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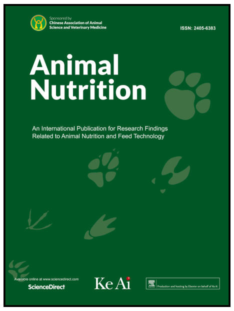

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Defatted black soldier fly (*Hermetia illucens*) in pikeperch (*Sander lucioperca*) diets: Effects on growth performance, nutrient digestibility, fillet quality, economic and environmental sustainability

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Abstract

The use of insect meal in aquafeed formulations has recently gained attention. Detailed knowledge about the inclusion levels for pikeperch (*Sander lucioperca*), a promising candidate for intensive aquaculture in Europe remains, however, fragmented. In the present study, 4 isoproteic (45% dry matter) and isoenergetic (21 MJ/kg) diets were formulated, including a control diet (H0) containing 30% fishmeal (FM) on an as fed basis and the other 3 diets in which FM protein was replaced by defatted black soldier fly meal (*Hemetia illucens*) (HIM) at 25%, 50%, and 100% (diet abbreviation H9, H18 and H36, corresponding to an inclusion level of 9%, 18% and 36%, respectively). The feeding trial was performed in triplicate groups of 50 juvenile pikeperch (mean weight, 68.7 g) fed with experimental diets for 84 d during which the growth performance, nutrient digestibility, fillet quality and economic and environmental sustainability of rearing pikeperch were evaluated. Our findings indicated that pikeperch fed H0, H9, and H18 groups displayed better results regarding growth performance indices, except for survival rate where no significant difference among groups was recorded ($P = 0.642$). A significantly lower organ-somatic index, including hepatosomatic, viscerosomatic and perivisceral fat index, was found in fish fed H18 groups than other groups ($P < 0.05$). Inclusion of HIM affected the digestibility of the nutrients and resulted in an almost linear reduction in the apparent digestibility coefficient of dry matter and protein. Concerning the fillet quality, dietary HIM negatively affected the protein and ash contents of the fish fillets, while the crude fat remained unchanged. Dietary HIM did not significantly modify total saturated, monounsaturated and polyunsaturated fatty acids in the fillets of fed pikeperch ($P > 0.05$) but did reduce total n-3 fatty acids ($P = 0.001$) and increased total n-6 ($P < 0.001$). Increasing inclusion levels of HIM reduced the environmental impacts associated with fish-in-to-fish out ratio but entailed heavy burdens on energy use and eutrophication. Low and moderate inclusion levels of HIM did not negatively affect land use and water use compared to an HIM-free diet ($P > 0.05$). The addition of HIM at a level as low as 9% elicited a similar carbon footprint to that of the control diet. The economic conversion ratio and economic profit index were negatively affected at increased insect meal inclusion levels. This study has shown that the incorporation of HIM in feed formulations for pikeperch is feasible at inclusion levels of 18% without adverse effects on growth performance parameters. The feasibility also highlighted the environmental benefits associated with land use and marine resources required to produce farmed fish.

Keywords: Alternative feed; Digestibility; Fish-in-to-fish-out ratio; Insect meal; Percids; Sustainability

1. Introduction

European aquaculture has recently been expanding to include new species such as pikeperch (*Sander lucioperca*) (Policar et al., 2019). In the wild, this carnivorous species feeds mainly on crustaceans and insects, and on fish at a later stage. It is an important food-fish for European inland aquaculture, and considerable efforts have been made to increase stock in fish farms (Steenfeldt et al., 2015; Policar et al., 2016).

In order to fully replace the natural diet with a formulated feed, pikeperch diets have to contain high levels of protein (43% to 50%) as recommended by Nyina-wamwiza et al. (2005). This requirement can be covered by marine fishmeal (FM), which is considered an optimal and nutritionally well-balanced ingredient for carnivorous fish (Oliva-Teles et al., 2015; Gasco et al., 2018). Nevertheless, FM sources are not endless; their market price is increasing and FM is therefore becoming unfavourable for commercial fish farming (FAO, 2020a).

It is well known that significant progress has been made over the past decade in reducing FM levels in commercial feeds for farmed fish (Gasco et al., 2019; Nogales-Mérida et al., 2019). Nowadays, various plant or animal-based alternatives are used for industrial aquafeedsto help decrease the dependency on FM and fish oil, with appropriate economic incentives to reduce the feed cost (Gasco et al., 2018). To be used in aquaculture, an alternative protein source needs to have certain nutritional characteristics, such as relatively high protein content, high nutrient digestibility, a balanced amino acid profile and low levels of fibre and anti-nutrients (Gasco et al., 2018). Plant proteins (i.e. soybean meal or plant protein concentrates) are frequently used (Fry at al., 2016), but are often associated with certain complications, mainly due to imbalances in the essential amino acid (EAA) profile, the presence of anti-nutritional factors or palatability problems (Mastoraki et al., 2020), consequently adversely affecting growth performance and/or fish health (Gai et al., 2012; Oliva-Teles et al., 2015). Processed animal proteins (PAPs), such as poultry by-products, blood or meat and bone meal, have also been included in aquafeeds, with promising results (Hua et al., 2019; Galkanda-Arachchige et al., 2020), even though their use is limited by legislation in Europe (Gasco et al., 2018) and by EAA deficiency, high ash content and variability in digestibility (Galkanda-Arachchige et al., 2020).

A great deal of attention has recently been paid to insects (Barragan-Fonseca et al., 2017; Gasco et al., 2019), which have already been proposed as an efficient and high-quality alternative protein source for poultry (Neumann et al., 2018; Secci et al., 2018; Gariglio et al., 2019; Pieterse et al., 2019; Yoo et al., 2019) and swine (Biasato et al., 2019; Chia et al., 2019). Insects are also a suitable source of protein and lipids for carnivorous fish (Lock et al., 2018) as a naturally available food in their environment. Insect meal has been shown to be a promising alternative to FM in aquaculture (Lock et al., 2018; Gasco et al., 2019; Nogales-Mérida et al., 2019) with optimal dietary sources of several vitamins and minerals (e.g. iron, potassium, calcium, magnesium etc.) (Gasco et al., 2018; Hawkey et al., 2021). Several insect species can be included successfully in carnivorous fish diets [e.g. for rainbow trout (*Oncorhynchus mykiss*) (Chemello et al., 2020), European sea bass (*Dicentrarchus labrax*) (Gasco et al., 2016), Atlantic salmon (*Salmo salar*) (Belghit et al., 2019) and gilthead seabream (*Sparus aurata*) (Piccolo et al., 2017)] or in omnivorous fish diets [e.g. for common carp (*Cyprinus carpio*) (Li et al., 2017) and Nile tilapia (*Oreochromis niloticus*) (Devic et al., 2018)], with the best results having been obtained from a partial replacement of FM. The most common insect species included as processed larva meal are mealworm (*Tenebrio molitor*), black soldier fly (*Hermetia illucens*, HI) and house fly (*Musca domestica*) (Lin and Mui, 2017; Magalhães et al., 2017; Ido et al., 2019; Chemello et al., 2020). In particular, HI larva meal seems to be one of the most promising insect-based PAP alternatives to FM. HI larva meal is rich in protein, with levels up to 60%. Even if lower in some EAAs compared to FM, HI larva meal has a well-balanced amino acid profile (Hawkey et al., 2021) and provides a good amount of minerals and vitamins (Li et al., 2016; Barragan-Fonseca et al., 2017; Magalhães et al., 2017; Renna et al., 2017; Devic et al., 2018, Nogales-Mérida et al., 2019). Moreover, black soldier fly larvae grown on low value organic can be an environmentally sustainable protein source (Danieli et al., 2019; Smetana et al., 2019; Gasco et al., 2020). Recent research has been conducted on the use of *H. illucens* meal in pikeperch (*Sander lucioperca*) showing that insect containing diets positively modulated the richness and diversity of fish intestinal microbiota without adverse effects in terms of intestinal histomorphology (Tran et al., 2021). To complement \ the cited study, the effects of different dietary inclusion levels of a partially defatted HI larva meal (HIM) in substitution of FM on the growth performance, digestibility, somatic indices, body and fillet proximate composition, economic indices and environmental sustainability of pikeperch juveniles has been evaluated and reported in this paper.

