Dietary inclusion of full-fat Hermetia illucens prepupae meal in practical diets for rainbow trout (Oncorhynchus mykiss): Lipid metabolism and fillet quality

Contents lists available at ScienceDirect

Aquaculture

journal homepage: http://ees.elsevier.com

Dietary inclusion of full-fat *Hermetia illucens* prepupae meal in practical diets for rainbow trout (*Oncorhynchus mykiss*): Lipid metabolism and fillet quality investigations

Leonardo Bruni [a,*], Basilio Randazzo [b], Gloriana Cardinaletti [c], Matteo Zarantoniello [b], Fabio Mina [c], Giulia Secci [a], Francesca Tulli [c], Ike Olivotto [b], Giuliana Parisi [a]

[a] Department of Agriculture, Food, Environment and Forestry, University of Florence, via delle Cascine 5, 50144 Florence, Italy
[b] Department of Life and Environmental Sciences, Polytechnic University of Marche, via delle Brecce Bianche, 60131 Ancona, Italy
[c] Department of Agricultural, Food, Environmental and Animal Science, University of Udine, via Sondrio 2, 33100 Udine, Italy

ARTICLE INFO

Keywords
Black soldier fly
Gene expression
qPCR
Quality
DHA
Docosahexaenoic acid

ABSTRACT

Insects are able to bio-convert organic by-products into a sustainable biomass for aquafeed formulation. Specifically, among several insect species, *Hermetia illucens* (H) is particularly interesting for its nutritious traits but, unfortunately, the lipidic fraction is poorly represented by polyunsaturated fatty acids n-3 and poses some limits in its application in aquafeed formulation.The present study undertook an interdisciplinary approach to explore the effects of three experimental diets containing increasing levels of full-fat H meal (H0 diet based on fishmeal and purified protein-rich vegetable ingredients; H25 and H50 diets containing 25% or 50% of full-fat H meal replacing fishmeal, respectively), on rainbow trout (*Oncorhynchus mykiss*) fed over a 98 days experimental period. The expression of genes related to lipid metabolism by RT-qPCR, liver histology, as well as the qualitative traits of fillets and fatty acid (FA) composition were investigated. Interestingly, *fads2* gene expression in pyloric caeca increased in fish fed diets containing the highest full-fat H meal inclusion (H50 > H0; $p < .05$). Liver histological examinations showed normal morphological aspect even though hepatic FA profiles seemed to resemble those of the diets. However, liver docosahexaenoic acid did not significantly differ between the dietary groups and showed a mean value of 11.07 g FA methyl esters/100 g total FA methyl esters. Despite the FA profile of the three diets differed depending on the H meal inclusion level, biometrics, fillet physical traits, total lipids and the overall FA profile were not jeopardised, not even eicosapentaenoic and docosahexaenoic acids.The overall results showed that the dietary full-fat H meal inclusion under study did not impair fish fillet quality, guaranteeing its nutritional value. Some effects on lipid metabolism were observed, as suggested by liver, pyloric caeca and mid intestine gene expression and liver FA profile. Future studies on the biological mechanisms behind the macroscopic traits of fish fed unprocessed insects are warmly encouraged.

1. Introduction

Sustainability has been set as a vital goal in every production process. Nowadays, insects have been deeply investigated as alternative protein source for aquafeed formulation (Lock et al., 2018; Nogales-Mérida et al., 2018; Sánchez-Muros et al., 2014), for their environmental, economic and societal benefits (Bosch et al., 2019; van Huis et al., 2013). Among others, the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae; H), is one the most studied and promising insect species for aquafeed formulation, especially for its nutritional characteristics that meet most of the fish nutritional requirements (Barragan-Fonseca et al., 2017; Barroso et al., 2014; Henry et al., 2015).

Nevertheless, insect lipids are primarily composed of saturated (SFA) and monounsaturated (MUFA) fatty acids (FAs), while polyunsaturated FAs n-3 (PUFAn-3) are scarce. This deficiency may affect fish welfare and the nutritional composition of the edible portion. In fact, fish are renowned for their high long-chain PUFAn-3 (LC PUFAn-3) content, which are mainly accumulated through the diet and, in some species, partly synthesised by endogenous production (especially in freshwater species) (Tocher, 2003). In humans, LC PUFAs, like eicosapentaenoic (EPA) and docosahexaenoic acids (DHA), are well known to reduce the risk of cardiovascular and inflammatory disorders and depression (Rosenlund et al., 2010) and thus one of the main goal of the aquaculture sector is to guarantee an adequate amount of these FAs in the final product. As a consequence, the aquaculture industry is con-

* Corresponding author.
E-mail address: leonardo.bruni@unifi.it (L. Bruni)

https://doi.org/10.1016/j.aquaculture.2020.735678
Received 12 April 2020; Received in revised form 25 June 2020; Accepted 30 June 2020
Available online xxx
0044-8486/

tinuously looking for "suitable, sustainable, and environmentally acceptable" dietary alternatives in order to reduce the use of conventional marine and vegetable sources and to guarantee fish physical, chemical and sensorial attributes. Recently, several feeding trials with different dietary inclusion levels of H meal have been performed on salmonids, showing that both digestibility and growth performance were not harshly affected (Cardinaletti et al., 2019; Lock et al., 2016; Renna et al., 2017). However, a potential effect of this new ingredient was evidenced on fillet qualitative traits (Belghit et al., 2018; Bruni et al., 2020; Mancini et al., 2018; Renna et al., 2017). To limit undesired outcomes on fillet FA composition, two approaches have been undertaken. On the one hand, prior to the administration to fish, insect larvae FA profile was tailored by rearing them on PUFAn-3-rich substrates, like *Ascophyllum nodosum* (Liland et al., 2017), microalgae (Truzzi et al., 2020) or fish material (Barroso et al., 2019, 2017; St-Hilaire et al., 2007). The second approach considered a thorough modulation of the lipidic ingredients of fish feed by augmenting the dietary fish oil inclusion level (Belghit et al., 2019a).

The FA profile of the fish end-product is the macroscopic manifestation of complex metabolic processes, mainly involving intestine and liver, occurring between feed ingestion, digestion, and lipid deposition in the fillet.

Nutrient absorption in fish takes place along the entire intestinal tract, usually decreasing along this same organ, in rainbow trout and Atlantic salmon (Bakke et al., 2010; NRC, 2011). The chyme contains emulsified lipids and lipid-soluble vitamins, which downstream are further emulsified by bile acids and consequently hydrolysed, absorbed and then re-esterified into triacylglycerols. The enzyme encoded by cluster of differentiation 36 (*cd36*) gene is involved in cellular FA uptake by enterocytes, hepatocytes and adipocytes (The UniProt Consortium, 2019). Pyloric caeca execute the absorption of several lipid components, such as free FAs, lysophospholipids and monoacylglycerols (Turchini et al., 2009); pyloric caeca are a site of *de novo* LC PUFA synthesis by means of a FA conversion pathway, involving elongase and desaturase enzymes, and are thought to play an important role in DHA synthesis (Bell et al., 2003). Scant investigations on mid intestine FA catabolism and *de novo* synthesis are found in the literature (Lazzarotto et al., 2018; Tacchi et al., 2012), while more grounded information is available on the role of liver. Among the numerous functions, hepatocytes receive nutrients from the intestine through the circulatory stream, are involved in lipids storage (Bakke et al., 2010; NRC, 2011) and are the main district for the FA metabolism (Tocher, 2003).

FA metabolism is governed by a substantial number of genes [among others, the peroxisome proliferator-activated receptors ($ppar_s$), FA desaturases ($fads_s$) and elongation of very long chain FAs ($elovl_s$)] regulated by endogenous and exogenous conditions like dietary lipid quantity and quality (Tocher, 2003), beside a tissue-specific modulation (Morash et al., 2009). $Ppar_s$ are transcriptional regulators, potentially expressed in all tissues, acting as regulators in lipid storage, mobilisation and fat burning, other than in glucose homeostasis, respiration, morphogenesis and inflammatory response (Janani and Ranjitha Kumari, 2015). Desaturation and elongation also can take place in fish liver with a pathway similar to that of other vertebrates (Tocher, 2003). EPA and DHA biosynthesis pathway starts with the essential FA C18:3n-3 and involves fad_s and $elovl_s$, working in turn until the production of C24:6n-3, that is finally β-oxidised to C22:6n-3, *i.e.*, DHA (Tocher, 2003). Specifically, rainbow trout elovl5's substrates are C18 and C20 PUFAs, while elovl2 is active on C20 and C22 PUFAs, therefore, elovl2 is the indispensable *elovl* enzyme for DHA production (Gregory and James, 2014). Alternatively, Oboh et al. (2017) have recently pointed out that the *fads2* enzyme also exhibits Δ4 activity in some fish species, but the authors did not specifically confirm these findings in salmonids. Nonetheless, salmonids have a more pronounced capacity of producing EPA and DHA in comparison to the other euryhaline or saltwater fish species, and this capacity is also modulated by the diet (Turchini et al., 2009).

The present study integrated the results on growth performance, gastrointestinal integrity and stress of rainbow trout (*Oncorhynchus mykiss*) fed practical diets including increasing full-fat H meal levels, published by Cardinaletti et al. (2019), by exploring the effects of this innovative ingredient on fillet qualitative traits and liver FA composition and histology. In addition, the expression of genes involved in lipid metabolism in liver, pyloric caeca and mid intestine, the main districts dedicated to FA absorption and metabolism, was investigated through real time PCR to question the physiological implications behind fillet lipid constitution when feeding this commercially important aquaculture fish species with this innovative ingredient.

