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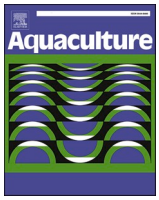

Performance and fillet traits of rainbow trout (*Oncorhynchus mykiss*) fed different levels of *Hermetia illucens* meal in a low-tech aquaponic system

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ABSTRACT

In this study, we evaluated the effects of dietary substitution of fishmeal (FM) with partially defatted *Hermetia illucens* meal (HI) on the growth, feed digestibility, gut morphology, and fillet quality of rainbow trout reared in a low-tech aquaponic system. A total of 173 rainbow trout (initial body weight: 156 g ± 39.8 g) were distributed among nine experimental aquaponic units. Three diets were fed to fish (three aquaponic units per dietary treatment) over a period of 76 days, i.e. H0 diet (control), H6 diet, and H12 diet containing 0 g/kg, 62 g/kg, or 124 g/kg of HI and 200 g/kg, 150 g/kg, or 100 g/kg of FM, respectively. We found that the apparent digestibility coefficients of diets were unaffected by the inclusion level of dietary HI. At the end of the trial, trout mortality was low (2.9%) and unaffected by dietary treatment. The specific growth rate was, however, lower in fish fed the H12 diet than in those fed H0 and H6 diets after 26 days (1.07% d^{-1} vs. 1.22% d^{-1}; $P < 0.001$) and at the end of the trial (0.81% d^{-1} vs. 0.88% d^{-1}; $P < 0.05$). In contrast, dietary inclusion of HI appeared to have no appreciable effect on the feed conversion ratio (on average 1.53), final weight (303 g), fish condition factor (1.40), viscerosomatic index (10.9%), or hepatosomatic index (1.22%). The inclusion of HI was, nevertheless, found to promote a 10% increase in the density of goblet cells in the gut of fish fed the H12 diet compared with those receiving the H0 diet ($P < 0.05$). With regards to fillet traits, redness and yellowness indices were lower in fish fed the H12 diet than in those fed the H0 diet. Although dietary HI had little effect on the proximate composition and fatty acid profile of fish, the proportions of C12:0 and C14:0 increased with HI dietary inclusion. In conclusion, fish growth and fillet quality were essentially unaffected by a 25% fish meal replacement with HI in isonitrogenous and isoenergetic diets (control diet containing 200 g/kg of fish meal), whereas at a replacement rate of 50%, we detected certain effects on gut histology and fillet colour and nutritional characteristics, which warrant further investigation.

1. Introduction

The global demand for food products is expected to grow by between 1.1% and 1.5% per year by 2050 (Alexandratos and Bruinsma, 2012). However, meeting these demands will necessitate an ever-increasing reliance on natural resources such as water, land, and nutrients, which are already unsustainably exploited by modern agricultural practices (Lennard and Goddek, 2019). To ensure sufficient food production and at the same time conserve natural resources and maintain environmental integrity, it will be necessary to develop alternative and more sustainable agricultural methods (Goddek et al., 2015; Maucieri et al., 2019). Aquaponics (AP), that combines recirculating aquaculture systems (RAS) with soilless hydroponic plant production and reduces water consumption (Verdegem et al., 2006; Endut et al., 2011), land use (Barbosa et al., 2015; dos Santos, 2016), and nutrient wastes (Nichols and Savidov, 2012; Graber and Junge, 2009; Wongkiew et al., 2017), can make an important contribution in this regard. In particular, aquaponic systems have relevant advantages with respect to nutrient cycling,

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by avoiding the discharge of fish effluents enriched with dissolved nitrogen and phosphorus into groundwater (van Rijn, 2013), and facilitating the fertilization of soilless crops with organic inputs derived from fish faeces (Goddek et al., 2015).

Fish feed serves as a primary nutritional source for aquaponic systems, providing nutrients that sustain fish, bacteria, and plants (Lennard and Goddek, 2019). The main protein sources in aquafeeds are fishmeal (FM) and soybean meal (Naylor et al., 2009; Turchini et al., 2009; Gasco et al., 2018). However, the use of both these nutrient sources raises important sustainability concerns on account of the increasing price of FM (Naylor et al., 2000; Tacon and Metian, 2018; FAO, 2018) and the environmental costs associated with protein-rich plant production (Foley et al., 2011). In this context, insect-based meals could represent a sustainable alternative source of protein for fish production (Mancini et al., 2018). Among insects with potential utility in this respect is the black soldier fly (*Hermetia illucens*; HI), which is considered one of the most promising species owing its low environmental impact, rearing requirements, and high adaptability to low-cost substrates such as manure, food by-products, and waste (Mancini et al., 2018; Gasco et al., 2020). Moreover, HI prepupae meal is characterized by an essential amino acid profile similar to that of FM (Henry et al., 2015). In addition, the production of these insects leaves a small ecological footprint, results in minimal releases of greenhouse gases and ammonia, and has limited need for arable land (Van Huiss, 2013; Makkar et al., 2014; Henry et al., 2015). Notably, the inclusion of processed insects in aquafeeds has recently been permitted in Europe (EC, 2017).

With regards to the species of fish used for AP, high-value species such as rainbow trout (*Oncorhynchus mykiss* Walbaum) have the potential to enhance the profitability and competitiveness of systems compared with the cyprinids and tilapias that are typically farmed using this technique (Palm et al., 2019). However, rainbow trout are notably more demanding in their requirements, particularly in terms of water temperature (7–18 °C) (Woynarovich et al., 2011) and dissolved oxygen (6–8 mg L^{-1}) (Timmons and Ebeling, 2013). To the best of our knowledge, there has to date been only a single study that has investigated the growth performance and quality of rainbow trout reared under aquaponic conditions (Birolo et al., 2020), although some commercial farms have already been established and are currently operational in Canada, Chile, and Colombia. Moreover, only two studies have investigated the inclusion of insect meal in aquafeed of fish raised in aquaponic systems, namely, the Nile tilapia (Kessens, 2016) and Siberian sturgeon (Zarantoniello et al., 2021).