2. Materials and methods

The feeding trial was conducted at the South Bohemia University, Faculty of Fisheries and Protection of Waters, in České Budějovice (The Czech Republic). The animal care and experimental protocols were designed and carried out and in accordance with the Czech and European Community Directive (2010/63/EU) on the protection of animals used for scientific purposes (ethic approval protocol number MSMT-6744/2018-2). The HIM provided by Hermetia Deutschland GmbH & Co. KG (Baruth / Mark, Germany) was obtained from larvae raised on plant by-products and partially defatted with a mechanical process performed using high pressure and without solvents. HIM composition is reported in Table 1.

2.1 Diet formulations

Four experimental diets, with increasing levels of HIM, were formulated: a control diet (H0) containing 30% FM, in which plant-based ingredients cover part of the protein requirements to mimic the current trend of using such materials in aquafeeds, and 3 diets in which HIM was used to substitute 25% (H9), 50% (H18) and 100% (H36) of the FM, thus leading to HIM inclusion levels of 9%, 18% and 36%, on an as fed basis, respectively. The diets were isonitrogenous (crude protein [CP]: 44.9% on an as fed basis), isolipidic (ether extract [EE]: 18.4% on an as fed basis) and isoenergetic (gross energy [GE]: about 20.71 MJ/kg as fed) to meet the nutritional requirements of juvenile pikeperch (Schulz et al. 2007, 2008). The extruded experimental feeds were prepared at the EXOT HOBBY s.r.o. facility (Cerna v Posumavi, Czech Republic). All dried ingredients, which were finely ground to 300 to 400 µm, were mixed in a feed mixer HLJ–700/C (Saibainuo, China), then 4% oil and water were sequentially blended in the feed mixer and the obtained mixture was then extruded, using a commercial dual-screw extruded SLG II 70 (Saibainuo, China), to form 3 mm pellets. The remainder of the lipid was added during vacuum coating. The pellets were dried to approximately 90% dry matter using a 7-layer air dryer KX-7-8D (Saibainuo, China). The pellets were vacuum packed and stored at −20°C until fed. The temperature and pressure during the feed production process ranged from 96 to 106°C and from 19 to 22 atm, respectively. A maximal temperature of 138 °C was used during the drying process, which lasted 25 to 30 min. Crystalline EAAs lysine and methionine were supplemented in the diets to ensure that the requirements of the pikeperch were met (Geay and Kestemont, 2015). The ingredients and the proximate composition of the experimental diets are reported in Table 1.

2.3 Facilities, fish and the feeding trial

The feeding trial lasted 12 wk and was conducted in a recirculation system (total volume 11,400 L), consisting of fifteen 250 L round conical plastic tanks (black walls, white bottom), a mechanical drum filter (AEM 15, AEM-Products V.O.F., Lienden, The Netherlands), sedimentation tanks (total volume 2,600 L, series of filtration sections Bioakvacit PP10) and a moving bed biofilter (volume 4,700 L, media BT10 Ratz Aqua & Polymer Technik, Remscheid, Germany). The water temperature was maintained at 23.1 ± 1.0 °C by conditioning the ambient air and using Eheim Jäger Thermocontrol 300 submerged heaters (Eheim GmbH & Co KG, Stuttgart, Germany); the photoperiod was set at 12 h light-12 h dark by controlling the light through the use of timers. Light intensity was set at 20 to 35 Lx on the water surface. The flow rate in each tank was approximately 200 L/h. Dissolved oxygen (8.6 ± 1.3 mg/L) and pH (6.98 ± 0.28) were monitored twice daily, at 08:00 and 16:00, using a HACH HQ 40 multi-meter (HACH Lange, Germany). Pure oxygen was distributed, using ceramic diffusors, in the header tank, whenever necessary. The ammonia, nitrate and nitrite concentrations were analysed by means of HACH, LCK 304, LCK 339 and LCK 341 kits, using a HACH DR2800 Spectrophotometer at 2-day intervals. The nitrite-N, nitrate-N, and ammonia-N concentrations were 0.42 ± 0.24, 78.88 ± 37.31 and 1.89 ± 0.58 mg/L, respectively.

The juvenile pikeperch used in the trial were obtained, according to the procedure described in Policar et al. (2013), from the own faculty source. Part of this stock was implanted with a PIT-tag (7 mm × 1.35 mm, Loligo Systems ApS) when juveniles reached a mean body weight of 52.51 ± 5.23 g (10 d before start of feeding trial). In order to perform the trial, a total of 750 juveniles (of which 450 were tagged) were individually weighed using a digital balance (Scout, Ohaus Corporation, The USA, d = 0.1 g) (initial body weight [IBW] of 68.7 ± 6.6 g) and randomly allotted to 15 tanks with a total of 50 fish per tank. The mean stocking density at the start of the trial was 13.17 ± 0.24 kg/m^3.

Moreover, the tagged fish were also measured after anaesthesia in an MS 222 bath (50 mg/L), (initial body length [IBL] ± 1 mm) to follow both the body weight and length over time. All the fish were acclimated to the rearing system for 10 d before the start of the trial and fed by a grower commercial feed EFICO Sigma 970 (crude protein: 54%, crude lipid: 18%, pellet size: 3 mm) (BioMar A/S, Brande, Denmark).

The pikeperch in each tank were fed 7 d, using a combination of automatic feeders (EHEIM Twins, 5 meals per day at 07:00, 09:00, 11:00, 13:00 and one hand feeding at the end of the day at 15:00). Feed distribution was stopped as soon as the fish stopped eating. After each meal, any uneaten pellets were siphoned off using a central bottom drain and counted to calculate the real total feed supply.

2.4 Growth parameters

On the first day and on day 21, 42, 63 and 84 of the experiment, a subsample of 30 tagged fish per tank was weighed (0.01 g) and measured (body length [BL] ± 1 mm). The fish were anesthetized during the measurements with a solution of MS 222 in the bath (50 mg/L). At the end of the trial, the fish were starved for 2 d, anesthetised, and individually weighed to record the final body weight (FBW). Moreover, the biomass of each tank was then determined through a bulk weighing of all the fish.

The obtained data were used to calculate the following variables:

- Survival(SR, %) = 100 – (Number of dead fish/Initial number of fish) × 100
- Weight gain(WG, %) = [(FBW (g) – IBW (g)/IBW (g)] × 100
- Specific growth rate (SGR, %/day) = [(lnFBW – lnIBW)/Number of feeding days] × 100

Feed intake (g/kg ABW per day) = Total feed consumed (g, DM)/Average body weight (kg)/Number of feeding days (Guerreiro et al., 2020)

Feeding rate (FR, %/day) = [Total feed supplied (g, DM) × 100/Number of feeding days]/[$e^{(\ln FBW + \ln IBW) \times 0.5}$] (Lock et al., 2018)

Protein efficiency ratio (PER) = WG (g)/Total protein fed (g, DM)

Where ABW is average body weight and calculated as (Initial body weight + Final body weight)/2; SD is the standard deviation of the fish subsample.

At the end of the experiment, 7 individuals were taken from each replicate (tank) to be measured and their viscera, liver and perivisceral fat were weighed (± 0.01 g) to determine the viscerosomatic (VSI), hepatosomatic (HSI) and perivisceral fat indices (PFI). All the fish were filleted, by a person experienced in filleting, to calculate the fillet yield (FY). The collected data were used to calculate the following parameters:

Fulton's condition factor（K) = (FBW/FBL^3) × 100

Hepatosomatic index (HSI, %) = 100 × Liver weight (g)/Fish weight (g)

Viscerosomatic index (VSI, %) = 100 × Viscera weight (g)/Fish weight (g)

Perivisceral fat index (PFI, %) = 100 × Perivisceral fat weight (g)/Fish weight (g)

Fillet yield (FY, %) = 100 × Fillet weight (g)/BW.

Where FBL is final total body length (mm). The right and left fillets of 5 fish per tank (15 fish/treatment) were stored at -20 °C for subsequent proximate composition analyses. Moreover, 3 fish per tank (9 fish/treatment) were sampled and stored at -20 °C for a whole-body composition (WBC) assessment.