2. Material and methods

2.1. Ethical statement, diets, fish rearing and tissue sampling

All procedures for animal handling and care were accomplished according to the guidelines of the European Union (Directive 2010/63/EU, 2010) and Italian law (D.L. 26/2014) and the experimental protocol was approved by the Ethical Committee of the University of Udine (Prot. N. 1/2018). The fish feeding trial was performed at the experimental facility of the Agricultural, Food, Environmental and Animal Sciences Department of the University of Udine (Italy) at the following conditions.

As previously described by Cardinaletti et al. (2019), three isonitrogenous, isolipidic, and isoenergetic diets were prepared (Table 1) in order to test one control diet containing fishmeal and purified protein-rich vegetable ingredients (H0) and two experimental diets where 25% (H25) or 50% (H50) of the fishmeal had been replaced by full-fat H meal. Each diet was assigned to three fish groups made of 30 juvenile rainbow trout (*Oncorhynchus mykiss*) each, with an initial body weight of 137.3 ± 10.5 g. Specimens were randomly allocated to nine 1 m^3 square fiberglass tanks and fed over 98 days as described in Cardinaletti et al. (2019). At the end of the feeding trial, after a 10-h fasting period to ensure that the intestinal tract still partially contained feed residues so that the tissues were metabolically active, fish were euthanised with MS-222 (300 mg/L) and the organs sampled as follows: from nine fish per dietary treatment, fillets were allocated to physical analyses, while liver, pyloric caeca and mid intestine (corresponding to the tract immediately behind the anterior segment to the ileorectal valve) were immediately excised, put in individual plastic tubes, frozen in liquid nitrogen and then stored at −80 °C for gene expression analyses. Subsamples of liver were quickly fixed in Bouin's solution (Merk Sigma Aldrich, Milan, Italy) for histological analysis; subsamples of liver and fillets were assigned to physical and chemical analyses.

2.2. Gene expression analyses

2.2.1. RNA extraction and cDNA synthesis

To enable the analysis of a number of genes, subsamples of liver (L), pyloric caeca (C) and mid intestine (M) samples were utilised to perform total RNA extraction using RNAzol® RT reagent (Sigma-Aldrich®, R4533) following the manufacturer's instructions. Total RNA extracted was eluted in 20 μL of RNase-free water (Qiagen). Final RNA concentration was determined by the NanoPhotometer® P-Class (Implen, München, Germany). RNA integrity was verified by GelRed™ staining of 28S and 18S ribosomal RNA bands on 1% agarose gel. RNA was stored at −80 °C until use. Finally, 2 μg of RNA were used for cDNA synthesis, employing the High Capacity cDNA Reverse Transcription Kit (Bio-Rad, Milan, Italy) following the manufacturer's instructions.

Table 1
Ingredients (g/kg), proximate composition (g/100 g), total lipids (g/100 g) and fatty acid profile (% of total FAMEs) of the experimental diets (from Cardinaletti et al. (2019) and further deepened in the present study).

	Diet		
	H0	H25	H50
Ingredients			
Chile prime fish meal [1]	420	315	210
Protein-rich vegetable ingredients [2]	110	156	200
H meal [3]	–	105	210
Wheat flour [4]	290	268	255
Fish oil	70	40	28
Palm oil	70	75	56
Mineral [5] and Vitamin [6] supplements	20	20	20
Binder	20	20	20
L-Methionine	–	1	1
Proximate composition [7]			
Moisture	4.24 ± 0.03	5.49 ± 0.03	5.31 ± 0.18
Crude Protein, CP	40.27 ± 0.45	39.98 ± 0.37	40.16 ± 0.39
Ether Extract, EE	18.63 ± 0.27	18.56 ± 0.14	17.68 ± 0.20
Ash	14.30 ± 0.28	14.20 ± 0.23	14.13 ± 0.31
Gross Energy (MJ/kg)	22.10 ± 0.11	22.30 ± 0.03	21.28 ± 0.06
Total lipids	19.76 ± 0.09	18.94 ± 0.27	19.08 ± 0.51
Fatty acids [7]			
C10:0	tr	0.36 ± 0.03	0.74 ± 0.04
C12:0	0.12 ± 0.05	6.49 ± 0.68	13.05 ± 1.37
C13:0	tr	tr	tr
C14:0	2.89 ± 0.16	4.02 ± 0.31	5.68 ± 1.05
C15:0	0.42 ± 0.09	0.43 ± 0.08	0.54 ± 0.26
C16:0	23.63 ± 1.63	24.11 ± 2.81	21.62 ± 1.21
C16:1n-9	0.11 ± 0.04	0.13 ± 0.05	0.18 ± 0.02
C16:1n-7	3.24 ± 0.36	3.88 ± 0.91	5.25 ± 0.84
C17:0	0.47 ± 0.11	0.48 ± 0.04	0.59 ± 0.13
C18:0	5.57 ± 0.74	5.44 ± 1.11	5.08 ± 0.23
C18:1n-9	26.25 ± 0.55	27.85 ± 1.65	24.97 ± 1.76
C18:1n-7	1.29 ± 0,38	0.99 ± 0.16	0.98 ± 0.31
C18:2n-6	10.11 ± 1.53	8.95 ± 1.46	8.03 ± 1.49
C18:3n-3	1.59 ± 0.13	1.13 ± 0.23	0.95 ± 0.35
C20:0	0.38 ± 0.07	0.42 ± 0.14	0.45 ± 0.07
C20:1n-9	0.95 ± 0.35	0.86 ± 0.22	0.94 ± 0.20
C20:2n-6	0.17 ± 0.01	0.10 ± 0.001	tr
C20:3n-6	0.12 ± 0.04	tr	tr
C20:4n-6	0.73 ± 0.74	0.49 ± 0.13	0.41 ± 0.06
C20:3n-3	0.15 ± 0.07	0.10 ± 0.14	tr
C20:5n-3, EPA	6.85 ± 0.24	4.24 ± 0.49	3.12 ± 0.74
C22:0	0.19 ± 0.03	0.27 ± 0.01	0.36 ± 0.07
C22:1n-9	0.56 ± 0.13	0.39 ± 0.05	0.35 ± 0.08
C22:6n-3, DHA	13.42 ± 0.89	8.24 ± 0.36	5.97 ± 0.72
C24:1n-9	0.47 ± 0,38	0.37 ± 0.08	0.38 ± 0.08
SFA	33.76 ± 0.14	42.13 ± 4.51	48.24 ± 1.67
MUFA	32.87 ± 1.23	34.46 ± 0.61	33.04 ± 1.45
PUFAn-3	22.01 ± 0.45	13.71 ± 0.22	10.11 ± 0.33
PUFAn-6	11.33 ± 2.20	9.68 ± 1.56	8.62 ± 1.50
n-3/n-6	1.99 ± 0.43	1.44 ± 0.25	1.19 ± 0.17

tr: fatty acids below of 0.1% of total FAMEs.
SFA = C10:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0 + C22:0 + C24:0.
MUFA = C16:1n-9 + C16:1n-7 + C18:1n-9 + C18:1n-7 + C20:1n-9 + C22:1n-9 + C24:1n-9.
PUFAn-3 = C18:3n-3 + C20:3n-3 + C20:5n-3 + C22:6n-3.
PUFAn-6 = C18:2n-6 + C18:3n-6 + C20:2n-6 + C20:3n-6 + C20:4n-6.
[1] Bioceval GmbH & Co. KG Cuxhaven, Germany.
[2] Protein-rich vegetable ingredients: blend of pea protein concentrate (Lombarda trading srl, Casalbuttano & Uniti, Cremona, Italy) and wheat gluten meal (Sacchetto SpA, Turin, Italy) in 1:1 ratio.
[3] SmartBugs srl (Treviso, Italy).
[4] Consorzio Agrario (Pordenone, Italy).
[5] Mineral supplement composition (% mix): $CaHPO_4 \times 2H_2O$, 78.9; MgO, 2.725; KCl, 0.005; NaCl, 17.65; $FeCO_3$, 0.335; $ZnSO_4 \times H_2O$, 0.197; $MnSO_4 \times H_2O$, 0.094; $CuSO_4 \times 5H_2O$, 0.027; Na_2SeO_3, 0.067.

[6] Vitamin supplement composition (% mix): thiamine HCL Vit B1, 0.16; riboflavin Vit B2, 0.39; pyridoxine HCL Vit B6, 0.21; cyanocobalamin B12, 0.21; niacin Vit PP, 2.12; calcium pantothenate, 0.63; folic acid, 0.10; biotin Vit H, 1.05; myoinositol, 3.15; stay C Roche, 4.51; tocopherol Vit E, 3.15; menadione Vit K3, 0.24; Vit A (2500 UI/kg diet), 0.026; Vit D3 (2400 UI/kg diet), 0.05; choline chloride, 83.99.

[7] Values reported as mean of triplicate analyses.

2.2.2. Real-time qPCR

Prior to qPCR reactions, all primer pairs were used in gradient reactions in order to determine the optimal annealing temperatures; control cDNA samples were pooled and used for this purpose. Then, PCR efficiency for each primer pair was determined using 10-fold serial dilutions of pooled liver, pooled pyloric caeca and pooled mid intestine cDNA samples.

qPCRs were performed in duplicate with SYBR® Green in an iQ5 iCycler thermal cycler (both from Bio-Rad, CA, USA). For each sample, reactions (10 μL) were set on a 96-well plate by mixing 1 μL cDNA diluted 1:20, 5 μL of 2× concentrated SYBR® Green as the fluorescent intercalating agent, 0.2 μM forward primer, and 0.2 μM reverse primer. The thermal profile for all reactions was: 3 min at 95 °C, followed by 45 cycles of 10 s at 95 °C, 20 s at a variable temperature depending on the primer annealing temperature (Table 2) and 20 s at 72 °C. Fluorescence was monitored at the end of each cycle. In all cases, dissociation curve analysis showed a single pick.