Thus, in this study, we aimed to assess the growth performance, diet digestibility, gut morphometry, and product quality of trout reared in a low-tech aquaponic system and fed different levels of HI meal.

2. Materials and methods

The growth trial conducted in the present study was performed at the Experimental Farm of the Department of Agronomy, Food, Natural Resources, Animal, and Environment of the University of Padova (Legnaro, Padova, Italy). Fish diets were prepared and a digestibility trial was carried out at the Experimental Facility of the Department of Agricultural, Forest and Food Sciences of the University of Torino (Torino, Italy). The study was approved by the Ethical Committee for Animal Experimentation (Organismo preposto al Benessere degli Animali) of the University of Padova (project no. 6/2018; prot. n. 15132 approved on 25/01/2018). The digestibility trial protocol was approved by the Ethical Committee of the University of Torino (Prot. N. 143,811). Animals were handled in accordance with the principles stated by the EU Directive 2010/63/EU regarding the protection of animals used for experimental and other scientific purposes. The research staff involved in animal handling were animal specialists (PhD or MS in Animal Science) and veterinary practitioners.

2.1. Equipment

The trial was performed at the farm of the University of Padova (Northeast Italy, 45°20′N; 11°57′E; 6 m a.s.l.) in a plastic greenhouse with 50% shading. The experimental system consisted of nine independent units, each of which comprised a main tank containing fish (volume 500 L; height 0.80 m) and a sedimenter (volume 100 L; height 0.60 m) (Fig. 1). These units also included two tanks containing plants (volume 275 L each; height 0.35 m), filled with 225 L of expanded clay (LECA Laterlite, Solignano, Italy), which received water from the main tank containing fish and acted both as biofilter and substrate for plant growth, as well as a water storage tank (volume 50 L; height 0.45 m), in which water derived from the tanks containing plants was collected and subsequently recirculated to the main tank containing fish via the operation of a single pump (Newa Jet 1700; NEWA Tecno Industria Srl, Loreggia, Italy). The tanks used in the present trial were made of high-density polyethylene (HDPE). The aquaponic units were designed to be "low-tech", as they were characterized by: i) a simple hydroponic section which acted also as a biofilter; ii) no energy utilization to regulate water temperature; iii) very low environmental control, i.e. the absence of probes and systems for the continuous evaluation of water and for remote management, and the absence of devices for water sanitation, such as UV and ozone chamber systems.

Water flow throughout the system was guaranteed by overflow (from the main tank with fish to the tanks with plants, and thereafter to the storage tank). A flow rate of 120 L h^{-1} permitted complete water turnover at 5-h intervals, and the oxygenation of water was facilitated by a porous stone connected to an aerator (Scubla D100; Scubla Srl, Remanzacco, Italy), which was placed within the main tanks containing fish.

2.2. Plants, fish, and experimental diets

Each of the two designated plant tanks contained seven strawberries (*Fragaria* × *ananassa* Duch.) plantlets (germinated in peat pots) bearing five to seven fully developed leaves, for a total of 14 plants per experimental unit. An ever-bearing cultivar was used for fruit ripening from April to August, and during the experimental period, ripe strawberries were collected daily.

A total of 173 rainbow trout (initial body weight: 156 g ± 39.8 g) were purchased from a commercial farm. A different number of fish per

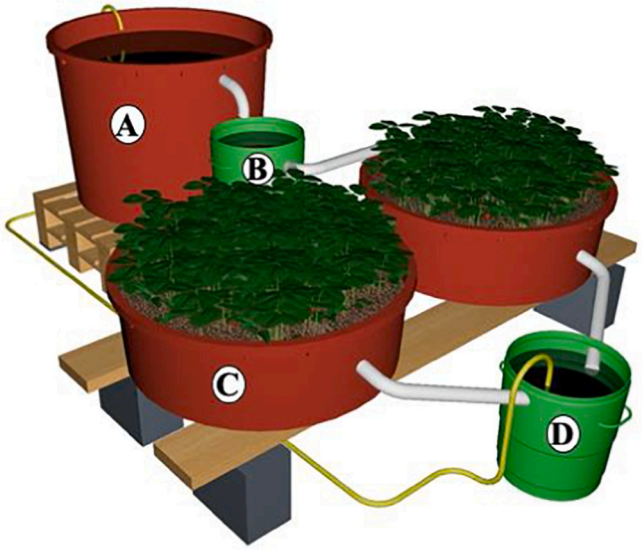

Fig. 1. A 3D sketch of one of the nine aquaponic experimental units. Tank containing fish (A); Sedimenter (B); Tank containing plants (C); Water storage tank (D).

tank (19–20) was allocated to the main tanks to balance the initial biomass among three experimental groups to be fed different diets (6.074 ± 0.69 kg m^{-3} per tank in H0 group; 6.026 ± 0.55 kg m^{-3} in H6 group; 6.073 ± 0.46 kg m^{-3} in H12 group) for 76 days.

The three dietary treatments assessed in the present study were based on partially defatted HI larval meal (Mutatec, Caumont-sur-Durance, France), which was used to replace different proportions of a standard FM (0%, 25%, and 50%) in diets H0, H6, and H12, respectively. These three dietary treatments were formulated as follows: diet H0, the control diet containing 200 g/kg FM and no HI; diet H6, containing 150 g/kg FM and 62 g/kg HI; and diet H12, containing 100 g/kg FM and 124 g/kg HI (Table 1). Owing to the lower crude protein concentration of HI meal (60.5% DM) compared with that of FM (74.3% DM), the HI meal was included at higher rates than the those of the substituted FM. Furthermore, the level of included gelatinized starch was slightly modified. The HI larvae were commercially reared on plant by-products and partially defatted using a mechanical process. However, no other information was provided by the producer regarding either the rearing substrate or the processing methodologies, as this information is deemed confidential.