2.5 Digestibility trial

Seventy-five day after the start of the trial, faeces were collected daily for 7 d using settling columns placed at the bottom of the tanks. After each meal, any uneaten feed was collected, as reported in section 2.3. One hour after each feeding, the faeces accumulated in each settling column were collected, centrifuged (3,000 × *g*), pooled for each tank and stored at -20 °C until they were freeze dried for analyses. The apparent digestibility coefficients of the dry matter (ADC_{DM}), crude protein (ADC_{CP}) and ether extract (ADC_{EE}) of the 4 experimental diets were measured using the indirect acid-insoluble ash (AIA) method, with 1% celite (Fluka, Switzerland) added to the diets as an inert marker, and then calculated according to Renna et al. (2017). Celite is a common and reliable indigestible marker used to assess nutrient digestibility in fish (Da et al., 2013; Chemello et al., 2020; Caimi et al., 2021). This marker was found to not leak from faeces throughout a 24 h cycle and therefore feasible to recover in adequate quantities in the faeces (Sales et al., 2001).

2.6 Proximate composition of the HIM, diets, fish and fillets

The HIM and feed samples were analysed as reported in Renna et al. (2017). The diets were ground finely using a cutting mill (MLI 204; Bühler AG, Uzwil, Switzerland) and the analyses were performed according to AOAC International 2000. Samples were dried in the oven at 105 ^{o}C to reach constant weight for dry matter (AOAC no.934.01), then crude protein was estimated using the Kjeldahl method (AOAC no.984.13), ash content measured (AOAC no.942.05) by incinerating the samples in a muffle furnace at 550 °C, and crude fat determinated by the Soxhlet extraction method following the procedure AOAC no. 2003.05 (AOAC, 2003). The gross energy content was determined using an adiabatic calorimetric bomb (C7000; IKA, Staufen, Germany). Chitin was estimated according to Finke (2007). All the feed analyses were performed in duplicate. Fatty acid profile was determined as described in detail by Sampels et al. (2014) by methylating lipid with boron triflouoride-methanol complex (BF3),dissolving in 0.5 mL of hexane and storing under normal atmosphere at –80 °C until gas chromatography analysis. Fatty acid methyl esters were determined using a gas chromatograph. Analysis of the amino acid composition of the experimental diets was performed in triplicate, using an automatic amino acid analyzer AAA 400 (INGOS Prague) based on dye-forming reaction of amino acids using ninhydrin as an oxidizing agent (Stejskal et al. 2019).

The whole-fish (*n* = 9) and fillets (*n* = 15) that had been stored for analysis were individually ground using a Braun FP3131WH grinder and then freeze-dried. Proximate composition and gross energy tests were performed using the same methods as those used for the experimental feeds.

The lipid quality indices were calculated according to Chen and Liu (2020) as follows:

Atherogenicity index (AI) = [C12:0 + (4 × C14:0) + C16:0]/ΣUFA

Thrombogenicity index (TI) = (C14:0 + C16:0 + C18:0)/[(0.5×ΣMUFA) + (0.5 × Σn6 PUFA) + (3 × Σn3 PUFA) + (n3/n6)]

Unsaturation index (UI) = 1 × (% monoenoics) + 2 × (% dienoics) + 3 × (% trienoics) + 4 × (% tetraenoics) + 5 × (% pentaenoics) + 6 × (% hexaenoics)

2.7 Economic analyses and environmental sustainability of the experimental diets

An economic conversion ratio (ECR) and an economic profit index (EPI) were calculated for each tested group to determine the relative efficacy of the tested diets and their subsequent benefits, using the following formulas [Moutinho et al., (2017)]:

ECR (€/kg of fish) = Feed conversion ratio × D_P

EPI (€/fish) = (WG × S_P) – (WG × D_P)

Where D_P is the price of the diet (€/kg of diet) and S_P is the selling price (€7.58/kg)

The per kilogram cost (in €), excluding labour and taxes, of all the used components bought from commercial retailers was as follows: FM = €1.48; HIM = €3.50; wheat meal = €0.60; fish oil = €1.33; mineral mixture = €0.51; vitamin mixture = €3.90; soy concentrate = €1.50; corn gluten meal = €0.37; soybean meal = €0.33; merigel = €0.75; fish oil =€1.33; soybean oil = €0.58; vitamin premix = €3.90; mineral premix = €0.51; L-methionine = €6.00; L-lysine = €1.50. The followed prices of the diets were calculated: H0 = €0.97; H9 = €1.17; H18 = €1.36 and H36 = €1.75. The sales price of pikeperch was calculated as €7.58/kg based on published price report (FAO 2020b) and personal communication with 2 European fish farms who produce pikeperch in RAS systems. The fish-in-to-fish-out ratio (FIFO) was used as a practical measure of the quantity of live fish from capture fisheries required for each kilogram of farmed pikeperch. This indicator was calculated as follows (Tacon and Metian, 2008):

$FIFO = (L_{FM} + L_{FO})/(Y_{FMw} + Y_{FOw}) \times$ Feed conversion ratio

Where L_{FM} is the level of FM in the diet; L_{FO} is the level of fish oil in the diet; Y_{FMw} is the FM yield from wild fish; Y_{FOw} is the fish oil yield from wild fish.

The simulated environmental impacts associated with 1 kg farmed pikeperch production were calculated according to Tran et al. (2022a) as a multiplication between environmental impacts of the diet and respective Feed Conversion Ratio. Six environmental impact categories of experimental diets, including global warming potential (GWP, kg CO_2 equivalent [eq.]), energy use (EU, kg oil eq.), acidification (kg SO_2 eq.), eutrophication (kg P eq.), land use (m^2 arable land [a.]) and water use (WU, m^3), were calculated based on the life cycle assessment database for animal feed ingredients (GFLI, 2022). These categories for black soldier fly (*H. illucens*) were retrieved from Smetana et al. (2019). Environmental impacts were calculated as follows:

Environmental impact (GWP, EU, WU) per kilogram of feed = Environmental impact (GWP, EU, WU)/kg ingredient (GFLI, 2022 database) × Inclusion levels of ingredients in pikeperch diet

Environmental impact (GWP, EU, WU) per kilogram of fish produced = Environmental impact (GWP, EU, WU) per kilogram ofkg feed × Feed conversion ratio

2.8 Statistical analysis

All data were tested for homogeneity of variance using the Cochran, Hartley and Bartlett tests. The effects of the diet on the growth performance, somatic indices, whole body proximate composition, FIFO, ECR and EPI were analysed separately, by means of one-way ANOVA, followed by the Tukey test.

The effects of the diet on composition of the pikeperch fillets were tested, by means of Kruskal–Wallis non-parametric analysis, using the median test and multiple pair wise comparisons by ranks. Differences were considered significant at $P < 0.05$. The data were expressed as the mean ± SD, and statistical analyses were performed using STATISTICA 12.0.

3. Results

The fish readily accepted the feeds and the survival rate was high, with no significant differences between treatments. At the end of the experiment, the FBW, WG and SGR, were found to be lower in the H36 group, while these parameters were not significantly different in the remaining groups. Clear differences in fish growth appeared between H36 and the other dietary treatments after 42 d of the trial. Consequently, the H36 group displayed significantly higher FR and feed intake than H0, H9 and H18 (Table 4).

Significant differences ($P < 0.05$) were highlighted for K, somatic and perivisceral indices and fillet yields (Table 5). The K of fish fed H36 was lower than H0 and H18, but similar to H9 groups. Similar trends were observed for the HSI and VSI of the fish fed the dietary treatments. HSI and VSI were lower in H18 than in H0 and H36, while H9 presented intermediate values. As far as PFI was concerned, H18 showed the lowest result ($P < 0.05$) of all the treatments. The only significant difference ($P < 0.05$) in FY was found in H18 and H36, with H36 having the lowest yield.

The ADC values of the nutrients are presented in Table 6. Differences ($P < 0.05$) were recorded for all the parameters, with the lowest values of DM and CP digestibility being recorded for the H36 diet. A decreasing trend of nutrient digestibility was generally observed for increasing inclusion levels of HIM, except for ether extract digestibility, where only the H36 diet differed from the other diets.

The inclusion of HIM significantly affected the whole-body DM, CP, EE and energy content ($P < 0.05$). The whole-body composition for DM, CP and energy content were markedly reduced in H36, compared to H9 ($P = 0.043$, 0.026, and 0.007, respectively). The whole-body

EE content was significantly lower in the H36 and H18 groups (P = 0.006) than in H9 while the ash content showed no significant differences (Table 7).

