebrafish on black soldier fly (*Hermetia illucens*): Biometric, histological, spectroscopic, biochemical, and molecular implications. *Zebrafish*, *15*, 404–419. https://doi.org/10.1089/zeb.2017.1559

Vargas-Abúndez, A. J., Randazzo, B., Foddai, M., Sanchini, L., Truzzi, C., Giorgini, E., Gasco, L., & Olivotto, I. (2019). Insect meal based diets for clownfish: Biometric, histological, spectroscopic, biochemical and molecular implications. *Aquaculture*, *498*, 1–11. https://doi.org/10.1016/j.aquaculture.2018.08.018

Walter, H. E. (1984). Proteinases: Methods with hemoglobin, casein and azocoll as substrates. *Methods of Enzymatic Analysis*, *5*, 270–277. H.J. Bergmeyer. Verlag Chemie, Weinham.

Wang, Y., Li, C., Pan, C., Liu, E., Zhao, X., & Ling, Q. (2019). Alterations to transcriptomic profile, histopathology, and oxidative stress in liver of pikeperch (*Sander lucioperca*) under heat stress. *Fish and Shellfish Immunology*, *95*, 659–669. https://doi.org/10.1016/j.fsi.2019.11.014

Wu, Y., & Zhou, Q. (2013). Silver nanoparticles cause oxidative damage and histological changes in medaka (*Oryzias latipes*) after 14 days of exposure. *Environmental Toxicology and Chemistry*, *32*, 165–173. https://doi.org/10.1002/etc.2038

How to cite this article: Melenchón F, Larrán AM, de Mercado E, et al. Potential use of black soldier fly (*Hermetia illucens*) and mealworm (*Tenebrio molitor*) insectmeals in diets for rainbow trout (*Oncorhynchus mykiss*). *Aquacult Nutr.* 2020;00:1–15. https://doi.org/10.1111/anu.13201