Relative quantification of the expression of genes involved in fish lipid metabolism (*fads2*, *elovl1*, *elovl2*, *pparα*, *pparβ*, *pparδ*, *pparγ* and *cd36*) was performed using *arp* and *60S* as housekeeping genes to standardise the results (Table 2). The primer sequences were designed using Primer-BLAST tool available in NCBI (http://www.ncbi.nlm.nih.gov/). Data were analysed using the iQ5 optical system software version 2.0, including Genex Macro iQ5 Conversion and Genex Macro iQ5 files (Bio-Rad, CA, USA). Modification of gene expression was reported in relation to controls.

2.3. Liver analyses: Hepatosomatic index, histology and fatty acid characterisation

Liver samples (n = 9) were fixed by immersion in Bouin's solution (Sigma-Aldrich, Milan, Italy) and stored at 4 °C for 24 h. Samples were washed three times with ethanol (70%) for ten minutes and preserved in the same ethanol solution. Samples were then dehydrated in crescent ethanol solutions (80, 95 and 100%), washed with xylene and embedded in solid paraffin (Bio-Optica, Milan, Italy). Solidified paraffin blocks were cut with a microtome (Leica RM2125 RTS, Nussloch, Germany) and 5 μm sections were stained with Mayer's haematoxylin and eosin Y (Sigma-Aldrich, Milan, Italy) according to Giorgini et al. (2018) and Randazzo et al. (2015). Sections were observed using a Zeiss Axio Imager.A2 (Oberkochen, Germany) microscope and images acquired by mean of a combined colour digital camera Axiocam 503 (Zeiss, Care Zeiss, Oberkochem, Germany). Furthermore, to ascertain the extent of fat accumulation in liver, a quantitative analysis was performed on a substantial number of histological sections from each experimental group in triplicate (n = 9). Non-evaluable areas, such as blood vessels, were not considered. The percentage of fat fraction (PFF) on the total tissue areas was calculated using the ImageJ software setting a homogeneous threshold value as described in Zarantoniello et al. (2020).

Other five livers per group were weighed in order to calculate the hepatosomatic index (HSI) as follows:

$$\mathrm{HSI} = \left[\text{liver weight/total body weight (g)}\right] \times 100.$$

Then, the same five livers were analysed for total lipid contents as well as FA profile, following the methods described by Folch et al. (1957) and Secci et al. (2018), respectively. The ratio of FA products:precursors was utilised to assess the desaturating and elongating activities, as proposed by Renaville et al. (2013).

2.4. Physical analyses, lipid composition and oxidative status of fillets

Fillets from nine fish per diet were allocated to the physical analyses. The colour of the fillets was measured on triplicate positions (cranial, medial and caudal) on both fish sides with a CHROMA METER CR-200 (Konica Minolta, Singapore Japan) following the CIELab system (CIE, 1976) and recording L* (lightness), a* (redness index) and b* (yellowness index) parameters. Colour values were recorded and ΔE between pairs of samples was calculated according to the following formula:

$$\Delta E_{(\beta-\alpha)} = \left[\left(L^*_\beta - L^*_\alpha\right)^2 + \left(a^*_\beta - a^*_\alpha\right)^2 + \left(b^*_\beta - b^*_\alpha\right)^2\right]^{0.5.}$$

where α and β represent alternatively the mean colour values of H0, H25 or H50.

The values of pH and water holding capacity (WHC) were measured as well. The pH value was measured on triplicate fillet positions (cranial, medial and caudal) by a pH-meter SevenGo SG2™ (Mettler-Toledo, Schwerzenbach, Switzerland). Afterwards, fillets were skinned, homogenised and utilised to determine WHC (Iaconisi et al., 2018) and chemical composition, as described below.

Table 2
Primer pair sequences and annealing temperature conditions for genes used in real-time PCR.

Gene	3′ primer sequence		Annealing temperature (°C)
	Forward	Reverse	
cd36	TCAAGCGTTGTCTGTAGTGAGT	CCCAGTAGCGTAAATTGCACA	58.2
pparα	AGTCGAGTAACGGCTCTGAAGG	CCGACACTCCAGGTTGAGAGA	60.0
pparβ	ATCAGCAGGAGAAGGGGAGTAG	GGAGACGATGTCTGGGACAGAT	58.2
pparδ	TCCTGTTTCCTGTGAGTGGGA	CCAGTCAGCACATTGCCATTTC	56.0
pparγ	GCCCTTATCGCCTTCTCAGT	AGAGCTGGCGTCTGTGTAAG	56.0
fads2	GCCCTACCATCACCAACACC	AAACTCATCGACCACGCCAG	60.0
elovl1	TTGCCCAAGCAGGATACCAA	ATTCATGCGTCTTGGGTGTTC	59.2
elovl2	TGGATGGGTCCCAGAGATGA	AGAAGGACAAGATCGTGAGGC	56.0
arp	GAAAATCATCCAATTGCTGGA	CTTCCCACGCAAGGACAGA	60.0
60S	AGCCACCAGTATGCTAACCAG	TGTGATTGCACATTGACAAAA	60.0

Total lipids were extracted from the fillets (n = 5) following the method described by Folch et al. (1957), then they were gravimetrically quantified. The FA profile of each lipid extract was determined as FA methyl esters (FAME) using a Varian 430 gas chromatograph (Agilent, Palo Alto, CA, USA) set as described in Secci et al. (2018). Once obtained the FA profile, the ratio of FA products:precursors was utilised to assess the desaturating and elongating activities of fillets, as proposed by Renaville et al. (2013).

The following nutritional indices were also calculated:

Atherogenicity index (AI) (Ulbricht and Southgate, 1991):

$$\frac{C12:0 + (4 \times C14:0) + C16:0}{MUFA + PUFAn-6 + PUFAn-3}$$

Thrombogenicity index (TI) (Ulbricht and Southgate, 1991):

$$\frac{C14:0 + C16:0 + C18:0}{(0.5 \times MUFA) + (0.5 \times PUFAn-6) + (3 \times PUFAn-3) + \left(\frac{n-3}{n-6}\right)}$$

- hypocholesterolaemic/Hypercholesterolaemic FA ratio (h/H) (Santos-Silva et al., 2002):

$$\frac{C18:1n-9 + C18:2n-6 + C18:3n-3 + C20:4n-6 + C20:5n \cdot}{C14:0 + C16:0}$$

- PUFAn-3/PUFAn-6 ratio

Finally, 2 g of homogenised fillet were utilised to determine the secondary lipid oxidation products (thiobarbituric acid reactive substances, TBARS) according to Vyncke (1970). The absorbance at 532 nm was read with a 50 Scan spectrophotometer equipped with Cary Win UV software (Varian, Palo Alto, CA, USA) on two technical replicates for each sample.

2.5. Statistical analysis

The statistical software package Prism6 (GraphPad Software, La Jolla, California, USA, www.graphpad.com) was used to analyse q-PCR data with a one-way ANOVA. Significance was set at $p \leq .05$; residuals df and F values are also reported in the text. Results are presented as means ± standard deviation.

Data about PFF, chemical composition, physical traits, nutritional indices and TBARS content were assessed for normality (Shapiro-Wilk test) and homoscedasticity (Levene's test). If normality and homoscedasticity were not met, a boxcox transformation was performed. Then, a one-way ANOVA followed by a Tukey's test were performed using the free software environment R (R Core Team, 2018), with significance set at $p \leq .05$; residuals df and F values are also reported in the text. Results are presented as means and pooled standard error of the mean (SEM), if not otherwise stated.

3. Results

3.1. Gene expression

The gene expression of *cd36*, *ppar*$_s$ and some genes related to LC PUFA biosynthetic pathways was analysed (Fig. 1a–h). Following the dietary H meal inclusion, pyloric caeca *cd36* gene expression increased, while mid intestine *pparα*, liver *pparδ* and the *pparβ* gene expression showed faint decreasing trends; *pparγ* seemed to be moderately stable across the three different dietary regimes; no significant differences were detected ($p > .05$). The *fads2* gene expression in the pyloric caeca significantly increased in fish fed the highest dietary H meal inclusion (H50 *vs* H0; $p < .05$, df = 9, F = 4.468). For the same target gene, a similar trend ($p > .05$) was also observed in the mid intestine (M). Finally, no significant differences in the expression of both *elovl1* and *elovl2* were observed in the different tissues under study (liver, pyloric caeca and mid intestine).

3.2. Analyses on livers

The calculated HSI lined up at 1.08, 1.04 and 1.13% ($p > .05$) for H0, H25 and H50 dietary groups, respectively.

The analysis of liver parenchyma did not allow to observe obvious abnormal morphological aspect. Conversely, results evidenced a variable degree of lipid accumulation in the liver of the three experimental groups (Fig. 2). The most appreciable visual differences were observed in H50 group (Fig. 2c) in comparison to H0 and H25 (Fig. 2a and b, respectively). These results were confirmed by the statistical quantification of the PFF, which showed a significantly ($p < .05$, df = 2, F = 2.14) higher liver fat accumulation in H50 (54.41 ± 1.5%, mean ± standard deviation) respect to both H0 (43.2 ± 0.9%) and H25 (42.49 ± 1.5%).