The experimental diets were prepared at the experimental facility of DISAFA. The ground ingredients were individually weighed (KERN PLE-N v.2.2; KERN & Sohn GmbH, Balingen-Frommern, Germany; d: 0.01) and subsequently mixed with fish oil using a blender (Brevetti S.A.G.A., Milano, Italy). From 250 to 500 mL kg^{-1} of water was added to the mixture to facilitate the pelleting process. The pelletizing was performed using a meat grinder (LABOR 32; Rheninghaus Factory, San Mauro Torinese, Italy). The pellets (3.0 mm) were subsequently dried (50 °C for 48 h) and stored in black bags at −20 °C until use. A total of 20 kg were prepared per each diet before the start of the trial.

The diets were isonitrogenous [crude protein: 44% dry matter (DM)], isolipidic (crude lipid: 17% DM), and isoenergetic (gross energy: 21 MJ/kg). The diets were formulated according to rainbow trout requirements (NRC, 2011). The chemical compositions of the HI and experimental diets are listed in Table 1; the fatty acid composition of the experimental diets is reported in Table 2.

Table 1
Components (g/kg as fed) and proximate composition (% DM) of the experimental diets and *Hermetia illucens* meal (HI).

	HI	Diets		
		H0	H6	H12
Ingredients				
Fishmeal (CP 73% DM)		200	150	100
Hermetia illucens larva meal		0	62	124
Gelatinized starch, D500		150	138	126
Corn gluten meal		119	119	119
Soybean (SB) meal		215	215	215
SB protein concentrate		70	70	70
Porcine haemoglobin		30	30	30
Wheat flour		55	55	55
Fish oil		70	70	70
Soybean oil		70	70	70
Hydrolysed krill		5	5	5
Mineral premix[1]		2.5	2.5	2.5
Vitamin premix[2]		2.5	2.5	2.5
DL-methionine		8	8	8
L-lysine		3	3	3
Proximate composition				
Dry matter, %	94.0	93.6	93.5	93.5
Crude protein, %DM	60.5	44.7	44.1	44.0
Crude lipid, %DM	7.43	17.0	16.7	17.3
Ash, %DM	10.8	6.40	6.12	6.00
Gross energy, MJ/kg DM	21.5	22.6	23.0	21.8

H0, H6 and H12 diets, fishmeal replaced with *Hermetia illucens* meal (HI) at 0%, 25% and 50%, respectively.

[1] Mineral premix (mg/kg diet): bicalcium phosphate 500 g, calcium carbonate 215 g, marine salt 40 g, potassium chloride 90 g, magnesium chloride 124 g, magnesium carbonate 124 g, iron sulfate 20 g, zinc sulfate 4 g, copper sulfate 3 g, potassium iodide 4 mg, cobalt sulfate 20 mg, manganese sulfate 3 g, sodium fluoride 1 g (Granda Zootecnici, Cuneo, Italy).

[2] Vitamin-premix (mg/kg diet): DL-αtocopherolacetate, 60 IU; sodium menadione bisulfate, 5 mg; retinylacetate, 15,000 IU; DL-cholecalciferol, 3000 IU; thiamin, 15 mg; riboflavin, 30 mg; pyridoxine, 15 mg; Vitamin B12, 0.05 mg; nicotinic acid, 175 mg; folic acid, 500 mg; inositol, 1000 mg; biotin, 2.5 mg; calcium panthotenate, 50 mg; choline chloride, 2000 mg (Granda Zootecnici, Cuneo, Italy).

2.3. Water quality

Throughout the trial, water lost via evapotranspiration from each unit was recorded daily and manually replenished. Dissolved oxygen and water temperatures were recorded daily, whereas pH, redox potential, electric conductivity, and chlorophyll content in the fish tanks were monitored at weekly intervals using a portable multi-parameter apparatus (HQ40d Portable Multi-Parameter Meter; Hach Lange GmbH, Germany). Similarly, total ammonia nitrogen (TAN) was measured once a week using an Ammonia Rapid Kit (Megazyme; Astori Tecnica, Poncarale, Italy).

During the trial period, the daily loss of water due to evapotranspiration averaged 7.86 L d^{-1}, which represented 1.31% of the total water contained in each unit, without differences among groups (data not reported in tables). Similar values for the physicochemical properties of water were obtained among all experimental groups: average water temperature 19.4 ± 1.7 °C, dissolved oxygen 7.96 ± 0.61 mg L^{-1}, pH 7.4 ± 0.2, and TAN 0.13 ± 0.10 mg L^{-1}. Additionally, on average, water chlorophyll levels were 76.0 ± 22.5 μg L^{-1}, electrical conductivity 976 ± 171 dS cm^{-1} and redox potential −30.5 ± 18.2 mV (data not reported in tables).

2.4. In vivo recordings

The health and mortality of fish were monitored daily. Fish were individually weighed at the beginning of the trial (0 d), and thereafter once a month at 26, 59, and 76 days (end of the rearing period) of the trial, after being anaesthetized in a separate tank with 10 mg L^{-1} of clove oil containing 87% eugenol. Fish were fasted for 48 h before and 24 h after weighing.

Fish were fed by hand twice daily (08:00 and 15:00), 6 days a week,

Table 2
Fatty acid profile (% of total FAME) of the experimental diets.

