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This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1877179> since 2024-12-04T10:32:01Z

Published version:

DOI:10.1016/j.aninu.2022.06.022

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Journal Pre-proof

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PII: S2405-6545(22)00124-X

DOI: <https://doi.org/10.1016/j.aninu.2022.06.022>

Reference: ANINU 656

To appear in: *Animal Nutrition Journal*

Received Date: 24 August 2021

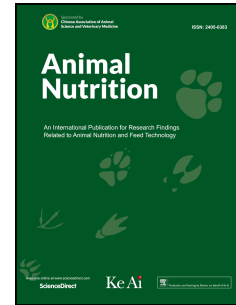
Revised Date: 13 May 2022

Accepted Date: 12 June 2022

Please cite this article as: Stejskal V, Tran HQ, Prokesová M, Zare M, Gebauer T, Policar T, Caimi C, Gai F, Gasco L, Defatted black soldier fly (*Hermetia illucens*) in pikeperch (*Sander lucioperca*) diets: Effects on growth performance, nutrient digestibility, fillet quality, economic and environmental sustainability, *Animal Nutrition Journal*, <https://doi.org/10.1016/j.aninu.2022.06.022>.

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

4

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18

19

20 Abstract

21 The use of insect meal in aquafeed formulations has recently gained attention. Detailed
22 knowledge about the inclusion levels for pikeperch (*Sander lucioperca*), a promising candidate
23 for intensive aquaculture in Europe remains, however, fragmented. In the present study, 4
24 isoproteic (45% dry matter) and isoenergetic (21 MJ/kg) diets were formulated, including a
25 control diet (H0) containing 30% fishmeal (FM) on an as fed basis and the other 3 diets in which
26 FM protein was replaced by defatted black soldier fly meal (*Hermetia illucens*) (HIM) at 25%,
27 50%, and 100% (diet abbreviation H9, H18 and H36, corresponding to an inclusion level of
28 9%, 18% and 36%, respectively). The feeding trial was performed in triplicate groups of 50
29 juvenile pikeperch (mean weight, 68.7 g) fed with experimental diets for 84 d during which the
30 growth performance, nutrient digestibility, fillet quality and economic and environmental
31 sustainability of rearing pikeperch were evaluated. Our findings indicated that pikeperch fed
32 H0, H9, and H18 groups displayed better results regarding growth performance indices, except
33 for survival rate where no significant difference among groups was recorded ($P = 0.642$). A
34 significantly lower organ-somatic index, including hepatosomatic, viscerosomatic and
35 perivisceral fat index, was found in fish fed H18 groups than other groups ($P < 0.05$). Inclusion
36 of HIM affected the digestibility of the nutrients and resulted in an almost linear reduction in
37 the apparent digestibility coefficient of dry matter and protein. Concerning the fillet quality,
38 dietary HIM negatively affected the protein and ash contents of the fish fillets, while the crude
39 fat remained unchanged. Dietary HIM did not significantly modify total saturated,
40 monounsaturated and polyunsaturated fatty acids in the fillets of fed pikeperch ($P > 0.05$) but
41 did reduce total n-3 fatty acids ($P = 0.001$) and increased total n-6 ($P < 0.001$). Increasing
42 inclusion levels of HIM reduced the environmental impacts associated with fish-in-to-fish out
43 ratio but entailed heavy burdens on energy use and eutrophication. Low and moderate inclusion
44 levels of HIM did not negatively affect land use and water use compared to an HIM-free diet
45 ($P > 0.05$). The addition of HIM at a level as low as 9% elicited a similar carbon footprint to

46 that of the control diet. The economic conversion ratio and economic profit index were
47 negatively affected at increased insect meal inclusion levels. This study has shown that the
48 incorporation of HIM in feed formulations for pikeperch is feasible at inclusion levels of 18%
49 without adverse effects on growth performance parameters. The feasibility also highlighted the
50 environmental benefits associated with land use and marine resources required to produce
51 farmed fish.

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

54

55

56

57 **1. Introduction**

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

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

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

82 with promising results (Hua et al., 2019; Galkanda-Arachchige et al., 2020), even though their
83 use is limited by legislation in Europe (Gasco et al., 2018) and by EAA deficiency, high ash
84 content and variability in digestibility (Galkanda-Arachchige et al., 2020).