Although by means of Folch et al. (1957)'s extraction method no differences in the hepatic total lipid content between dietary treatments were observed ($p > .05$), it enabled FA profile detection, which was significantly influenced by the diets (Table 3). In particular, the fatty acid C18:2n-6 was the highest in the livers of fish fed H25 diet and the lowest in H0 ($p < .01$, df = 27, F = 7.748). With the exception of EPA, all the 20 carbon atom-long FAs were significantly higher in H50 dietary treatment than in the H0 group. DHA did not significantly differ between groups and showed a mean value of 11.07 g FAME/100 g total FAME. Overall, SFA were abundantly present in all the dietary treatments (47.25 g FAME/100 g total FAME) and they were not significantly different between the three dietary treatments, whilst both MUFA and PUFAn-6 increased with the increasing inclusion of H meal ($p < .05$, df = 27, F = 3.906; $p < .01$, df = 27, F = 8.207, respectively) in the diet, and PUFAn-3 tended to show an opposite trend. The PUFAn-3/PUFAn-6 ratio was significantly lower ($p < .001$, df = 27, F = 18.21) in the liver of fish fed H50 and H25 diets compared to H0 livers.

The products:precursors ratios did not highlight any significant difference ($p > .05$).

3.3. Analyses on fillets

As shown in Table 4 the total lipids and the FA profile of fillets were not significantly affected by the different dietary regimes, except for SFAs. Indeed, total SFAs, C12:0 and C14:0 increased with the increasing dietary inclusion of H meal ($p < .01$, df = 12, F = 7.35; $p < .001$, df = 12, F = 28.2; $p < .01$, df = 12, F = 11.35, respectively), while C16:0 showed an opposite trend ($p < .01$, df = 12, F = 8.478). The oleic acid (C18:1n-9) showed a decreasing trend ($p > .05$) following the increasing dietary H meal inclusion. Independently to the dietary composition, fillet C18:2n-6 showed a mean value of 11.41 g FAME/100 g total FAME between groups. The sum of EPA + DHA was in average 14.92 g FAME/100 g total FAME. Products:precursors ratios showed that C18:4n-3/C18:3n-3 ratio was higher in H25 and H50 than in H0 group.

As displayed in Table 5, TI and the n-3/n-6 ratio were not significantly affected by the tested dietary treatments, whilst the AI was higher in H50 than in H25 and H0 groups ($p < .001$, df = 12, F = 23.21). The h/H ratio showed the lowest ($p < .05$, df = 12, F = 4.306) value recorded in the fillets from fish fed the H50 diet and the highest one in the H25 dietary group, with H0 showing intermediate values. As shown in Fig. 3, the levels of malondialdehyde-equivalents of the fillets from fish fed the H25 and H50 diets presented lower values than H0 fillets ($p < .05$, df = 24, F = 16.59).

Fillets exhibited similar physical traits between dietary treatments (Table 6). The yellowness index (b*) was the only parameter af-

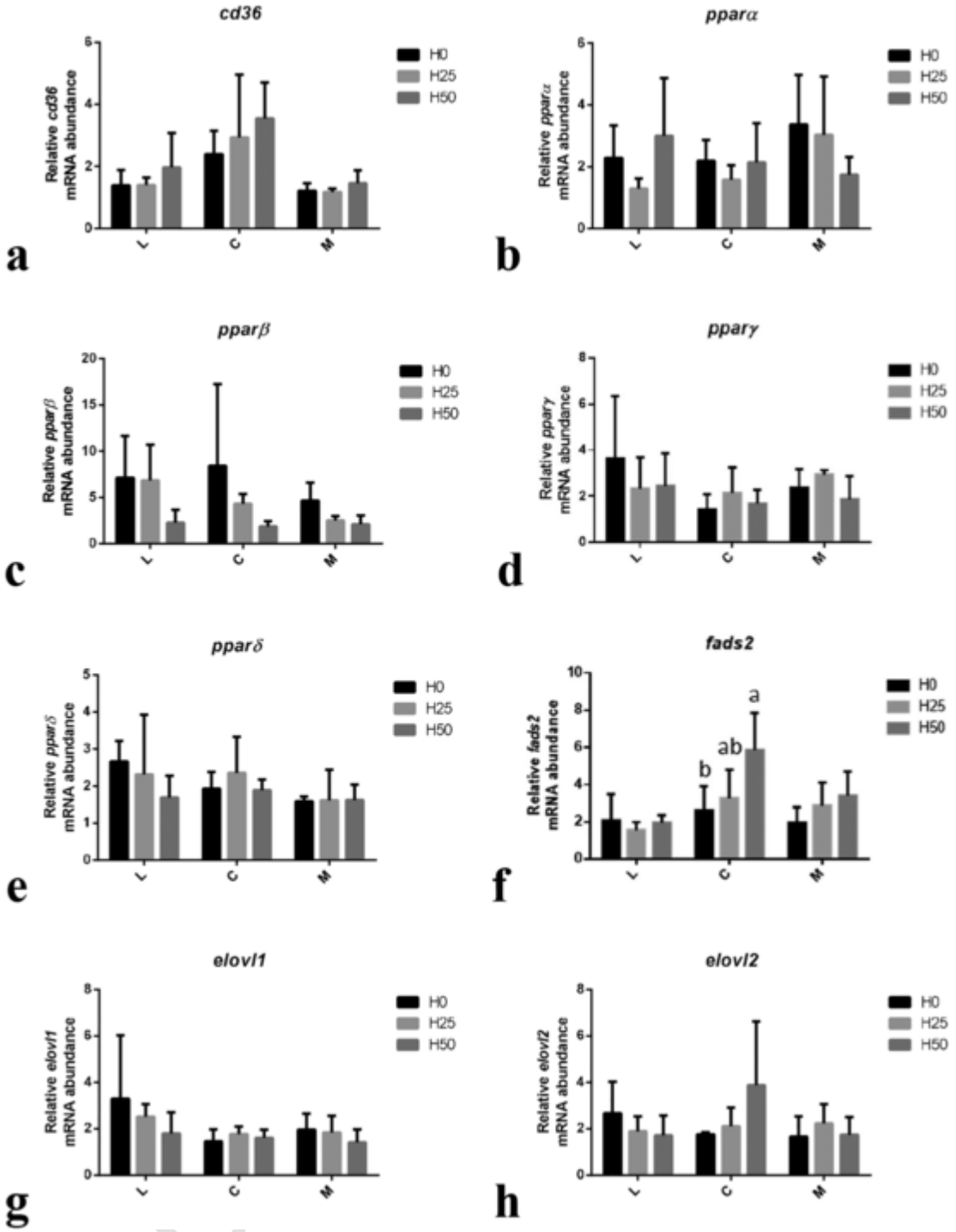


Fig. 1. Relative mRNA abundances of genes related to the lipid metabolism along the gastrointestinal tract (L, liver; C, pyloric caeca; M, mid intestine). a: *cd36*; b to e: *ppar*$_s$; f: *fads2*; g and h: *elovl*$_s$. Bars indicate standard deviation.

fected by the diet, reaching the lowest value in fillets from fish fed H25 diet and the highest one in H0 diet ($p < .05$, df = 27, F = 4.649). ΔE calculations showed the following results: H0 *vs* H25: 2.28, H0 *vs* H50: 1.23, H25 *vs* H50: 1.37.

4. Discussion

Rainbow trout is an economically important species worldwide and an important freshwater species in the European Countries, with Turkey, Chile, Norway, Peru, China and Italy being the first six producers in the world, accounting for 60% of the global production (FAO, 2019). Feed plays a major role for the further virtuous and sustainable development of aquaculture, and each single ingredient performs as a main actor in the definition of the end-product quality. The evaluation of fish quality is a *sine qua non* both for business outcome and for human nutrition and health. In fact, aquaculture products are particularly renowned for their high LC PUFAn-3 content, mainly drawn by the fish from the diet and, in the case of Salmonids and freshwater species, from a relatively small endogenous production (Tocher, 2003). Among the LC PUFAs, EPA and DHA are the most valued FAs for their benefits on human health (Rosenlund et al., 2010).

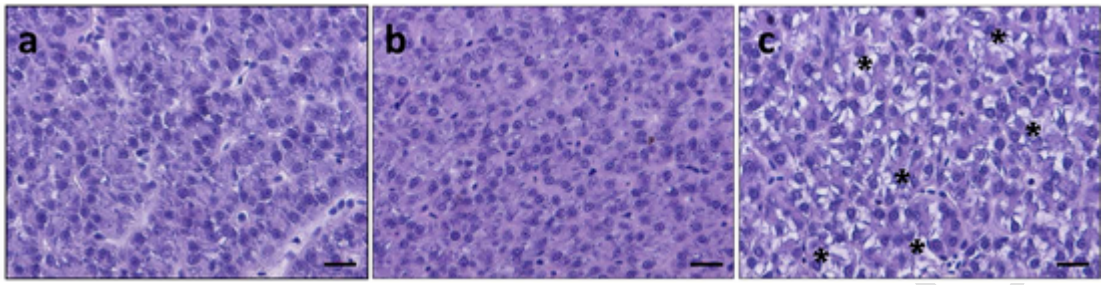


Fig. 2. Liver histology of rainbow trout fed experimental diets including increasing levels of full-fat *Hermetia illucens* prepupae meal: 0%, H0 (a); 25%, H25 (b) and 50%, H50 (c). Asterisks in figure c indicate fat accumulation in the liver parenchyma stained with periodic acid of Shiff (PAS). Scale bars: 10 μm.