	Diet		
	H0	H6	H12
C12:0	0.15	2.37	3.81
C14:0	5.90	6.16	6.64
C16:0	19.6	20.1	23.6
C18:0	3.96	3.44	4.22
C16:1n-7	5.88	5.54	5.67
C18:1n-9	15.8	15.9	18.6
C18:1n-7	2.16	1.99	2.09
C18:2n-6	24.2	24.2	27.1
C18:3n-3	3.54	3.37	2.45
C20:4n-6	0.37	0.33	0.23
C20:5n-3	5.91	5.18	3.16
C22:6n-3	3.99	3.76	2.44
SFA[1]	31.62	34.08	40.99
MUFA[1]	24.82	24.34	27.14
PUFA[1]	43.56	41.58	31.88
∑n-3	16.02	14.55	9.56
∑n-6	25.07	24.95	20.97
∑n-6/n-3	1.58	1.74	2.22
PUFA/SFA	1.38	1.22	0.78

H0, H6, and H12 diets, fishmeal replaced with *Hermetia illucens* meal (HI) at 0%, 25%, and 50%, respectively. FAME: fatty acid methyl esters; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

[1] Including minor FAs.

until visually assessed apparent satiation. The amount of feed was recorded daily and the specific growth rate (SGR) and feed conversion ratio (FCR) were calculated as follows:

$$\text{SGR}\left(\%\text{d}^{-1}\right) = [(\text{Log}_e \text{ final weight} - \text{Log}_e \text{ initial weight})/\text{No.of days}] \times 100$$

$$\text{FCR} = \text{weight of dry feed distributed/net wet weight gain of fish.}$$

2.5. In vivo digestibility

An in vivo digestibility trial was performed to assess the apparent digestibility coefficient (ADC) of nutrients. Two hundred and sixteen rainbow trout (100.6 ± 8.53 g) were allocated to 12 cylinder-conical tanks of 250 L (four tanks per treatment, 18 fish per tank) connected to a flow-through open system (tank water inflow: 8 L min^{-1}) supplied with artesian well water (constant temperature: 13 ± 1 °C; dissolved oxygen level: 8.5 mg L^{-1}). After 14 days of adaptation to the experimental diets, the fish were fed by hand to apparent satiety twice daily (08:00 and 15:00), 6 days per week. ADC values were obtained using the indirect acid-insoluble ash method. To this end, 1% Celite ® (Fluka, St. Gallen, Switzerland) was added to the diets as an inert marker to replace 1% wheat meal. Faeces were collected daily from each tank for four consecutive weeks, using a continuously operated automatic device (Choubert's system; Chemello et al., 2020), and then freeze-dried and frozen (−20 °C) until used for analyses. The ADC values of dry matter, crude protein, ether extract, and gross energy were calculated as described by Caimi et al. (2020).

2.6. Recordings at the time of fish slaughter

At the end of the trial, all fish were slaughtered after fasting for 24 h. Fish were caught as for previous weighing and then stunned by a percussion applied to the head of manually restrained fish using a plastic knob. Dead fish were weighed and total and standard lengths were measured, from which Fulton's condition factor (K) was calculated as follows:

$$\text{K} = \left(\text{fish weight}/\text{total length}^3\right) \times 100$$

The fish were then dissected, and the carcasses were weighed. The somatic indices and carcass and fillet yields were calculated as follows:

$$\text{Hepatosomatic index (HSI, \%)} = (\text{liver weight}/\text{fish weight}) \times 100$$

$$\text{Viscerosomatic index (VSI, \%)} = (\text{viscera weight}/\text{fish weight}) \times 100$$

$$\text{Carcass yield (\%)} = (\text{carcass weight}/\text{slaughter weight}) \times 100$$

$$\text{Fillet yield (\%)} = (\text{fillet weight}/\text{slaughter weight}) \times 100$$

The skin was separated from the fillets and the L* a* b* colour indices (CIE, 1976) were measured at three points on the dorsal side of the right fillets using a Minolta CM–508C spectrophotometer (Minolta Corp., Ramsey, NJ, USA). The colour difference (ΔE) between pairs of fillet samples was calculated according to Mokrzycki and Tatol (2011).

Both the right and left fillets were minced. The right fillets were freeze-dried, placed under vacuum in plastic bags, and stored at 4 °C until used for analysis of the proximate composition of meat. The left fillets were stored under vacuum at −18 °C until used for analysis of meat fatty acid profiles.

2.7. Proximate composition and fatty acid analysis

The experimental diets, freeze-dried fillets, and collected faeces were analysed according to AOAC (2000) methods to determine the contents of dry matter (934.01), ash (967.05), and crude protein (2001.11). Ether extract contents were measured after acid hydrolysis treatment (EC, 1998). The gross energy content of diets was assessed using an adiabatic calorimetric bomb (C7000; IKA, Staufen, Germany), and the inert marker content in diets and faeces was analysed using the acid-insoluble ash method, as described by Atkinson et al. (1984).

For the fatty acid composition of diets and fillets, the fat was extracted based on accelerated solvent extraction (Application Note 334; ASE®, Dionex, Sunnyvale, CA, USA) using two extraction cycles. The extracted lipids were initially transmethylated as fatty acid methyl esters (FAMEs). Prior to methylation, an internal standard (13:1 methyl ester) was added to the extract. After centrifugation, the supernatant was subjected to two-dimensional gas chromatography (GC × GC) using an Agilent 7890A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with a modulator (Agilent G3486A CFT, CA, USA), an automatic sampler (Agilent 7693, CA, USA), and an FID detector connected to chromatography data system software (Agilent ChemStation, CA, USA). A Supelco SP 2560 column (80 m × 0.18 mm internal diameter, 0.14-μm film thickness; Sigma-Aldrich, St. Louis, MO, USA) was used as the first capillary column with hydrogen gas as a carrier. As the second capillary column, we used a J&W HP 5 ms column (3.8 × 0.25 mm internal diameter, 0.25-μm film thickness; Agilent Technologies) with hydrogen again being used as the carrier. Fatty acids were identified by comparing with the retention times of the standard FAME mixture (Supelco 37 component FAME Mix, 47,885 – U). Individual FAMEs were expressed as a percentage of the total volume of the eluted FAMEs.