The chemical composition and fatty acid profiles of the fillets of the fish fed the experimental diets is reported in Table 8. Although EE remained unaffected by the treatments, the inclusion of HIM significantly altered the DM, CP and ash content ($P < 0.05$). In details, DM was lower in H36 than in H9 ($P < 0.05$). The CP of the fillets was improved in H9, compared to H0 (+2.5%) and H36 (+5.2%) ($P < 0.05$). The total replacement of FM by HIM decreased the ash content, while H0, H9 and H18 did not show any correlation with this parameter.

The total amount of saturated fatty acids (SFA) in the pikeperch fillets was not influenced by the diet. The lauric acid (C12:0) and myristic acid (C14:0) values of the fillets gradually increased as the insect meal inclusion increased.

Palmitic acid (C16:0) was the predominant SFA, with a significantly higher content in the H9 group than in the H36 group (Table 8). Stearic acid (C18:0) was also present at high levels, but dietary insect meal inclusion showed no effect. Other SFAs made up less than 3% of the total fatty acids. The total monounsaturated fatty acid (MUFA) level was not influenced by the feeds with different insect meal inclusion levels. Oleic acid (C18:1n9) was the predominant MUFA in all the experimental groups, but the insect meal inclusion level showed no effect. Moreover, no difference was found for the total polyunsaturated fatty acids (PUFA) between the experimental groups. Docosahexaenoic acid (DHA, C22:6n3) was the predominant PUFA, with similar levels in the H0, H9 and H18 groups. The H36 group showed a significantly lower relative content than H0 and H9 (P = 0.001). The second most abundant PUFA was linoleic acid (C18:2n6), which showed a higher level in H36 than in the other diets. A significant difference also emerged between groups for the n3:n6 ratio ($P < 0.001$) as well as for UI (P = 0.003), AI (P = 0.002) and TI (P = 0.003). On the contrary, the C18:2n6, C18:3n3, MUFA, PUFA+MUFA and n6 values for the fillets were numerically lower than those of the experimental insect-based feeds.

The effects of the insect meal inclusion level on the pikeperch diets, as observed for some environmental parameters and economic aspects, are shown in Table 9. The increased inclusion level of HIM increased the cost of the diet and had an adverse effect on ECR and EPI. However, the inclusion of HIM progressively improved the fish-in-fish-out ratio ($P < 0.001$). Environmental impacts associated with one kg pikeperch production were HIM-dose dependent. Dietary HIM significantly elevated eutrophication and energy use ($P < 0.001$), while acidification and land use remained comparable among the control, H9, and H18 groups ($P > 0.05$). At an inclusion level as low as 9%, dietary insect meal entailed similar GWP as the control diet, while increasing HIM levels caused a significant burden on GWP ($P < 0.001$). It is worth noting that low to moderate inclusion levels of HIM (9% and 18%) required a similar amount of water to produce one kg pikeperch compared to HIM-free diet ($P > 0.05$), but the higher inclusion (36%) created a higher water demand ($P < 0.001$).

4. Discussion

Insect meal has been identified as one of the most promising potential alternative protein sources for aquafeeds in the coming decades (Hua et al., 2019). The inclusion of insect meal at appropriate levels in aquatic animal diets has shown a good response, in terms of growth performance and feed utilisation (Gasco et al., 2019; Hua, 2021). In addition, the use of dietary insect meal entails environmental benefits associated with the use of forage fish (FIFO) (Stejskal et al., 2020) and, from a life cycle assessment viewpoint, on climate change, acidification, human toxicity, marine ecotoxicity and abiotic depletion (Smárason et al., 2017).

4.1 Growth performance, condition factor, somatic indices, and digestibility of the diets

The growth performance of juvenile pikeperch in the present study, measured as specific growth rate (SGR) (range 0.76% to 0.95%/d), was comparable to the 0.77%/d in earlier findings (Zakęś et al., 2008) but slightly lower than that reported previously [1.14% to 1.24 %/d (Jarmołowicz et al., 2012)] and [(1.1% to 2.1%/day (Wang et al., 2009)]. The discrepancy could be attributed to the different fish sizes utilised in these studies; in fact larger fish, such as those utilised in our study, usually have lower SGR compared to fingerlings utilised in the other trials (Wang et al., 2009; Jarmołowicz et al., 2012). A meta-analysis concerning the effects of FM replacement by insect meal on the growth performance of fish conducted by Hua (2021) revealed that possible inclusions up to 33% and 25% full and defatted HIM, respectively, ensured a similar growth response to that of fish fed FM-based diets. Our results are consistent with that finding and have confirmed that an 18% inclusion threshold (which, in our research, led to 50% FM substitution) was possible for pikeperch. Previous studies that included HIM also reported a threshold over the 13.2% to 40% range (or 25% to 50% FM substitution) (St-Hilaire et al., 2007; Sealey et al., 2011; Renna et al., 2017; Dumas et al., 2018; Terova et al., 2019) for rainbow trout (*Oncorhynchus mykiss*), whilst 14.8% to –25%, or a 100% substitution level, was applied, with no adverse effects, to SGR in Atlantic salmon (Lock et al., 2016; Belghit et al., 2019). Similarly, 10.6% to 14% levels, or 100% FM substitution, were found to be possible for omnivorous common carp (*Cyprinus carpio*), without any negative effects on SGR (Li et al., 2017; Zhou et al., 2018). Feeding Nile tilapia (*Oreochromis niloticus*) with a dietary HM of 8% (Devic et al., 2018) or 30% (Muin et al., 2017) was also found to be successful.

Increasing the dietary HIM inclusion to 36% (100% FM substitution) depressed the growth performance of pikeperch, as shown by the significantly lower WG, FW and SGR in H36 than in the control diet. Hua (2021) reported that the negative effect on fish growth, caused by increasing levels of insect meal, could refer to a nutritional imbalance. Such a worsening of the performance parameters was supported by the general decrease in the digestibility coefficients recorded as the HIM inclusion increased. In addition, an increasing dietary inclusion of HIM reduced essential fatty acid components, PUFA and MUFA (Table 3), which play important roles in the growth and health-promoting effects of aquatic animals (Turchini et al., 2009). The presence of chitin, a non-protein nitrogen, in the cuticle of insects (Henry et al., 2015), could be a factor that impairs the growth rate of pikeperch fed H36. An analysis of chitin revealed a content in the HIM of 5.34% as it is, leading to dietary inclusions of 0.47%, 0.97% and 1.93% for H9, H18 and H36, respectively. These values are similar to the ones reported in the study of Stejskal et al. (2020). Previous studies pointed out a reduction in the SGR of turbot (Kroeckel et al., 2012) fed 17% HIM as a replacement of 20% FM. In contrast, feeding increasing levels of HIM did not affect the SGR of European perch (Stejskal et al., 2020) or Atlantic salmon (Belghit et al., 2018) fed diets containing 40% and 60% of HIM, respectively. The detrimental effect of chitin on the growth performance of fed organisms could be due to the compromise of protein digestibility related to its capacity to reduce the activity of proteolytic enzymes that break down peptides into aminoacids or bind proteins (Henry et al., 2015; Weththasinghe et al., 2021) and the induction of stress in fish (Gopalakannan and Arul, 2006). This is illustrated by a decreasing condition factor (K), which is known to reflect the growth rate of fish (Mahadevan et al., 2020). K is an index of the health and metabolic status of fish; the lower K value in pikeperch fed H36 could possibly be the result of a synergic effect, considering that fish in this group were smaller and less fatty in respect the other groups. Conversely, fish in the H18 group showed a higher K value due to the different metabolism of fat as shown by the HSI an VSI indices.

One criterion that should be considered concerning the possibility of introducing alternative ingredients to FM in aquafeeds is palatability, which can influence the feed intake and other physiological characteristics of fed organisms (Galkanda-Arachchige et al., 2020). HIM appeared to be palatable to pikeperch as a higher feed intake was recorded for the H36 group compared with HIM inclusion levels up to 18%, where a similar feed intake was recorded. These results are in contrast to those observed for Jian carp (*Cyprinus carpio*) (Li et al., 2017), rainbow trout (Renna et al., 2017), Japanese seabass (*Lateolabrax japonicus*) (Wang et al., 2019), and European perch (Stejskal et al., 2020) where a decreased palatability was observed with increasing HIM inclusion level. Interestingly, our results indicated that HIM inclusions of 9% and 36% did not affect the somatic indices (VSI, HSI and PFI), while HIM inclusion of 18% significantly reduced these parameters. In fish metabolism, the liver plays a key role and HSI is often used to assess the effect of diet on liver functionality (Dernekbaşı, 2012; Chemello et al., 2020). In salmonids, values between 1% and 2% are considered standard for HSI while lower or higher values could indicate issues such as oxidized feed, disorders in lipid and glucose metabolism, or vitamin deficiency (Pearce et al., 2003). In our study, all the fish groups recorded HSI values in the range considered normal for salmonids, therefore an HIM inclusion level up to 36% in pikeperch feeds could be tolerated without negative impacts on lipid and glucose metabolism.