Table 3
Total lipids (g/100 g liver), fatty acid profile (% total FAMEs) and products:precursors ratios (gray background) in livers of rainbow trout fed experimental diets including increasing levels of full-fat *Hermetia illucens* prepupae meal (0%, H0; 25%, H25; 50%, H50).
The following FAs were used for calculating the classes of FAs but they are not listed because below 1% of total FAME: C14:1n-5, C15:0, C16:1n-9, C16:2n-4, C17:0, C16:3n-4, C17:1, C18:2n-4, C18:3n-6, C18:3n-4, C18:3n-3, C18:4n-3, C20:0, C20:1n-11, C20:1n-7, C20:3n-3, C20:4n-3, C22:0, C22:1n-11, C22:1n-9, C22:5n-3.
[1]SEM: standard error of the mean.
[2]ns: not significant ($p > .05$); a, b, c as superscript letters indicate significantly different means at $p < .05$ (*); $p < .01$ (**); $p < .001$ (***).
[3]UFA: unsaturated fatty acids.

H. illucens, one of the most promising insects for being an innovative, sustainable and nutritious ingredient for aquafeed formulation, is unfortunately scant of LC PUFAs (Barroso et al., 2014). Typically, fish fed with insects mirror the FA profile of the administered diets (Belghit et al., 2019b; Iaconisi et al., 2018; Renna et al., 2017) and for this reason in some trials dietary fish oil was increased when H meal was used as ingredient (Belghit et al., 2019a; Sealey et al., 2011). Cardinaletti et al. (2019) reared rainbow trout with diets including increasing full-fat H meal levels but, to counterbalance the increased lipidic component supplied by full-fat H meal, fish oil was parallelly decreased to induce rainbow trout ability to efficiently use FAn-3 and thus fulfil environmental sustainability. This led to a decreased dietary amount of PUFAs and a parallel increase in SFAs.

Specific FAs have different physiological functions in fish, including energy production, membrane structure and functionality,

Table 4
Total lipids (g/100 g fillet), fatty acid profile (% of total FAMEs) and products:precursors ratios (gray background) in fillets of rainbow trout fed experimental diets including increasing levels of full-fat *Hermetia illucens* prepupae meal (0%, H0; 25%, H25; 50%, H50).
The following FAs were used for calculating the classes of FAs but they are not listed because below 1% of total FAME: C14:1, C15:0, C16:2n-4, C17:0, C16:3n-4, C17:1, C16:4n-1, C18:2n-6 trans, C18:2n-4, C18:3n-6, C18:3n-4, C18:4n-3, C18:4n-1, C20:0, C20:1n-11, C20:1n-9, C20:2n-6, C20:3n-6, C20:3n-3, C20:4n-3, C22:0, C22:1n-11, C22:1n-9, C22:4n-6, C21:5n-3, C22:5n-3, C24:0, C24:1.
[1]SEM: Standard error of the mean.
[2]ns: not significant ($p > .05$); a, b, c as superscript letters indicate significantly different means at $p < .05$ (*); $p < .01$ (**); $p < .001$ (***).
[3]UFA: unsaturated fatty acids.

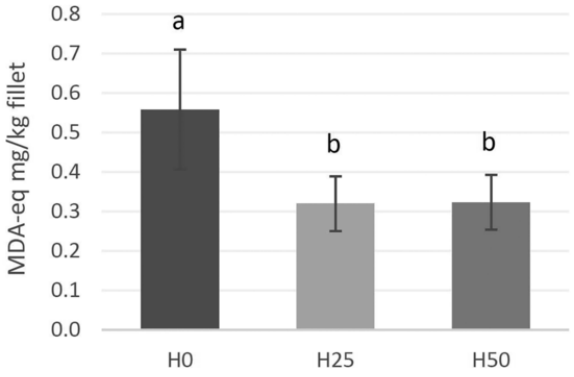
	Diet			SEM[1]	*p-value*[2]
	H0	**H25**	**H50**		
Total lipids	5.13	4.42	4.87	0.36	*ns*
C12:0	0.26[c]	2.15[b]	5.00[a]	0.57	***
C13:0	7.23	8.52	9.27	0.86	*ns*
C14:0	1.68[b]	1.94[b]	2.66[a]	0.14	**
C16:0	15.47[a]	13.51[b]	13.89[b]	0.30	**
C16:1n-7	2.13	2.10	2.15	0.08	*ns*
C18:0	3.59	3.44	3.49	0.05	*ns*
C18:1n-9	25.35	24.69	23.79	0.83	*ns*
C18:1n-7	2.32	2.16	1.88	0.10	*ns*
C18:2n-6	11.79	11.69	10.77	0.45	*ns*
C18:3n-3	2.07	1.85	1.66	0.08	*ns*
C20:1n-11	1.45[a]	1.24[ab]	1.15[b]	0.05	*
C20:4n-6	0.85	1.11	1.10	0.05	*p=0.0568*
C20:5n-3, EPA	2.58	2.38	2.19	0.09	*ns*
C22:6n-3, DHA	12.70	13.03	11.87	0.71	*ns*
SFA	28.46[b]	30.44[b]	35.09[a]	0.90	**
MUFA	29.77	30.95	29.66	1.01	*ns*
PUFAn-6	13.11	14.50	13.61	0.41	*ns*
PUFAn-3	18.48	19.76	17.97	0.75	*ns*
EPA+DHA	15.28	15.41	14.06	0.77	*ns*
SFA/UFA[3]	0.45[b]	0.47[ab]	0.58[a]	0.02	*
C18:4n-3/C18:3n-3	0.21[b]	0.29[a]	0.29[a]	0.01	**
C20:5n-3/C18:3n-3	1.26	1.33	1.37	0.08	*ns*
C22:6n-3/C18:3n-3	6.25	7.50	7.43	0.64	*ns*
C20:4n-6/C18:2n-6	0.07	0.10	0.10	0.01	*ns*

Table 5
Nutritional indices in fillets of rainbow trout fed experimental diets including increasing levels of full-fat *Hermetia illucens* prepupae meal (0%, H0; 25%, H25; 50%, H50).

	Diet			SEM [1]	*p-value* [2]
	H0	H25	H50		
AI	0.34 [b]	0.36 [b]	0.48 [a]	0.019	***
TI	0.25	0.23	0.26	0.008	*ns*
h/H	3.30 [ab]	3.63 [a]	3.18 [b]	0.080	*
n-3/n-6	1.41	1.42	1.35	0.089	*ns*

AI: atherogenicity index; TI: thrombogenicity index; h/H: hypocholesterolaemic/Hypercholesterolaemic fatty acid ratio.
[1] SEM: standard error of the mean.
[2] ns: not significant ($p > .05$); a, b as superscript letters indicate significantly different means at $p < .05$ (*); $p < .001$ (***).

Fig. 3



Fillet TBARS content of rainbow trout fed experimental diets including increasing levels of full-fat *Hermetia illucens* prepupae meal (0%, H0; 25%, H25; 50%, H50). Values are expressed as mg of malondialdehyde-equivalent/kg of fillet (MDA-eq/kg fillet). Bars indicate standard deviation.

Table 6
Fillet physical parameters of rainbow trout fed experimental diets including increasing levels of full-fat *Hermetia illucens* prepupae meal (0%, H0; 25%, H25; 50%, H50).

	Diet			SEM [1]	*p-value* [2]
	H0	H25	H50		
pH	6.59	6.67	6.59	0.02	*ns*
WHC [3], %	91.66	93.89	91.82	0.52	*ns*
Colour					
L*	48.42	46.79	47.61	0.43	*ns*
a*	0.31	1.08	0.32	0.18	*ns*
b*	5.57 [a]	4.21 [b]	4.43 [ab]	0.21	*

[1] SEM: standard error of the mean.
[2] ns: not significant ($p > .05$); a, b as superscript letters indicate significantly different means at $p < .05$ (*).
[3] WHC: water holding capacity.

eicosanoid biosynthesis, transcriptional control, and lipid homeostasis (Conde-Sieira and Soengas, 2017; Monroig et al., 2018; Tocher, 2015, 2003). In fish, differently from other vertebrates, energy is mainly obtained from C16:0 and MUFA (*e.g.* C18:1n-9, C20:1n-9, C22:1n-11), seldom from EPA and only in specific cases from DHA(Tocher, 2003). As regards membrane structure and functionality, FA length and degree of unsaturation orchestrate membrane's biophysical properties, such as fluidity (Tocher, 2015). Eicosanoids are bioactive molecules involved in different biological functions such as immune inflammatory response, renal and neural function, cardiovascular tone, and reproduction (Monroig et al., 2018; Tocher, 2003). They originate from C20:3n-6, C20:4n-6 and EPA; EPA derivatives are usually less biologically-active than the C20:4n-6 ones. In addition, it should be stated that EPA and C20:3n-6 compete with C20:4n-6. Finally, FAs and particularly PUFAs play a role on their own homeostasis at a transcriptional level (Conde-Sieira and Soengas, 2017; Monroig et al., 2018; Tocher, 2015).

Rainbow trout has a malleable lipid metabolism. Liver and intestine play a pivotal role to the digestive physiology and to the end-product nutritional characteristics. Specifically, intestine and liver control dietary lipid absorption, storage and *de novo* synthesis (Tocher, 2003). Several laboratory techniques allowing the assessment of fish physiological response to new diets exist. While biometric indices and histology are able to detect possible alterations in fish organs, molecular biology offers a quick and precocious vision into fish metabolism. For instance, gene expression can be helpful to precociously detect fish physiological responses to this new ingredient (Cardinaletti et al., 2019; Li et al., 2020; Zarantoniello et al., 2020, 2018). Although specimens in Cardinaletti et al. (2019) and in the present paper came from the same growth trial, the former work focused on growth performance, gastrointestinal integrity and stress, and showed a non-significant downward flection of biometrics measurements and no significant differences in growth biomarkers (*igf1*, *mstn1a*) analysed in the liver. The present study combined the use of chemical analysis to assess fillet qualitative aspects and the use of molecular biology tools to investigate some of the lipid metabolism mechanisms behind.