2.8. Gut histological analyses

At slaughter, we selected 36 fish (4 fish per tank, 12 fish per dietary treatment), based on treatment-wise average live weight, and dissected to sample gut tissue. From these, a 2-cm sample was taken from the proximal intestine (the tract between the pyloric sphincter and the ileum-rectal valve) (Verdile et al., 2020), and the annexed pyloric caeca were removed. Samples were washed in phosphate-buffered saline (PBS) solution and approximately 1 cm was fixed in paraformaldehyde in PBS (0.1 M, pH 7.4), dehydrated, and embedded in paraffin. Four 4-μm sections of per sample were obtained using a microtome and stained with haematoxylin/eosin for morphometric evaluation. Intestinal villus lengths were measured using NIH ImageJ software (Rueden et al., 2017), according to the procedure described by Hampson (1986), with 20 measurements being obtained from each gut sample. The goblet cells identified on 10 different villi per trout were counted along 300 μm of the villus surface.

2.9. Statistical analysis

The data obtained for growth performance, gut morphology, ADC, slaughter results, and fillet quality of fish were analysed via a one-way ANOVA, with the experimental diet (H0, H6, and H12) serving as the main effect. The PROC GLM procedure of SAS (Statistical Analysis System, 2013) was used for all analyses. Bonferroni's test was used for the comparison of means, with differences among means assumed to be statistically significant at $P \leq 0.05$. The data obtained for fillet fatty acid profiles were preliminarily assessed for normal distribution using the Shapiro-Wilk test and the PROC UNIVARIATE procedure of SAS.

3. Results

3.1. Plant productivity, fish production, and in vivo digestibility

With regards to plant productivity, strawberries yielded on average 38 ± 19 fruits m^{-2} with an average fruit weight of 7.4 ± 1.4 g, which did not differ significantly among dietary treatments (Fig. 2).

Among the experimental trout, only five fish died during the course of treatment (four fed the H0 diet and one fed the H6 diet) without any prior visible symptoms of disease, whereas four fish (one and three from

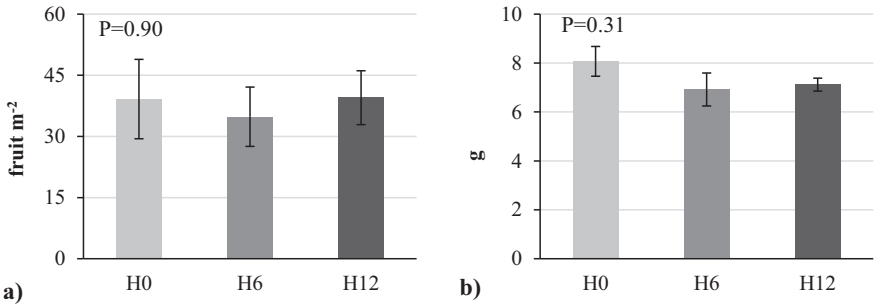

Fig. 2. Number of fruits (a) and fruit weight (b) (means ± SE) of strawberries cultivated in an aquaponic system with trout fed diets with percentage replacements of fishmeal with *Hermetia illucens* (HI) meal (0%, 25% and 50% replacement for H0, H6 and H12 diets, respectively).

the H0 and H6 diets, respectively) were discarded at weight controls during the trial, given the lack of difference between their initial and final live weights.

During the initial 26 days of the trial, SGR was lower in fish fed the H12 diet than in those receiving the H0 and H6 diets (−12.3% on average; $P < 0.001$) (Table 3). Similarly, at the end of the trial, the SGR of H12 fish was lower than that of either H0 or H6 fish (−7.9%; $P = 0.035$). In contrast, we recorded no significant differences among the experimental groups with respect to final live weight (304 g on average), FCR (1.53), final fish biomass (11.0 kg m^{-3}), or biomass growth (5.28 kg m^{-3}).

Similarly, no significant differences were detected among dietary treatments in terms of the ADC of dry matter (89.0%, on average) or crude protein (96.4%), ether extract (98.0%), or gross energy (93.2%) contents (Table 4).

Table 3
Growth performance of rainbow trout.

	Diet			*P*-value	RMSE
	H0	H6	H12		
Total fish per treatment (n)	53	53	58		
Tanks (n)	3	3	3		
Fish weight (g)					
0 days	158	160	154	0.681	40
26 days	218	221	202	0.150	53
59 days	291	293	270	0.195	75
76 days	310	313	285	0.142	82
Specific growth rate (% d^{-1})					
0–26 days	1.22[b]	1.22[b]	1.07[a]	<0.001	0.26
26–59 days	0.87	0.84	0.86	0.714	0.23
59–76 days	0.35	0.38	0.32	0.135	0.18
0–76 days	0.88[b]	0.87[b]	0.81[a]	0.035	0.16
Feed conversion ratio					
0–26 days	1.21	1.23	1.27	0.799	0.13
26–59 days	1.54	1.60	1.50	0.642	0.12
59–76 days	2.28	2.37	2.77	0.396	0.43
0–76 days	1.50	1.54	1.55	0.785	0.10
Biomass (kg m^{-3})					
0 days	5.60	5.67	5.95	0.644	0.48
26 days	7.69	7.80	7.82	0.951	0.54
59 days	10.3	10.4	10.4	0.973	0.80
76 days	11.0	11.1	11.0	0.980	0.82
Biomass growth (kg m^{-3})					
0–26 days	2.09	2.13	1.87	0.391	0.23
26–59 days	2.61	2.56	2.62	0.981	0.34
59–76 days	0.67	0.73	0.60	0.607	0.15
0–76 days	5.35	5.41	5.08	0.734	0.54

H0, H6, and H12 diets, fishmeal replaced with *Hermetia illucens* meal (HI) at 0%, 25%, and 50%, respectively. RMSE: root mean square error.