4.2 Whole body and fillet composition

No consistent trends were observed with the composition of the body of pikeperch fed graded levels of HIM among the low and medium inclusion levels. However the pikeperch fed the H36 diet, except for the ash content, showed a significantly different composition than other groups. This pattern could be explained by considering feed nutrient digestibility, as the lower body nutrient content recorded in the pikeperch fed H36, compared to the other groups, could be attributed to a decline in nutrient digestibility as reported in other fish trials carried out in several species fed increasing content of insect meal (Coutinho et al. 2021). Furthermore, the detrimental effect of chitin on protein digestibility is well known (Henry et al., 2015; Gasco et al., 2016).

The fat content of the fillets in our study was dietary HIM-independent and ranged from 0.81% to 0.88%, which was higher than the range (0.20% to 0.58%) reported for pikeperch farmed in RAS, pond-RAS and in a pond system (Policar et al., 2016), or controlled rearing conditions (Schulz et al., 2005) with values of 0.6% in fish fed diets with different dietary lipid composition. However, the protein content in the fillets was comparable with the data from these studies.

The FA profile in the pikeperch fillets reflects those of the corresponding diets, as reported for finfish species (Turchini et al., 2009). The major effect of dietary partially defatted HIM on the muscle profile of pikeperch was a significant increase in total n6 constituents, especially linoleic acid (C18:2n6), and a significant decrease of total n3 fatty acids (especially C22:6n3). A similar phenomenon was also observed in previous studies carried out on juvenile pikeperch fed with feed supplemented with vegetable oils, such as linseed and peanut (Kowalska et al., 2010).

Another pronounced trend was observed for the fish muscle saturated fatty acids, lauric and myristic acids, which increased significantly with insect meal dietary inclusion. A similar pattern was also reported for rainbow trout fed increasing levels of defatted HIM (Renna et al., 2017). However, these differences in lauric and myristic acids seem to be too mild to alter the total SFA across the fed groups. Interestingly, the considerably lower lauric acid content in the fish fillets than in the feed may attributed to a prioritised energy utilisation of this FA (Renna et al., 2017) in pikeperch. PUFAs are significant components of muscle lipids in pikeperch, and they were found to range from 50.2% to 57.0% (Guler et al., 2007). These fatty acids were found to be high in our study (55% to 57% total detectable fatty acids) and independent of the administered diets. Compared to data reported for sander farmed in a different system (PUFAs, 34% to 44%) (Policar et al., 2016), the present study has shown relatively higher percentages of these fatty acids. DHA and EPA are important fatty acids that play vital roles in human health. DHA was found to be predominant in our study, ranging from 28% to 32% of the total detected fatty acids, and was affected by dietary HIM. An HIM inclusion of 18% maintained the DHA content relative similar to the FM group. The percentage of EPA instead varied by 4.5% to 4.9%, regardless of the dietary HIM. The DHA values are higher than those previously published for pikeperch (Policar et al., 2016; Kowalska et al., 2010). Therefore, using HIM at moderate inclusion levels, in combination with a marine oil source, could be a good way of enhancing the beneficial fatty acids of pikeperch for human nutrition.

4.3 Economic analysis and environmental sustainability

There is a general lack of economic analysis on insect meal inclusion in aquafeeds (Arru et al., 2019; Stejskal et al., 2020). The current study has revealed that increasing inclusion levels of HIM resulted in elevated ECR and reduced EPI, which is consistent with recent findings for European perch (Stejskal et al., 2020). Arru et al. (2019) revealed low profitability as a result of insect meal (*T. molitor*) inclusion in farmed seabass aquafeeds. This economic insufficiency could mainly be due to the uncompetitive price of insect meal vs. FM (IPIFF, 2018; Arru et al., 2019). Fortunately, insect meal production is increasing globally (IPIFF, 2018; Gasco et al., 2020) and the price of insect meal is thus expected to be comparative with that of FM in the near future (Arru et al., 2019; Hua et al., 2019). In the meantime, the marketing of seafood products with socially and environmentally sustainable feed ingredients, such as insect meal, could improve consumers' perceptions and their willingness to pay (Zander and Feucht, 2018). Together with the economic aspects, the environmental impacts associated with aquafeeds are of critical concern (Ghamkhar and Hicks, 2020). Our study has shown that dietary HIM has negative impacts on the environment associated with eutrophication and energy use. On the other hand, an inclusion level of up to 18% resulted in comparable acidification and land use with the control diet. Our study also highlighted the benefits of using insect meal HIM in the diet for pikeperch at a moderate inclusion level (18%) in terms of water resource use relative to an HIM-free diet. The high variability in environmental impact indices following replacement of FM by HIM could be attributed to the percentage of HIM vs. FM ingredients and slight modification of wheat meal across experimental diets. Indeed, the larger impact of HIM production, associated with energy use, GWP, eutrophication, and land use, than those of FM, has been confirmed (Salomone et al., 2017; Smetana et al., 2019, Tran et al., 2022b). Recent studies employing life cycle assessment have demonstrated that feeding arctic char (*Salvelinus alpinus*) with dietary HIM also entailed a heavier environmental burden of EU than insect-free diets, while multiple benefits were reported for abiotic depletion, acidification, eutrophication, the global warming potential, the human toxicity potential and the marine aquatic ecotoxicity potential (Smárason et al., 2017). Similar findings were reported for rainbow trout fed dietary *T. molitor* (Le Feon et al., 2019).

Although insect meal inclusion entails more environmental impacts than improvements, Le Feon et al. (2019) found a positive effect on the use of biotic resources and water. In addition to water use, we also found similarities in land use among H0, H9, and H18. In other words, the low to moderate inclusion level of HIM did not negatively affect the environmental impact indices associated with the most limited natural resources – water and land. This phenomenon could be associated with the change in wheat meal inclusion levels across experimental diets. It is well acknowledged that the production of wheat meal among plant ingredients requires a significantly higher amount of water and arable land than FM (GFLI, 2022; Silva et al., 2018; Smetana et al., 2019). Therefore, a substantial decrease in wheat meal following FM replacement by HIM to ensure nutrient balance, in combination with slightly higher water use and land use from production of HIM over FM (Samuel-Fitwi et al., 2013; Smetana et al., 2019), could result in comparable impacts on these natural resources among the control, H9, and H18 groups. Additionally, feed conversion ratio was reported to be responsible for the environmental impacts of the aquaculture system (Bohnes et al., 2019) and for that associated with one kg pikeperch production in the present study. As illustrated by the comparable feed conversion ratio, 3 diets, H0, H9, and H18, were efficiently utilized by pikeperch (Tran et al., 2021). However, despite a gradual decrease in wheat meal, a significantly higher feed conversion ratio following 100% replacement FM with HIM did not improve environmental impacts on pikeperch production. It is apparent that although an FM-free diet with the addition of HIM did not benefit pikeperch aquaculture in terms of either production performance or environmental consequences, elimination of FM originated from marine resources in aquafeed could be beneficial for the marine ecosystem as indicated by FIFO. In the present study, replacement of FM by HIM significantly improved the FIFO as less marine fish forage was required to produce the live weight of farmed fish (Tacon and Metian, 2008; Naylor et al., 2009). The same result has been reported for European perch (Stejskal et al., 2020) and for Siberian sturgeon (Rawski et al., 2021). We found that FIFO could be decreased by 40.1% in pikeperch fed an insect-based diet, without affecting the growth performance (group H18). From a global perspective, an increasing use of fish by-products and other FM alternatives could be a strategic way of ensuring the environmental sustainability of the aquaculture industry (Hua et al., 2019; Cottrell et al., 2020; Gasco et al., 2020), thereby reducing FM, and the fish oil proportion in aquafeeds. Consequently, the global FIFO is expected to reduce considerably in the coming decades (Kok et al., 2020). Since aquaculture is increasingly dependent on terrestrial crops and forage fish as feed inputs, and thereby damaging to aquatic ecosystems and fisheries (Smith et al., 2011; Troell et al., 2014), the use of insect meal could provide a promising alternative to tackle the growth of aquaculture in an era that has limited natural and marine fishery resources.