Although no significant differences were noticed when it comes to *cd36* and *ppar* gene expression, interesting trends were noticed. A slight increase in pyloric caeca *cd36* gene expression was noticed upon the increase of dietary H meal inclusion. In a similar manner, Li et al. (2019) described an increased expression of *cd36* in the proximal intestine of pre-smolt Atlantic salmon fed a diet containing H meal, having simultaneously raised dietary fish oil content and diminished rapeseed oil. Underlining that *cd36* is a multifunctional receptor binding ligands involved in different physiological processes (The UniProt Consortium, 2019) and that the function of *cd*36 in rainbow trout has not been deeply studied yet, the observed increasing trend cannot be directly attributed to H meal only, because the interaction with the other ingredients could have played a major role on *cd36* modulation.

Also *ppar*$_s$ regulate different signalling pathways related to lipid metabolism (Kortner et al., 2013). Belghit et al. (2018) compared the effect on Atlantic salmon liver gene expression of replacing 85% of dietary protein with H larvae meal, and/or replacing all the vegetable oils with two different H oils. Differently from the present study, Belghit et al. (2018) found that *pparα* was not affected by the different diets and that *pparγ* was downregulated in the diets containing H meal, irrespective of the dietary oily source. Although their diets contained the same amount of LC PUFA, the H meal diets contained a slightly higher amount of total lipids than that of the ones lacking insect. This element might possibly have contributed to the modulation of *ppar*$_s$ in a different way than that of the present study, as *ppar*$_s$ are regulated by both the FA type and their overall amount (Tocher, 2003).

It should be remarked that pyloric caeca are a significant site of DHA synthesis in rainbow trout (Bell et al., 2003). In the present study, pyloric caeca *elovl2* expression tended to increase with the increasing amount of the dietary H meal inclusion. In addition, an increased *fads2* expression was noted in pyloric caeca and possibly mid intestine; their production could have contributed to the uniform DHA content in livers and fillets belonging to the three different dietary groups, overcoming the lack in the diets containing insect. Moreover, the investigation on FA products:precursors ratios hinted that a higher deposition of C18:4n-3 in fillets of fish fed H meal was realised, suggesting that elongase and desaturase enzymes were active. A direct enzymatic activity assessment would be a useful tool to evaluate the

real outcome of gene expression and to explain the endogenous mechanisms of PUFA biosynthesis.

As fillet DHA content surprisingly did not reflect dietary content, it seemed clear that endogenous elongase and desaturase enzymes changed the dietary FA profile and improved DHA deposition in the muscle. Zarantoniello et al. (2019, 2018) used zebrafish as experimental model to test the effects of diets equivalent to those tested in the present study. Similarly to the present study, the authors observed an increased *elovl2* gene expression in the 50% group after 21 days of feeding trial (Zarantoniello et al., 2018); after six months of feeding, in the same dietary treatment, *fads2* gene expression was upregulated (Zarantoniello et al., 2019). The authors also found a significant decrease in EPA after six months, while the DHA content in zebrafish belonging to the 0 or 25% groups was even and significantly higher than the content of 50% zebrafish (Zarantoniello et al., 2019, 2018). Usually, elongase and desaturase genes are upregulated when fish are fed vegetable oil-based diets, possibly for the deprivation of LC PUFA or the increased content of C18:3n-3, as reviewed by Tocher (2015). A similar mechanism could explain our findings.

The HSI commonly indicates the general status of metabolic activity and energy reserves. Some previous studies (Belforti et al., 2015; Sealey et al., 2011) evidenced a lower HSI in rainbow trout fed with *Tenebrio molitor* or H meal, in comparison to trout fed fishmeal-based control diets. The lack of a significant effect of the experimental diets here tested on rainbow trout HSI represents a promising result. In spite of this, hepatic histological analyses, performed to evaluate lipid accumulation or steatosis, showed a significantly higher PFF in H50 livers in comparison to those of the other two groups. Belghit et al. (2019a) did not find different size distribution of hepatic lipid droplets of Atlantic salmon fed diets with fishmeal or where fishmeal had been replaced by H meal, nor the hepatic triacylglycerol concentrations were dissimilar; the administered diets were isolipidic and contained very similar amounts of the different FA classes, specifically, SFA and PUFAn-3 were to some extent higher while MUFA and PUFAn-6 were slightly lower in the diet containing H meal in comparison to the control diet. On the contrary, Zarantoniello et al. (2019, 2018) fed zebrafish isolipidic diets equivalent to those tested here and showed that liver of the H25 and H50 groups accumulated a higher amount of lipids than the H0 group. An associations between high n-6/n-3 ratio and steatosis was found (Zarantoniello et al., 2020, 2019, 2018). Besides, comparing H0 and H50 diets we point out that SFA increased from 33.75 to 48.24% of total FAMEs, while PUFAn-3 fell from 22.01 to 10.11% of total FAMEs. Reviews on rodents show that PUFAn-3-rich diets reduce intrahepatic triglyceride content and steatosis, while diets poor in PUFAn-3 fuel steatosis (Di Minno et al., 2012). Also, saturated free FAs possibly have an impact on lipotoxicity, which is thought to be fought by increasing intrahepatic triglyceride accumulation, a method to dispose of excess FAs (Leamy et al., 2013).

As revealed by gas-chromatography, total SFA content in liver was not different between the dietary treatments although C12:0 and C14:0 significantly increased in H25 and H50 dietary groups, counterbalanced by the decrease in C16:0. On the other hand, Belghit et al. (2018) noticed a decreased liver triacylglycerol and C12:0 content (~1.5% of total FAs, regardless of the administered diet) when freshwater Atlantic salmon was fed diets containing H derivatives and a high fish oil level, in comparison to the control diet. It can be thus assumed that fish of the present study accumulated C12:0 rather than oxidising it. As concerns FAs of the n-6 series, their presence in the livers mainly derived from endogenous production as they could be found in little amounts in the experimental diets. Since no different *elovl1* and *elovl2* expression was observed in the liver of the three groups and the calculated FA products:precursors indices resulted unaffected, the equal level of DHA in liver of the three groups might suggest that DHA was accumulated in the liver after a synthesis in pyloric caeca or mid intestine by elongase and desaturase enzymes. Data on the overall upregulation of *fads2* and *elovl2* in H25 and H50 pyloric caeca seemed to support this hypothesis.

As molecular markers produce changes in the composition of the end-product, quality-oriented parameters of fillets, such as their nutritional value and oxidative status, were investigated. The main results are encouraging and partly contradicting some earlier findings. In fact, only the increase in fillet SFA content parallel to the increasing H meal inclusion levels agreed with the findings of similar studies (Borgogno et al., 2017; Iaconisi et al., 2018; Stadtlander et al., 2017; Zarantoniello et al., 2019). Conversely, and surprisingly, all the other lipid classes, primarily the PUFAn-3 as well as DHA amounts, did not reflect the dietary content, thus resulting in a well-balanced FA profile of the fillets regardless the dietary treatment.

Standing on the improved DHA deposition in the muscle of H-fed specimens and the parallel increased pyloric caeca *elovl2* and *fads2* expression as well as the possible increased mid intestine *fads2* expression, it seemed clear that endogenous elongase and desaturase enzymes were effective in modifying the dietary FA profile. The question to be answered is why the present results substantially differed from the available literature. Indeed, although Zarantoniello et al. (2019, 2018) observed an increased *elovl2* and *fads2* gene expression in zebrafish fed an H50 diet totally equivalent to that tested here compared to H0 and H25 groups, the DHA content in zebrafish belonging to the H0 or H25 groups were significantly higher than the content of H50 zebrafish after 180 days of feeding trial. Contrariwise, the DHA content was significantly higher in the muscle of juvenile Jian carp (*Cyprinus carpio* var. Jian) fed a diet where H larvae oil replaced soybean oil (at 25, 50, 75 or 100% levels) than in the muscle of the control group (Li et al., 2016). Despite the authors did not explain the possible causes of this unexpected effect, it is of interest that H oil could play a key-role in promoting elongase and desaturase activity in rainbow trout. In view of the contrasting findings mentioned, despite elongase and desaturase gene upregulation is usually associated to severe deprivation of dietary LC PUFA (Tocher, 2015), the equal amount of fillet PUFAn-3 content in contrast to the decrease dietary PUFAn-3 content cannot be easily explained. Other factors must play a role in muscle lipid storage constitution, like fish age, specific content of single dietary FAs, free FAs, *etc.* Further investigation should address these factors, as well as analyse the direct elongase and desaturase enzyme activity and serum biochemical indices.

The nutritional and oxidative status of the fillets were assessed calculating AI, TI, h/H, n-3/n-6 ratios and TBARS content. The overall fillet FA profile was mirrored in the nutritional indices. For instance, both AI and h/H were worsened in H50 group due to the high SFA content, while the stability of the other lipid classes (especially PUFAn-3) can be the cause of the rather stable TI and n-3/n-6 ratio values. Our findings did not agree with the available literature. Indeed, fillets of rainbow trout fed 0, 25 or 50% substitution levels of fishmeal with partially defatted H meal showed that PUFA/SFA ratio, AI, TI and n-3/n-6 ratio were negatively affected by insect inclusion in the diet (Renna et al., 2017). To conclude, the positive results obtained here pave the way to new opportunities for the study and use of full-fat H larvae meal.

A limited number of studies analysed the TBARS content in fish fed diets containing insect meal. Similarly to the present results, Secci et al. (2019) found a decreasing trend following the increasing H meal inclusion. This finding could be primarily attributed to the higher SFA content in the fillets belonging to H25 and H50 groups. However, at a closer look, recent works underlined that dietary H meal seemed to ameliorate the antioxidant defence of fish by increasing serum catalase activity (Li et al., 2017) or glutathione S-transferase, ethoxyresorufin O-deethylase and total glutathione in rainbow trout liver and kidney (Elia et al., 2018).