Table 4
Apparent digestibility coefficient (ADC) of nutrients in experimental diets.

	Diets			P-value	RMSE
	H0	H6	H12		
Dry matter (%)	88.6	88.7	89.6	0.31	0.84
Crude protein (%)	96.8	96.3	96.2	0.12	0.36
Ether extract (%)	97.8	97.7	98.4	0.51	0.40
Gross energy (%)	93.5	93.0	93.2	0.65	0.59

H0, H6, and H12 diets, fishmeal replaced with *Hermetia illucens* meal (HI) at 0%, 25%, and 50%, respectively. RMSE: root mean square error.

3.2. Gut histological analysis

Dietary treatment had no significant effect on proximal gut villus height, the average of which was 505 ± 73.5 μm (Fig. 3a). However, the density of goblet cells was significantly higher in fish fed the H12 diet than in those fed the H0 diet (7.45 vs. 8.36 cells × 300 μm^{-1}; $P < 0.05$), with intermediate values being obtained for fish fed the H6 diet (Fig. 3b).

3.3. Biometric traits and flesh quality of fish

Dietary treatment had no significant effect with respect to Fulton's condition factor (average 1.40), VSI (10.9%), HSI (1.22%), carcass yield (88.2%), fillet weight (156 g), or fillet yield (57.8%) (Table 5). In contrast, fillet redness (a*) and yellowness (b*) were significantly lower in fish fed the H12 diet than in those fed the H0 diet (−58% and − 19%, respectively; $P < 0.001$; Table 5).

We detected no significant differences among the experimental groups for fillet chemical composition: average moisture (73.0%), crude protein (21.2%), and crude lipids (4.17%) (Table 6). In terms of fatty acid profiles, fish fed the H12 diet were found to have higher proportions of C12:0 (1.33% vs. 0.42%; $P < 0.001$) and C14:0 (4.38% vs. 3.96%; $P < 0.01$) compared with those fed the H0 diet, with intermediate values being recorded for fish fed the H6 diet, whereas an opposite trend was observed for C18:1 n-7 (2.31% vs. 2.18%; $P < 0.001$). In contrast, dietary treatment was found to have no significant effect on the proportions of eicosapentaenoic acid (EPA, C20:5 n-3: 2.72% on average), docosahexaenoic acid (DHA, C22:6 n-3: 6.01%), and saturated (32.6%), monounsaturated (30.0%), n-3 (13.3%), or n-6 (23.1%) fatty acids (Table 6).

4. Discussion

From the perspective of AP, the rainbow trout is not considered among the most suitable of species for culturing, primarily due to its high water quality requirements (Molony, 2001). Nevertheless, some successful commercial farms are currently operational outside of Europe, and the results obtained in the present study confirm the

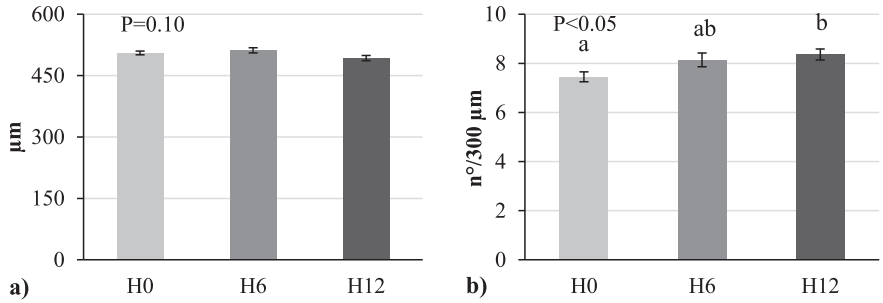

Fig. 3. Villus height (a) and goblet cell density (b) (means ± SE) in the proximal intestine of rainbow trout cultured in aquaponics and fed diets with different percentage replacements of fishmeal with *Hermetia illucens* (HI) meal (0%, 25% and 50% of replacement for H0, H6 and H12 diets, respectively).

Table 5
Morphometric and somatic indices, slaughter results, and fillet characteristics of trout.

	Diets			P-value	RMSE
	H0	H6	H12		
Fish (n)	53	53	58		
Total length (mm)	276	281	273	0.267	25
Standard length (mm)	252	255	249	0.463	23
K	1.43	1.39	1.37	0.101	0.13
VSI (%)	10.8	11.0	10.8	0.850	1.4
HSI (%)	1.22	1.24	1.19	0.703	0.32
Carcass weight (g)	267	277	254	0.260	73
Carcass yield (%)	87.6	88.2	88.9	0.607	6.6
Fillets (n)	24	24	24		
Weight (g)	158	162	148	0.553	44
Yield (%)	56.7	58.5	58.1	0.611	6.3
Colour					
L*	39.7	40.3	40.9	0.164	2.2
a*	-1.21^{b}	-1.54^{ab}	-1.91^{a}	<0.001	0.57
b*	8.69^{b}	7.50^{ab}	7.01^{a}	<0.001	1.26

H0, H6, and H12 diets, fishmeal replaced with *Hermetia illucens* meal (HI) at 0%, 25%, and 50%, respectively. K: Fulton's condition factor; VSI: viscerosomatic index; HIS: hepatosomatic index; RMSE: root mean square error.

Table 6
Fillet proximate composition, fatty acid profile, and dietary indices of trout.