Future research should be focused on optimising the level of inclusion of insect meal in fish diets and the fine tuning of insect-based diets. Moreover, long-term studies focusing on growing fish to higher marketable size (more than 700g) in combination with sensory and textural analyses of the final product should be carried out to explore the full potential and gaps of insect-based diets for pikeperch throughout their whole life cycle. Information on the effect of insect meal on the physical characteristics of extruded feeds in aquafeeds for different fish species is still lacking, and more research and new methods to establish the correct insect meal digestibility of such fish feeds are therefore needed (Arru et al., 2019; Papáček et al., 2020).

This investigation is the first on the potential of HI larva meal for *S. lucioperca*. The main findings of the present work are that the inclusion of HIM to levels of up to 18% (equivalent to a 50% substitution of FM in the diet), did not affect the biometry, fillet yield, or the nutritional quality of pikeperch, except for the fat content which was lower. Both hepatosomatic index and perivisceral fat index were even improved by the inclusion of HIM up to 18%. Feeding HIM to pikeperch improved the FIFO, that led to the use of less forage fish from marine ecology to produce farmed fish and conserved more water resources than an insect-free diet. In economic terms, at present, HIM does not seem to be a price-competitive ingredient for pikeperch feeds.

5. Conclusion

This study has shown that the incorporation of HI meal in the feed formulations of pikeperch for inclusion levels of up to 18% did not affect most of the growth parameters considered. Moreover, the use of such feeds is associated with a reduction in reliance on marine resources and freshwater use. On the other hand, certain limitations have emerged, such as the production cost, decreased digestibility of protein and dry matter as well as increased impact on greenhouse gas production, energy use, and eutrophication.

Author contributions

Francesco Gai, **Laura Gasco** and **Vlastimil Stejskal** conceived and designed the experiment. **Hung Quang Tran**, **Christian Caimi**, **Laura Gasco** and **Vlastimil Stejskal** prepare the diets, performed the trial and collected the experiments data. **Hung Quang Tran**, **Markéta Prokesová**, **Tatyana Gebauer**, **Tomas Policar** and **Christian Caimi** carried out the laboratory analyses. **Vlastimil Stejskal** performed the statistical analysis. **Hung Quang Tran**, and **Vlastimil Stejskal** analyzed and interpret the data. **Hung Quang Tran**, **Francesco Gai**, **Laura Gasco** and **Vlastimil Stejskal** wrote the first draft of the manuscript. All authors critically reviewed the manuscript for intellectual content and gave final approval for the version to be published.

Declaration of competing interests

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.

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Table 1. Ingredients and proximate composition (%, as fed) of the HIM and of the experimental diets.

Item	Fishmeal	HIM	H0	H9	H18	H36
Ingredients						
Herring fishmeal[1]			30	22.5	15	0
HIM[2]			0.0	9.0	18.0	36.0
Soybean protein concentrate			7.5	7.5	7.5	7.5
Corn gluten meal			17.0	17.0	17.0	17.0
Soybean meal			15.0	15.0	15.0	15.0
Wheat meal			8.0	6.5	5.0	2.0
Merigel			6.0	6.0	6.0	6.0
Fish oil			6.0	6.0	6.0	6.0
Soybean oil			6.0	6.0	6.0	6.0
Vitamin mixture[3]			1.0	1.0	1.0	1.0
Mineral mixture[4]			1.0	1.0	1.0	1.0
DL-Methionine			0.7	0.7	0.7	0.7
L-Lysine			0.8	0.8	0.8	0.8
Celite[5]			1.0	1.0	1.0	1.0
Proximate composition[6]						
DM	94.0	91.0	94.3	94.9	94.5	94.8
CP (N × 6.25)	71.2	54.5	44.8	45.2	44.7	45.1
EE	9.4	8.5	18.9	18.2	18.9	17.4
Ash	14.0	7.6	8.7	8.6	8.1	7.4
Chitin[7]		5.34	-	0.47	0.97	1.93
NFE[8]	4.1	24.06	27.60	27.53	27.33	28.17
Gross energy[9], MJ/kg	21.22	20.20	21.05	20.36	20.32	21.06

HIM = defatted *Hermetia illucens* larva meal; DM = dry matter; CP = crude protein; EE = ether extract; NFE = nitrogen free extracts.

[1] Purchased from FF SKAGEN A/S (Skagen, Denmark).

[2] Purchased from Hermetia Deutschland GmbH & Co. KG (Baruth/Mark, Germany).

[3] Vitamin mixture (IU or mg/kg diet): DL-α tocopherol acetate, 60 IU; sodium menadione bisulphate, 5 mg; retinyl acetate, 15,000 IU; DL-cholecalciferol, 3,000 IU; thiamin, 15 mg; riboflavin, 30 mg; pyridoxine, 15 mg; B_{12}, 0.05 mg; nicotinic acid, 175 mg; folic acid, 500 mg; inositol, 1,000 mg; biotin, 2.5 mg; calcium panthotenate, 50 mg (purchased from Granda Zootecnici S.r.l., Cuneo, Italy).

[4] Mineral mixture (g or mg/kg diet): dicalcium phosphate, 500 g; calcium carbonate, 215 g; sodium salt 40, g; potassium chloride, 90 g; magnesium chloride, 124 g; magnesium carbonate,

124 g; iron sulphate, 20 g; zinc sulphate, 4 g; copper sulphate, 3 g; potassium iodide, 4 mg; cobalt sulphate, 20 mg; manganese sulphate, 3 g; sodium fluoride, 1 g (purchased from Granda Zootecnici S.r.l., Cuneo, Italy).

[5] Celite, a source of acid-insoluble ash.

[6] Values are reported as the mean values of duplicated analyses.

[7] Estimated as ADF – ADFN.

[8] Calculated as 100 − (CP + EE + Ash + Chitin).

[9] Determined by means of a calorimetric bomb.

Table 2. Amino acid content (% of protein) of the fishmeal, defatted black soldier fly *Hermetia illucens* and the experimental diets.

Item	FM	HIM	Experimental diets[1]			
			H0	H9	H18	H36
Σ Essential amino acids	46.2	54.3	50.8	46.1	48.8	47.2
Arginine	6.2	5.6	4.4	3.8	4.5	4.2
Histidine	2.4	3.0	2.7	2.5	2.5	2.3
Isoleucine	4.2	5.1	3.7	3.5	3.8	3.8
Leucine	7.2	7.9	9.2	8.4	8.9	8.7
Lysine	7.5	6.6	9.8	8.8	9.2	8.3
Methionine	2.7	2.1	3.4	2.6	2.7	2.3
Phenylalanine	3.9	5.2	4.6	4.2	3.9	3.9
Tyrosine	3.1	6.9	3.6	3.7	3.6	4.2
Threonine	4.1	3.7	5.3	4.8	5.1	4.8
Valine	4.9	8.2	4.1	3.8	4.6	4.7
Σ Non-essential amino acids	42.5	44.0	46.5	44.2	43.8	45.5
Alanine	6.3	7.7	5.4	5.2	6.1	6.5
Aspartic acid	9.1	10.0	7.9	7.2	7.9	7.7
Glycine	6.4	5.7	4.2	3.8	3.9	3.8
Glutamic acid	12.6	10.9	15.7	14.6	15.3	14.9
Proline	4.2	6.6	9.2	9.6	6.4	8.3
Serine	3.9	3.1	4.1	3.8	4.2	4.3
Total amino acids	88.7	98.3	97.3	90.3	92.6	92.7

FM = herring fish meal; HIM = *Hermetia illucens* meal;

[1] H0, H9, H18, H36 represent experimental diets where fishmeal was replaced by HIM at 0%, 25%, 50% and 100%, respectively.

Table 3. Fatty acid composition (% of the total fatty acids) of fishmeal, defatted black soldier fly (*Hemetia illucens*) and the experimental diets.