Finally, results on the fillet physical traits, agreeing with most of the results from similar studies using defatted insect meals (Iaconisi

et al., 2018; Mancini et al., 2018), showed that pH, WHC, lightness index and redness index were not affected by the different dietary treatments, while yellowness index was significantly lower in H25 in comparison to H0. Colour variations are difficult to interpret as they depend on the presence of dietary pigments (especially vegetable pigments), on rancidity in the case of the stored products, on the punctual readings of the colourimeter. Therefore, it is difficult to explain unambiguously the reason of the increased yellowness index (b*) in H25 fillets. The ΔE result worth considering is the couple H0 *vs* H25, which was 2.28. This value is considered just above the threshold enabling unexperienced observers noticing colour differences (Mokrzycki and Tatol, 2011). Nevertheless, following Sharma (2003)'s indications, a $\Delta E = 2.28$ is below the limit enabling an unexperienced observer noticing the differences. Presumably, a test with trained panellists could cast light on the actual perceived difference.

5. Conclusions

The dietary full-fat H meal influenced rainbow trout lipid metabolism, as shown by qPCR gene expression analysis and liver histological examinations. Nonetheless, the final qualitative traits of the fillets were not negatively affected, and a nutritious final product was guaranteed. In the light of the differences between previous literature and the present positive results, further studies on the biological mechanisms behind the macroscopic traits of fish fed insects are warmly encouraged, for instance by directly assessing elongase and desaturase enzyme activities and serum biochemical indices, as well as by discerning the impact of other possible factors, as fish age, specific content of single dietary FAs, free FAs, *etc.*

Author contributions

Conceptualization and methodology: FT, IO, GP; Sampling, data collection and curation: LB, BR, GC, GS; Laboratory analyses: LB, BR, MZ, FM, GS; Statistical analyses and data visualisation: LB, BR; Writing-original draft: LB, GS; Writing-review and editing: LB, BR, GC, GS, FT, IO, GP; Funding acquisition: FT, IO, GP.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the SUSHIN (SUstainable fiSH feeds INnovative ingredients) project funded by Ager (AGER2-SUSHIN Cod 2016-0112), by Fondi di Ateneo (ex-60%) project to GP, by Fondi di Ateneo project to IO and by Pj_libera Tulli project to FT.

References

Bakke, A M, Glover, C, Krogdahl, Å, 2010. Feeding, digestion and absorption of nutrients. In: Fish Physiology. Elsevier Inc, pp. 57–110. doi:10.1016/S1546-5098(10)03002-5.

Barragan-Fonseca, K B, Dicke, M, van Loon, J J A, 2017. Nutritional value of the black soldier fly (*Hermetia illucens* L.) and its suitability as animal feed – a review. J. Insects as Food Feed 3, 105–120. doi:10.3920/JIFF2016.0055.

Barroso, F G, de Haro, C, Sánchez-Muros, M-J, Venegas, E, Martínez-Sánchez, A, Pérez-Bañón, C, 2014. The potential of various insect species for use as food for fish. Aquaculture 422–423, 193–201. doi:10.1016/j.aquaculture.2013.12.024.

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. J. Food Compos. Anal. 62, 8–13. doi:10.1016/j.jfca.2017.04.008.

Barroso, F G, Sánchez-Muros, M J, Rincón, MÁ, Rodriguez-Rodriguez, M, Fabrikov, D, Morote, E, Guil-Guerrero, J L, 2019. Production of n-3-rich insects by bioaccumulation of fishery waste. J. Food Compos. Anal. 82, 103237. doi:10.1016/j.jfca.2019.103237.

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 fillets. Ital. J. Anim. Sci. 14, 670–676. doi:10.4081/ijas.2015.4170.

Belghit, I, Liland, N S, Waagbø, R, Biancarosa, I, Pelusio, N, Li, Y, Krogdahl, Å, Lock, E-J, 2018. Potential of insect-based diets for Atlantic salmon (*Salmo salar*). Aquaculture 491, 72–81. doi:10.1016/j.aquaculture.2018.03.016.

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. doi:10.1016/j.aquaculture.2018.12.032.

Belghit, I, Waagbø, R, Lock, E-J, Liland, N S, 2019. Insect-based diets high in lauric acid reduce liver lipids in freshwater Atlantic salmon. Aquac. Nutr. 25, 343–357. doi:10.1111/anu.12860.

Bell, M V, Dick, J R, Porter, A E A, 2003. Pyloric ceca are significant sites of newly synthesized 22:6n-3 in rainbow trout (*Oncorhynchus mykiss*). Lipids 38, 39–44. doi:10.1007/s11745-003-1029-5.

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. J. Sci. Food Agric. 97, 3402–3411. doi:10.1002/jsfa.8191.

Bosch, G, van Zanten, H H E, Zamprogna, A, Veenenbos, M, Meijer, N P, van der Fels-Klerx, H J, van Loon, J J A, 2019. Conversion of organic resources by black soldier fly larvae: legislation, efficiency and environmental impact. J. Clean. Prod. 222, 355–363. doi:10.1016/j.jclepro.2019.02.270.

Bruni, L, Belghit, I, Lock, E-J, Secci, G, Taiti, C, Parisi, G, 2020. Total replacement of dietary fish meal with black soldier fly (*Hermetia illucens*) larvae does not impair physical, chemical or volatile composition of farmed Atlantic salmon (*Salmo salar* L.). J. Sci. Food Agric. 100, 1038–1047. doi:10.1002/jsfa.10108.

Cardinaletti, G, Randazzo, B, Messina, M, Zarantoniello, M, Giorgini, E, Zimbelli, A, Bruni, L, Parisi, G, Olivotto, I, Tulli, F, 2019. Effects of graded dietary inclusion level of full-fat *Hermetia illucens* prepupae meal in practical diets for rainbow trout (*Oncorhynchus mykiss*). Animals 9, 1–21. doi:10.3390/ani9050251.

CIE, 1976. Official Recommendations on Uniform Colour Space, Colour Difference Equations and Metric Colour Terms. CIE Publ. No. 15 1976.

Conde-Sieira, M, Soengas, J L, 2017. Nutrient sensing systems in fish: impact on food intake regulation and energy homeostasis. Front. Neurosci. 10, 1–21. doi:10.3389/fnins.2016.00603.

Di Minno, M N D, Russolillo, A, Lupoli, R, Ambrosino, P, Di Minno, A, Tarantino, G, 2012. Omega-3 fatty acids for the treatment of non-alcoholic fatty liver disease. World J. Gastroenterol. 18, 5839. doi:10.3748/wjg.v18.i41.5839.

Directive 2010/63/EU, 2010. No 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. OJ L 276, 20.10.2010. pp. 33–79 Official Journal of the European Union.

Elia, A C, Capucchio, M T, Caldaroni, B, Magara, G, Dörr, A J M, Biasato, I, Biasibetti, E, 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. doi:10.1016/j.aquaculture.2018.07.009.

FAO, 2019. Fishery and aquaculture statistics. In: Global aquaculture production 1950–2017 (FishstatJ), in: FAO Fisheries and Aquaculture Department [Online]. Rome. Updated 2019. http://www.fao.org/fishery/statistics/software/fishstatj/en.

Folch, J, Lees, M, Stanley, G H S, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.

Giorgini, E, Randazzo, B, Gioacchini, G, Cardinaletti, G, Vaccari, L, Tibaldi, E, Olivotto, I, 2018. New insights on the macromolecular building of rainbow trout (*O. mykiss*) intestine: FTIR imaging and histological correlative study. Aquaculture 497, 1–9. doi:10.1016/j.aquaculture.2018.07.032.

Gregory, M K, James, M J, 2014. Rainbow trout (*Oncorhynchus mykiss*) Elovl5 and Elovl2 differ in selectivity for elongation of omega-3 docosapentaenoic acid. Biochim. Biophys. Acta Mol. Cell Biol. Lipids 1841, 1656–1660. doi:10.1016/j.bbalip.2014.10.001.

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. doi:10.1016/j.anifeedsci.2015.03.001.

Iaconisi, V, 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. doi:10.1016/j.aquaculture.2017.11.034.

Janani, C, Ranjitha Kumari, B D, 2015. PPAR gamma gene – A review. Diabetes Metab. Syndr. Clin. Res. Rev. 9, 46–50. doi:10.1016/j.dsx.2014.09.015.

Kortner, T M, Gu, J, Krogdahl, Å, Bakke, A M, 2013. Transcriptional regulation of cholesterol and bile acid metabolism after dietary soyabean meal treatment in Atlantic salmon (*Salmo salar* L.). Br. J. Nutr. 109, 593–604. doi:10.1017/S0007114512002024.

Lazzarotto, V, Médale, F, Larroquet, L, Corraze, G, 2018. Long-term dietary replacement of fishmeal and fish oil in diets for rainbow trout (*Oncorhynchus mykiss*): effects on growth, whole body fatty acids and intestinal and hepatic gene expression. PLoS One 13, e0190730. doi:10.1371/journal.pone.0190730.

Leamy, A K, Egnatchik, R A, Young, J D, 2013. Molecular mechanisms and the role of saturated fatty acids in the progression of non-alcoholic fatty liver disease. Prog. Lipid Res. 52, 165–174. doi:10.1016/j.plipres.2012.10.004.