	Diet			P-value	RMSE
	H0	H6	H12		
Fillets (n)	12	12	12		
Proximate composition					
Moisture (%)	72.8	73.0	73.1	0.84	1.2
Ash (%)	1.38	1.39	1.41	0.29	0.04
Protein (%)	21.2	21.3	21.2	0.72	0.4
Lipid (%)	4.24	4.14	4.12	0.97	1.35
Fatty acids (% of total FAME)					
C12:0	0.43^{a}	0.84^{b}	1.33^{c}	<0.001	0.26
C14:0	3.96^{a}	4.18^{ab}	4.38^{b}	<0.01	0.30
C16:0	22.3	22.2	22.6	0.77	1.4
C18:0	3.79	3.82	3.74	0.80	0.30
C16:1n-7	6.09	6.17	5.96	0.60	0.50
C18:1n-9	20.8	20.1	20.8	0.21	1.2
C18:1n-7	2.31^{b}	2.25^{ab}	2.18^{a}	<0.001	0.08
C18:2n-6	20.7	21.1	21.4	0.22	0.9
C18:3n-3	2.50	2.49	2.43	0.53	0.15
C20:4n-6	0.57	0.55	0.50	0.21	0.09
C20:5n-3	2.88	2.78	2.51	0.21	0.52
C22:6n-3	6.27	6.41	5.35	0.14	1.36
SFA[1]	31.9	32.5	33.5	0.10	1.8
MUFA[1]	30.4	29.5	30.0	0.35	1.4
PUFA[1]	37.7	38.0	36.5	0.35	2.5
∑n-3	13.8	13.8	12.2	0.14	2.2
∑n-6	22.9	23.2	23.3	0.45	0.9
∑n-6/n-3	1.73	1.72	1.93	0.10	0.26
PUFA/SFA	1.19	1.18	1.10	0.20	0.13

H0, H6, and H12 diets, fishmeal replaced with *Hermetia illucens* meal (HI) at 0%, 25%, and 50%, respectively. FAME: fatty acid methyl esters; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; RMSE: root mean square error. [1]Including minor FAs.

adaptability of trout to aquaponic conditions, even when a low level of technology (basic system components, no environmental control, no water sanitation) is adopted (Birolo et al., 2020). Indeed, we recorded a notably low fish mortality (2.9% on average) during the course of trial, which is consistent with the findings of previous studies that have examined the performance of European carp (*Cyprinus carpio*; Maucieri et al., 2019), rainbow trout (Birolo et al., 2020), and largemouth bass (*Micropterus salmoides*; Bordignon et al., 2020) using the same type of system. This is in spite of the fact that on some warm days during the trial, water temperatures reached peaks higher than the temperature considered optimal for rainbow trout (<20 °C; Ineno et al., 2005; Chen et al., 2015), although it remained lower than the limit of thermal tolerance for this species (24 °C; Bear et al., 2007). Otherwise, the dissolved oxygen concentration (6.08 to 10.2 mg L^{-1}) varied within a suitable range for rainbow trout growth and welfare (Ellis et al., 2002), plant nutrient absorption, and biofilter activity (Lennard and Goddek, 2019). Similarly, water pH, which ranged from 6.4 to 8.5 (average 7.38), was consistent with the optimal ranges of fish (6.5 to 8.5; Molony, 2001) and bacteria (6.5 to 8.0; Lennard and Goddek, 2019; Timmons et al., 2002). In addition, recorded values for the TAN content of water were found to be below the chronic toxicity threshold for rainbow trout (approx. 4 mg NH_3-N L^{-1}; Thurston et al., 1984).

With regards to production, the results obtained in AP can be similar to those achieved in stand-alone RAS (see Lennard and Goddek, 2019 for a review). However, we found that trout performance in the present trial was lower than that typical of open and RAS systems (Naderi et al., 2017; Zahedi et al., 2019). Nevertheless, SGR was found to be higher (+18%), whereas FCR was lower (−3%) than the values previously obtained for rainbow trout (from 141 g to 331 g) using the same aquaponic system (Birolo et al., 2020).

In terms of the composition of fish diets with respect to the replacement of FM with HI meals, the findings of a previous study on rainbow trout (179 to 541 g live weight over 78 days) revealed no significant differences in performance in response to an inclusion of 20%–40% partially defatted HI meal (Renna et al., 2017). Similar results have been reported for rainbow trout (137 g to 277 g live weight over 98 days) fed a full-fat HI meal (10.5% and 21%; Cardinaletti et al., 2019). Conversely, however, other authors have reported reductions in the FCR of rainbow trout juveniles in response to the provision of diets containing increasing proportions of defatted HI meal (from 6.5% to 26%; Dumas et al., 2018), and with the inclusion of HI prepupae at 30% (St-Hilaire et al., 2007) and 33% (Sealey et al., 2011). This is partially consistent with the impairment of growth performance observed during the first period of the present trial with respect to the highest HI rate. Comparatively, reduced fish growth performance during entire trials has

previously been reported in Nile tilapia farmed in AP (Kessens, 2016) and Siberian sturgeon (*A. baerii*) (Zarantoniello et al., 2021) fed diets containing 68% and 50% HI meal, respectively.

The reduction in SGR observed in the present study at the highest HI inclusion was, however, not found to be associated with any obvious differences in diet digestibility.