Fatty acids	FM	HIM	Experimental diets[1]			
			H0	H9	H18	H36
C12:0	0.35	43.70	0.04	1.61	2.57	6.18
C14:0	5.16	11.82	1.72	2.01	2.12	2.75
C16:0	21.64	16.34	10.27	10.68	10.52	10.62
C16:1	5.00	3.92	2.37	2.39	2.40	2.41
C18:0	4.45	2.69	2.99	3.02	3.03	2.81
C18:1n9	16.64	11	20.13	19.60	19.56	18.85
C18:1n7	1.67	0.38	20.62	19.60	19.79	19.45
C18:2n6	2.47	nd	25.76	25.41	25.10	24.18
C18:3n3	0.16	0.76	3.89	3.73	3.70	3.43
C20:1n9	1.25	nd	3.30	3.12	3.10	2.75
C20:3n3	4.26	nd	0.11	0.10	0.10	0.08
C20:4n6	0.17	nd	0.25	0.24	0.19	0.11
C20:5n3	0.99	nd	0.32	0.31	0.30	0.26
C22:5n6	9.72	nd	0.63	0.59	0.54	0.42
C22:6n3	1.00	nd	4.82	4.55	3.91	2.67
C23:0	nd	nd	0.50	0.80	0.86	0.81
Other	4.40	1.0	2.28	2.24	2.21	2.22
SFA	33.76	74.89	16.46	19.06	20.00	23.95
MUFA	29.30	15.43	47.09	45.36	45.48	44.02
PUFA	36.58	9.15	36.00	35.14	34.04	31.60
n3	31.83	0.76	9.14	8.69	8.01	6.44
n6	4.74	8.39	26.81	26.40	25.98	24.83
n3/n6	6.72	0.09	0.34	0.33	0.31	0.26

FM = herring fish meal; HIM = defatted black soldier fly (*Hemetia illucens*); nd = traces, < 0.05%; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

[1] H0, H9, H18, H36 represent experimental diets where fishmeal was replaced by HIM at 0%, 25%, 50% and 100%, respectively.

Table 4. Survival and growth performances of the pikeperch fed the experimental diets (mean ± standard deviation).

Item	Experimental diets[1]				*P*-value
	H0	H9	H18	H36	
IBW, g	69.0±6.5	67.5±7.0	68.4±5.7	69.9±7.0	0.092
BW21, g	91.3±12.1^{ab}	91.1±8.9a^{b}	91.2±10.3^{a}	87.6±9.8^{b}	0.031
BW42, g	111.8±18.0^{a}	109.3±12.9^{a}	110±16.8^{a}	102.4±13.6^{b}	0.001
BW63, g	128.5±21.8^{a}	129.6±20.3^{a}	127.1±20.8^{ab}	119.0±18.6^{b}	0.005
FBW, g	154.3±24.5^{a}	152.3±24.2^{a}	151.6±26.5^{a}	132.7±19.9^{b}	<0.001
SR, %	96±2.0	97.3±3.1	96.7±1.2	94±5.3	0.642
WG, %	122.0±2.5^{a}	126.1±17.4^{a}	121.9±6.5^{a}	86.9±6.7^{b}	0.004
SGR, %/d	0.95±0.20^{a}	0.96±0.21^{a}	0.93±0.22^{a}	0.76±0.17^{b}	<0.001
Feed intake (g/kg ABW per day)	10.65±0.27^{b}	10.86±0.30^{b}	10.66±0.18^{b}	11.78±0.12^{a}	<0.001
Feed conversion ratio[2]	1.27±0.06^{b}	1.28±0.07^{b}	1.29±0.03^{b}	1.81±0.15^{a}	<0.001
FR, %/d	1.25±0.01^{b}	1.28±0.01^{b}	1.26±0.03^{b}	1.34±0.02^{a}	0.002
PER	1.66±0.08^{a}	1.64±0.09^{a}	1.64±0.04^{a}	1.16±0.10^{b}	<0.001

IBW = initial body weight; BW21 = body weight at day 21; BW42 = body weight at day 42; BW63 = body weight at day 63; FBW = final body weight; SR = survival rate; WG = weight gain; SGR = specific growth rate; ABW = average body weight; FR = feeding rate; PER = protein efficiency ratio.

^{a,b}Different letters within a row indicate significant differences ($P < 0.05$).

[1] H0, H9, H18, H36 represent experimental diets where fishmeal was replaced by defatted black soldier fly (*Hemetia illucens*) at 0%, 25%, 50% and 100%, respectively.

[2] Data published in the study (Tran et al., 2021).

Table 5. Condition factor, somatic indexes and fillet yield in the pikeperch fed the experimental diets (mean ± standard deviation, *n* = 21).

Item	Experimental diets[1]				*P*-value
	H0	H9	H18	H36	
K[2]	0.81±0.09^{a}	0.80±0.07^{ab}	0.81±0.09^{a}	0.78±0.06^{b}	0.019
HSI[3], %	1.41±0.36^{a}	1.20±0.27^{ab}	1.03±0.26^{b}	1.27±0.22^{a}	< 0.001
VSI[4], %	9.42±1.58^{a}	8.68±1.39^{ab}	7.54±0.95^{b}	8.79±1.73^{a}	< 0.001
PFI[5], %	5.16±1.42^{a}	4.64±1.27^{a}	3.92±0.74^{b}	4.58±1.40^{a}	0.019
FY[6], %	45.6±2.1^{ab}	46.1±2.2^{ab}	46.6±1.5^{a}	44.8±1.9^{b}	0.027

^{a,b}Different letters within a row indicate significant differences ($P < 0.05$).

[1] H0, H9, H18, H36 represent experimental diets where fishmeal was replaced by defatted black soldier fly (*Hemetia illucens*) at 0%, 25%, 50% and 100%, respectively.

[2] Fulton's condition factor（K) = [Final body weight (g)/Final body length (mm)^{3}] × 100.

[3] Hepatosomatic index (HSI) = 100 × Liver weight (g)/Fish weight (g).

[4] Viscerosomatic index (VSI) = 100 × Viscera weight (g)/Fish weight (g).

[5] Perivisceral fat index (PFI) = 100 × Perivisceral fat weight (g)/Fish weight (g).

[6] Fillet yield (FY) = 100 × Fillet weight (g)/BW.

Table 6. Apparent digestibility coefficient of the dry matter, proteins and ether extract of pikeperch fed the experimental diets (mean ± standard deviation, $n = 3$).

Item	Experimental diets[1]				P-value
	H0	H9	H18	H36	
ADC $_{DM}$	82.77 ± 0.77^{a}	81.64 ± 0.59^{ab}	80.86 ± 0.35^{b}	72.90 ± 0.16^{c}	0.001
ADC $_{CP}$	86.10 ± 0.62^{a}	84.35 ± 0.50^{b}	82.95 ± 0.16^{c}	70.75 ± 0.18^{d}	0.001
ADC $_{EE}$	84.15 ± 0.71^{a}	82.90 ± 0.55^{a}	83.15 ± 0.68^{a}	72.22 ± 0.17^{b}	0.001

ADC = apparent digestibility coefficient; DM = dry matter; CP = crude protein; EE = ether extract.

$^{a\text{-}d}$Different letters within a row indicate significant differences ($P < 0.05$).

[1] H0, H9, H18, H36 represent experimental diets where fishmeal was replaced by defatted black soldier fly (*Hemetia illucens*) at 0%, 25%, 50% and 100%, respectively.

Table 7. Proximate composition (homogenates of the whole body; g/100 g as it is) of the pikeperch fed the experimental diets (mean ± standard deviation, *n* = 9).

	Experimental diets[1]				
Item	H0	H9	H18	H36	*P*-value
DM	26.2±1.4^{ab}	27.0±1.8^{a}	25.7±0.9^{ab}	25.0±1.5^{b}	0.043
CP	16.8±0.6^{ab}	17±1.0^{a}	16.9±1.0^{ab}	15.9±0.7^{b}	0.026
EE	7.2±0.7^{ab}	7.8±1.6^{a}	6.2±0.7^{b}	6.4±0.7^{b}	0.006
Ash	3.8±0.2	3.8±0.4	4.0±0.4	3.8±0.3	0.597
Energy content, MJ/kg	0.63±0.04^{ab}	0.65±0.06^{a}	0.59±0.03^{ab}	0.57±0.06^{b}	0.007

DM = dry matter; CP = crude protein; EE = ether extract.