Li, S, Ji, H, Zhang, B, Tian, J, Zhou, J, Yu, H, 2016. Influence of black soldier fly (*Hermetia illucens*) larvae oil on growth performance, body composition, tissue fatty acid composition and lipid deposition in juvenile Jian carp (*Cyprinus carpio* var. Jian). Aquaculture 465, 43–52. doi:10.1016/j.aquaculture.2016.08.020.

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. Aquaculture 477, 62–70. doi:10.1016/j.aquaculture.2017.04.015.

Li, Y, Kortner, T M, Chikwati, E M, Munang'andu, H M, Lock, E-J, Krogdahl, Å, 2019. Gut health and vaccination response in pre-smolt Atlantic salmon (*Salmo salar*) fed black soldier fly (*Hermetia illucens*) larvae meal. Fish Shellfish Immunol. 86, 1106–1113. doi:10.1016/j.fsi.2018.12.057.

Li, Y, Kortner, T M, Chikwati, E M, Belghit, I, Lock, E, Krogdahl, Å, 2020. Total replacement of fish meal with black soldier fly (*Hermetia illucens*) larvae meal does not compromise the gut health of Atlantic salmon (*Salmo salar*). Aquaculture 520, 734967. doi:10.1016/j.aquaculture.2020.734967.

Liland, N S, Biancarosa, I, Araujo, P, Biemans, D, Bruckner, C G, Waagbø, R, Torstensen, B E, Lock, E-J, 2017. Modulation of nutrient composition of black soldier fly (*Hermetia illucens*) larvae by feeding seaweed-enriched media. PLoS One 12, e0183188. doi:10.1371/journal.pone.0183188.

Lock, E-J, Arsiwalla, T, Waagbø, R, 2016. Insect larvae meal as an alternative source of nutrients in the diet of Atlantic salmon (*Salmo salar*) post-smolt. Aquac. Nutr. 22, 1202–1213. doi:10.1111/anu.12343.

Lock, E-J, Biancarosa, I, Gasco, L, 2018. Insects as Raw Materials in Compound Feed for Aquaculture, in: Edible Insects in Sustainable Food Systems. Springer International Publishing, Cham, pp. 263–276. doi:10.1007/978-3-319-74011-9_16.

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. doi:10.1017/S1751731117003421.

Mokrzycki, W S, Tatol, M, 2011. Color Difference ΔE - A survey. M. G. V. 20. pp. 383–412.

Monroig, O, Tocher, D R, Castro, L F C, 2018. Polyunsaturated fatty acid biosynthesis and metabolism in fish. In: Polyunsaturated Fatty Acid Metabolism. Elsevier, pp. 31–60. doi:10.1016/B978-0-12-811230-4.00003-X.

Morash, A J, Bureau, D P, McClelland, G B, 2009. Effects of dietary fatty acid composition on the regulation of carnitine palmitoyltransferase (CPT) I in rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol. Part B Biochem. Mol. Biol. 152, 85–93. doi:10.1016/j.cbpb.2008.10.005.

Nogales-Mérida, S, Gobbi, P, Józefiak, D, Mazurkiewicz, J, Dudek, K, Rawski, M, Kierończyk, B, Józefiak, A, 2018. Insect meals in fish nutrition. Rev. Aquac. 1–24. doi:10.1111/raq.12281.

NRC, N R C, 2011. Nutrient Requirements of Fish and Shrimp. National academies press, Washington DC.

Oboh, A, Kabeya, N, Carmona-Antoñanzas, G, Castro, L F C, Dick, J R, Tocher, D R, Monroig, O, 2017. Two alternative pathways for docosahexaenoic acid (DHA, 22:6n-3) biosynthesis are widespread among teleost fish. Sci. Rep. 7, 3889. doi:10.1038/s41598-017-04288-2.

R Core Team R: A Language and Environment for Statistical Computing [WWW Document]. R Found. Stat. Comput. Vienna, AustriaURLhttps://www.r-project.org/2018

Randazzo, B, Abbate, F, Marino, F, Mancuso, M, Guerrera, M C, Muglia, U, Navarra, M, Germanà, A, 2015. Induction of mild enterocolitis in zebrafish *Danio rerio* via ingestion of *Vibrio anguillarum* serovar O1. Dis. Aquat. Org. 115, 47–55. doi:10.3354/dao02864.

Renaville, B, Tulli, F, Bruno, M, Tibaldi, E, Messina, M, 2013. Fatty acid desaturase 2 (FADS2) insertion/deletion polymorphism impact on muscle fatty acid profile in European grayling (*Thymallus thymallus*). Br. J. Nutr. 110, 1559–1564. doi:10.1017/S0007114513001049.

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. J. Anim. Sci. Biotechnol. 8, 57. doi:10.1186/s40104-017-0191-3.

Rosenlund, G, Corraze, G, Izquierdo, M, Torstensen, B, 2010. The effects of fish oil replacement on nutritional and organoleptic qualities of farmed fish. In: Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds. CRC Press, Boca Raton, FL, pp. 487–522. doi:10.1201/9781439808634-c15.

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. doi:10.1016/j.jclepro.2013.11.068.

Santos-Silva, J, Bessa, R J B, Santos-Silva, F, 2002. Effect of genotype, feeding system and slaughter weight on the quality of light lambs 1. Fatty acid composition of meat. In: Livest. Prod. Sci., 77. pp. 187–194. doi:10.1016/S0301-6226(02)00059-3.

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*. J. World Aquacult. Soc. 42, 34–45. doi:10.1111/j.1749-7345.2010.00441.x.

Secci, G, Bovera, F, Nizza, S, Baronti, N, Gasco, L, Conte, G, Serra, A, Bonelli, A, Parisi, G, 2018. Quality of eggs from Lohmann Brown classic laying hens fed black soldier fly meal as substitute for soya bean. Animal 1–7. doi:10.1017/S1751731117003603.

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

Sharma, G, 2003. Digital Color Handbook. CRC Press, Boca Raton, FL.

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

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. J. World Aquacult. Soc. 38, 309–313. doi:10.1111/j.1749-7345.2007.00101.x.

Tacchi, L, Secombes, C J, Bickerdike, R, Adler, M A, Venegas, C, Takle, H, Martin, S A M, 2012. Transcriptomic and physiological responses to fishmeal substitution with plant proteins in formulated feed in farmed Atlantic salmon (*Salmo salar*). BMC Genomics 13, 363. doi:10.1186/1471-2164-13-363.

The UniProt Consortium, 2019. UniProt: a worldwide hub of protein knowledge. Nucleic Acids Res. 47, D506–D515. doi:10.1093/nar/gky1049.

Tocher, D R, 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Rev. Fish. Sci. 11, 107–184. doi:10.1080/713610925.

Tocher, D R, 2015. Omega-3 long-chain polyunsaturated fatty acids and aquaculture in perspective. Aquaculture 449, 94–107. doi:10.1016/j.aquaculture.2015.01.010.

Truzzi, C, Giorgini, E, Annibaldi, A, Antonucci, M, Illuminati, S, Scarponi, G, Riolo, P, Isidoro, N, Conti, C, Zarantoniello, M, Cipriani, R, Olivotto, I, 2020. Fatty acids profile of black soldier fly (*Hermetia illucens*): influence of feeding substrate based on coffee-waste silverskin enriched with microalgae. Anim. Feed Sci. Technol. 259, 114309. doi:10.1016/j.anifeedsci.2019.114309.

Turchini, G M, Torstensen, B E, Ng, W-K, 2009. Fish oil replacement in finfish nutrition. Rev. Aquac. 1, 10–57. doi:10.1111/j.1753-5131.2008.01001.x.

Ulbricht, T L V, Southgate, D A T, 1991. Coronary heart disease: seven dietary factors. Lancet.. doi:10.1016/0140-6736(91)91846-M.

van Huis, A, Van Itterbeeck, J, Mertens, E, Halloran, A, Muir, G, Vantomme, P, 2013. Edible Insects: Future Prospects for Food and Feed Security. FAO Forestry Paper No. 171, Rome, FAO, p. 201.

Vyncke, W, 1970. Direct determination of the thiobarbituric acid value in trichloracetic acid extracts of fish as a measure of oxidative rancidity. Fette, Seifen, Anstrichm.. doi:10.1002/lipi.19700721218.

Zarantoniello, M, Bruni, L, Randazzo, B, Vargas, A, Gioacchini, G, Truzzi, C, Annibaldi, A, Riolo, P, Parisi, G, Cardinaletti, G, Tulli, F, Olivotto, I, 2018. Partial dietary inclusion of *Hermetia illucens* (black soldier Fly) full-fat prepupae in zebrafish feed: biometric, histological, biochemical, and molecular implications. Zebrafish 15, 519–532. doi:10.1089/zeb.2018.1596.

Zarantoniello, M, Randazzo, B, Truzzi, C, Giorgini, E, Marcellucci, C, Vargas-Abúndez, J A, Zimbelli, A, Annibaldi, A, Parisi, G, Tulli, F, Riolo, P, Olivotto, I, 2019. A six-months study on black soldier Fly (*Hermetia illucens*) based diets in zebrafish. Sci. Rep. 9, 8598. doi:10.1038/s41598-019-45172-5.

Zarantoniello, M, Zimbelli, A, Randazzo, B, Compagni, M D, Truzzi, C, Antonucci, M, Riolo, P, Loreto, N, Osimani, A, Milanović, V, Giorgini, E, Cardinaletti, G, Tulli, F, Cipriani, R, Gioacchini, G, Olivotto, I, 2020. Black soldier Fly (*Hermetia illucens*) reared on roasted coffee by-product and *Schizochytrium* sp. as a sustainable terrestrial ingredient for aquafeeds production. Aquaculture 518, 734659. doi:10.1016/j.aquaculture.2019.734659.