Although a number of previous studies on rainbow trout fed diets containing different inclusion levels of HI (Renna et al., 2017) and *Tenebrio molitor* meal (Belforti et al., 2015; Chemello et al., 2020; Rema et al., 2019) have not reported the effects on diet digestibility, other authors have noted that the apparent digestibility of major nutrients might be affected by the inclusion of insect meals in aquafeeds (for a review see Gasco et al., 2019). Furthermore, reductions in the digestibility of crude protein have been observed in trout fed diets containing high levels of HI (20%; Renna et al., 2017) and *T. molitor* meal (20%; Chemello et al., 2020; 50%, Belforti et al., 2015). To a large extent, such disparities among studies are assumed to be attributable to substantial differences in insect meal quality associated with the origin of raw materials and their processing, as well as differences in other compound feed ingredients and FM replacement rates (Gasco et al., 2019).

Defence-related responses of fish gut to insect-based diets have also been described in other fish, including the black tetra (*Gymnocorymbus ternetzi*; Leknes, 2014), rice field eel (*Monopterus albus*; Dai et al., 2007), and tiger barb (*Puntius tetrazona*; Leknes, 2014), which are assumed to be associated with an increase in the density of goblet cells, as has been observed in the gut of trout fed the highest percentage HI inclusion in the trials conducted by Elia et al. (2018) and Cardinaletti et al. (2019), as well as in the present trial. Additionally, a shortening of villus height in the gut of fish fed diets containing insects has been reported in the literature, indicating potential intestinal inflammation and a diminished absorptive surface (Li et al., 2017; Zhang et al., 2018; Cardinaletti et al., 2019). In contrast, consistent with the findings of the present study, other authors have observed no appreciable alterations in the proximal (Elia et al., 2018) or distal (Dumas et al., 2018) intestine of rainbow trout fed diets containing different inclusion levels of partially defatted HI meal for 78 and 84 days, respectively.

As to whether the replacement of FM with insect meals can affect product quality requires the evaluation of both the rheological and nutritional properties of fillets. With respect to rheological properties, consistent with the findings of the present study, certain changes in fillet colour have previously been detected in rainbow trout fed a diet containing 10.5% full-fat HI meal (Bruni et al., 2020) or 20% of defatted HI meal (Mancini et al., 2018), although other studies have reported no differences in trout fed full-fat mealworms (*T. molitor*; Iaconisi et al., 2019) or defatted HI meal (Renna et al., 2017). In the present study, we observed differences in colour indices of the fillets of trout fed the H0 and H12 diets ($\Delta E = 3.74$), which were within a range of colour difference ($3.5 < \Delta E < 5$) that can be appreciated even by inexperienced observers (Mokrzycki and Tatol, 2011). Such changes are conceivably attributable to the variations in feed pigments associated with the substitution of FM with HI meal, as has previously been observed in studies where FM is replaced by vegetable sources (Iaconisi et al., 2018; Tibaldi et al., 2015). However, the effects of HI meal pigments on fish fillets are poorly understood and may be dependent on multiple factors, including species, rearing substrates, and the processing of insects (Gasco et al., 2019; Larouche et al., 2019; Leni et al., 2019).

With regards to the influence of dietary HI inclusion on the nutritional quality of fillets, the findings of previous studies have been somewhat inconsistent. Some studies have reported changes in the lipid and dry matter contents of fish flesh (Renna et al., 2017; Sealey et al., 2011), whereas others have not (Dumas et al., 2018; Mancini et al., 2018). In the present study, we detected increases in C12:0 and C14:0 contents in the fillets of trout fed insect meal, as has previously been reported (Bruni et al., 2020; Mancini et al., 2018; Renna et al., 2017). Noteworthy, despite the lower dietary supply in insect-based diets, the EPA and DHA contents in fillets were apparently unaffected, which can probably be attributed to the fact that experimental diets contained the same amount of fish oil and that the partially defatted HI meal have low amounts of ether extract (approx. 7.5% DM).

Turning to other components of the evaluated system, namely, the plants cultivated in AP, the major nitrogen source for plants is the proteins in fish feed (Lennard and Goddek, 2019). Thus, it is reasonable to assume that the use of different protein sources (i.e., FM or HI meal) might have an influence on plant growth in instances where there is a change in ADC. However, we found no evidence to indicate that this was the case in the present study. Indeed, the yields obtained for strawberries were found to be comparable with those obtained in AP with different carp species (Roosta and Afsharipour, 2012), as well as in a closed hydroponics system (Talukder et al., 2019) and plastic bag cultivation (Saidimoradi et al., 2019).

Finally, based on the costs at the feed mill of the raw materials used for producing the diets, we calculated that diet H6 and H12 had a cost equal to 1.13 and 1.26 times the cost of diet H0. Thus, since fish biomass and strawberry production were not affected by the dietary treatment, we can state that the production cost of trout increased with the inclusion level of HI meal.

5. Conclusions

In the present study, we demonstrated the feasibility of rainbow trout production based on a low-tech aquaponic system with limited environmental control. With respect to the use of HI meals, we established that diets with the highest level of insect meal inclusion had a slight effect on fish growth performance and also promoted an increase in the density of gut goblet cells, thereby tending to indicate certain detrimental effects at the gut level, which clearly warrant further investigation. Moreover, the effects on fillet traits require careful evaluation in view of consumers' perception of fish quality. Overall, our findings indicate that rearing high-value fish species in aquaponic systems, combined with the substitution of dietary FM with insect meal, could contribute to enhancing the competitiveness and attractiveness of aquaponic products, provided that costs for insect meals will decrease.

Authors' contributions

GX, LG, and AT conceived, designed and supervised the experiment. GX acquired the financial support for the project. FB, MB, CM, CN, CC, and LG performed the in-vivo trial, registered the experimental data, collected and prepared samples for chemical and histological analyses. LG and CC performed the digestibility trial. CB and MB performed histological analyses of gut. FB and AT performed the statistical analyses, analysed and interpreted the data, and wrote the first draft of the manuscript. All authors critically reviewed the manuscript for intellectual content and gave final approval of the version to be published.

Declaration of Competing Interest

None.

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