^{a,b}Different letters within a row indicate significant differences ($P < 0.05$).

[1] H0, H9, H18, H36 represent experimental diets where fishmeal was replaced by defatted black soldier fly (*Hemetia illucens*) at 0%, 25%, 50% and 100%, respectively.

Table 8. Proximate composition (g/100 g as it) and fatty acid profiles (% of total fatty acids) of fillet of pikeperch fed the experimental diets.

	Experimental diets^{1}				*P*-value
Item	H0	H9	H18	H36	
Proximate composition					
DM	20.8±0.1^{ab}	21.3±0.6^{a}	21.0±0.8^{ab}	20.3±1.1^{b}	0.003
CP	19.7±0.7^{b}	20.2±0.3^{a}	19.9±0.5^{ab}	19.2±0.7^{c}	< 0.001
EE	0.86±0.21	0.81±0.21	0.88±0.27	0.83±0.20	0.791
Ash	1.10±0.06^{a}	1.10±0.11^{a}	1.09±0.12^{a}	1.01±0.04^{b}	< 0.001
Fatty acid profiles					
C12:0	0.02±0.01^{c}	0.23±0.09^{bc}	0.55±0.14^{a}	0.50±0.27^{ab}	<0.001
C14:0	1.15±0.21^{c}	1.26±0.13^{bc}	1.59±0.20^{a}	1.55±0.29^{ab}	0.001
C16:0	18.83±1.44^{ab}	19.25±0.57^{a}	18.75±0.65^{ab}	18.27±0.81^{b}	0.048
C16:1	2.17±0.31	1.84±0.30	2.15±0.39	2.08±0.42	0.112
C18:0	4.98±0.60	5.45±0.31	5.5±0.64	5.27±0.55	0.155
C18:1n9	13.15±1.65	11.86±1.24	13.02±1.08	13.3±1.72	0.072
C18:1n7	nd	nd	nd	nd	
C18:2n6	13.85±3.95^{b}	14.36±1.06^{b}	15.34±0.63^{b}	17.31±1.18^{a}	0.001
C18:3n3	1.66±0.47^{a}	1.47±0.13^{b}	1.67±0.13^{ab}	1.83±0.20^{a}	0.001
C20:1n9	1.64±0.08^{a}	1.43±0.13^{b}	1.46±0.06^{b}	1.57±0.12^{ab}	<0.001
C20:3n3	1.45±0.18^{a}	1.44±0.13^{a}	1.27±0.10^{ab}	1.25±0.16^{b}	0.002
C20:4n6	0.14±0.03	0.14±0.03	0.14±0.01	0.14±0.04	0.690
C20:5n3	4.88±0.61	4.95±0.56	4.53±0.22	4.89±0.54	0.252
C22:5n6	1.49±0.17^{b}	1.47±0.52^{ab}	1.41±0.45^{b}	1.82±0.36^{a}	0.009
C22:6n3	32.79±4.14^{a}	32.85±2.02^{a}	30.69±1.80^{ab}	28.37±2.67^{b}	0.001
C23:0	nd	nd	nd	nd	
SFA	25.66±1.93	26.88±0.65	27.04±0.95	26.21±0.82	0.078
MUFA	15.08±1.64	13.59±1.32	14.73±1.13	15.12±1.80	0.075
PUFA	56.75±1.41	57.21±1.47	55.57±1.91	56.16±2.19	0.185
PUFA+MUFA	71.82±1.76^{a}	70.8±0.71^{ab}	70.29±1.10^{b}	71.28±0.81^{ab}	0.029
n3	40.78±4.13^{a}	40.71±2.10^{a}	38.16±1.94^{ab}	36.33±2.84^{b}	0.001
n6	15.96±3.88^{b}	16.49±1.25^{b}	17.40±0.77^{b}	19.82±1.04^{a}	<0.001
n3/n6	2.88±1.53^{a}	2.49±0.29^{a}	2.20±0.16^{ab}	1.84±0.21^{b}	<0.001
UI	284.90±16.76^{a}	284.25±9.77^{a}	272.37±10.35^{ab}	267.05±13.74^{b}	0.003
AI	0.33±0.02^{b}	0.35±0.01^{ab}	0.37±0.02^{a}	0.35±0.02^{ab}	0.002
TI	0.18±0.01^{b}	0.19±0.01^{ab}	0.20±0.01^{a}	0.20±0.01^{a}	0.003

DM = dry matter; CP = crude protein; EE = ether extract; nd = traces, < 0.05%; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

$^{a\text{-}c}$Different letters within a row indicate significant differences ($P < 0.05$).

^{1}H0, H9, H18, H36 represent experimental diets where fishmeal was replaced by defatted black soldier fly (*Hemetia illucens*) at 0%, 25%, 50% and 100%, respectively.

[2] Unsaturation index (UI) = 1 × (% monoenoics) + 2 × (% dienoics) + 3 × (% trienoics) + 4 × (% tetraenoics) + 5 × (% pentaenoics) + 6 × (% hexaenoics).

[3] Atherogenicity index (AI) = [C12:0 + (4 × C14:0) + C16:0]/ΣUnsaturated fatty acids.

[4] Thrombogenicity index (TI) = (C14:0 + C16:0 + C18:0)/[(0.5×ΣMUFA) + (0.5 × Σn6 PUFA) + (3 × Σn3 PUFA) + (n3/n6)].

Table 9 Economic and environmental sustainability parameters of pikeperch fed the experimental diets (mean ± standard deviation, n = 3).

Item	Experimental diets[1]				*P*-value
	H0	H9	H18	H36	
Diet cost, €/kg	0.97	1.17	1.36	1.75	-
ECR[2], € /kg of fish	1.23 ± 0.06^{c}	1.50 ± 0.08^{bc}	1.75 ± 0.04^{b}	3.17 ± 0.27^{a}	<0.001
EPI[2], €/fish	1.06 ± 0.02^{a}	1.03 ± 0.02^{a}	1.00 ± 0.03^{a}	0.81 ± 0.03^{b}	<0.001
FIFO[2]	1.66 ± 0.08^{a}	1.33 ± 0.07^{b}	0.98 ± 0.02^{c}	0.40 ± 0.03^{d}	<0.001
Environmental impacts associated with 1 kg pikeperch production					
GWP, kg CO_2 eq.	2.59 ± 0.13^{c}	3.1 ± 0.17^{bc}	3.6 ± 0.09^{b}	6.45 ± 0.54^{a}	<0.001
Acidification, kg SO_2 eq.	11.67 ± 0.58^{b}	12.96 ± 0.71^{b}	14.24 ± 0.36^{b}	23.42 ± 1.96^{a}	<0.001
Eutrophication, kg P eq.	0.26 ± 0.01^{d}	0.98 ± 0.05^{c}	1.71 ± 0.04^{b}	4.44 ± 0.37^{a}	<0.001
Land use, m^{2}a	2.11 ± 0.11^{b}	2.23 ± 0.12^{b}	2.35 ± 0.06^{b}	3.61 ± 0.3^{a}	<0.001
Energy use, kg oil eq.	0.34 ± 0.02^{d}	0.53 ± 0.03^{c}	0.73 ± 0.02^{b}	1.58 ± 0.13^{a}	<0.001
Water use, m^3	0.036 ± 0.002^{b}	0.036 ± 0.002^{b}	0.036 ± 0.001^{b}	0.051 ± 0.004^{a}	<0.001

GWP = global warming potential; eq. = equivalent.

$^{a\text{-}d}$Different letters within a row indicate significant differences ($P < 0.05$).

[1] H0, H9, H18, H36 represent experimental diets where fishmeal was replaced by defatted black soldier fly (*Hemetia illucens*) at 0%, 25%, 50% and 100%, respectively.

[2] ECR = Feed conversion ratio × D_P;

EPI = (Weight gain × S_P) – (Weigth gain × D_P);

FIFO = (L_{FM} + L_{FO})/(Y_{FMw} + Y_{FOw}) × Feed conversion ratio;

Where D_P is the price of the diet (€/kg of diet) and S_P is the selling price (€7.58/kg); L_{FM} is the level of FM in the diet; L_{FO} is the level of fish oil in the diet; Y_{FMw} is the FM yield from wild fish; Y_{FOw} is the fish oil yield from wild fish.