85 A great deal of attention has recently been paid to insects (Barragan-Fonseca et al.,
86 2017; Gasco et al., 2019), which have already been proposed as an efficient and high-quality
87 alternative protein source for poultry (Neumann et al., 2018; Secci et al., 2018; Gariglio et al.,
88 2019; Pieterse et al., 2019; Yoo et al., 2019) and swine (Biasato et al., 2019; Chia et al., 2019).
89 Insects are also a suitable source of protein and lipids for carnivorous fish (Lock et al., 2018)
90 as a naturally available food in their environment. Insect meal has been shown to be a promising
91 alternative to FM in aquaculture (Lock et al., 2018; Gasco et al., 2019; Nogales-Mérida et al.,
92 2019) with optimal dietary sources of several vitamins and minerals (e.g. iron, potassium,
93 calcium, magnesium etc.) (Gasco et al., 2018; Hawkey et al., 2021). Several insect species can
94 be included successfully in carnivorous fish diets [e.g. for rainbow trout (*Oncorhynchus mykiss*)
95 (Chemello et al., 2020), European sea bass (*Dicentrarchus labrax*) (Gasco et al., 2016), Atlantic
96 salmon (*Salmo salar*) (Belghit et al., 2019) and gilthead seabream (*Sparus aurata*) (Piccolo et
97 al., 2017)] or in omnivorous fish diets [e.g. for common carp (*Cyprinus carpio*) (Li et al., 2017)
98 and Nile tilapia (*Oreochromis niloticus*) (Devic et al., 2018)], with the best results having been
99 obtained from a partial replacement of FM. The most common insect species included as
100 processed larva meal are mealworm (*Tenebrio molitor*), black soldier fly (*Hermetia illucens*,
101 HI) and house fly (*Musca domestica*) (Lin and Mui, 2017; Magalhães et al., 2017; Ido et al.,
102 2019; Chemello et al., 2020). In particular, HI larva meal seems to be one of the most promising
103 insect-based PAP alternatives to FM. HI larva meal is rich in protein, with levels up to 60%.
104 Even if lower in some EAAs compared to FM, HI larva meal has a well-balanced amino acid
105 profile (Hawkey et al., 2021) and provides a good amount of minerals and vitamins (Li et al.,
106 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

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

151

152 *2.3 Facilities, fish and the feeding trial*

153 The feeding trial lasted 12 wk and was conducted in a recirculation system (total volume
154 11,400 L), consisting of fifteen 250 L round conical plastic tanks (black walls, white bottom),
155 a mechanical drum filter (AEM 15, AEM-Products V.O.F., Lienden, The Netherlands),
156 sedimentation tanks (total volume 2,600 L, series of filtration sections Bioakvacit PP10) and a
157 moving bed biofilter (volume 4,700 L, media BT10 Ratz Aqua & Polymer Technik, Remscheid,

158 Germany). The water temperature was maintained at 23.1 ± 1.0 °C by conditioning the ambient
159 air and using Eheim Jäger Thermocontrol 300 submerged heaters (Eheim GmbH & Co KG,
160 Stuttgart, Germany); the photoperiod was set at 12 h light-12 h dark by controlling the light
161 through the use of timers. Light intensity was set at 20 to 35 Lx on the water surface. The flow
162 rate in each tank was approximately 200 L/h. Dissolved oxygen (8.6 ± 1.3 mg/L) and pH (6.98
163 ± 0.28) were monitored twice daily, at 08:00 and 16:00, using a HACH HQ 40 multi-meter
164 (HACH Lange, Germany). Pure oxygen was distributed, using ceramic diffusers, in the header
165 tank, whenever necessary. The ammonia, nitrate and nitrite concentrations were analysed by
166 means of HACH, LCK 304, LCK 339 and LCK 341 kits, using a HACH DR2800
167 Spectrophotometer at 2-day intervals. The nitrite-N, nitrate-N, and ammonia-N concentrations
168 were 0.42 ± 0.24 , 78.88 ± 37.31 and 1.89 ± 0.58 mg/L, respectively.

169 The juvenile pikeperch used in the trial were obtained, according to the procedure described in
170 Policar et al. (2013), from the own faculty source. Part of this stock was implanted with a PIT-
171 tag ($7 \text{ mm} \times 1.35 \text{ mm}$, Loligo Systems ApS) when juveniles reached a mean body weight of
172 52.51 ± 5.23 g (10 d before start of feeding trial). In order to perform the trial, a total of 750
173 juveniles (of which 450 were tagged) were individually weighed using a digital balance (Scout,
174 Ohaus Corporation, The USA, $d = 0.1$ g) (initial body weight [IBW] of 68.7 ± 6.6 g) and
175 randomly allotted to 15 tanks with a total of 50 fish per tank. The mean stocking density at the
176 start of the trial was 13.17 ± 0.24 kg/m³.

177 Moreover, the tagged fish were also measured after anaesthesia in an MS 222 bath (50 mg/L),
178 (initial body length [IBL] ± 1 mm) to follow both the body weight and length over time. All the
179 fish were acclimated to the rearing system for 10 d before the start of the trial and fed by a
180 grower commercial feed EFICO Sigma 970 (crude protein: 54%, crude lipid: 18%, pellet size:
181 3 mm) (BioMar A/S, Brande, Denmark).

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

187

188 *2.4 Growth parameters*

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

195 The obtained data were used to calculate the following variables:

- 196 • Survival(SR, %) = $100 - (\text{Number of dead fish} / \text{Initial number of fish}) \times 100$
- 197 • Weight gain(WG, %) = $[(\text{FBW (g)} - \text{IBW (g)}) / \text{IBW (g)}] \times 100$
- 198 • Specific growth rate (SGR, %/day) = $[(\ln \text{FBW} - \ln \text{IBW}) / \text{Number of feeding days}] \times$
 199 100

200 Feed intake (g/kg ABW per day) = $\text{Total feed consumed (g, DM)} / \text{Average body weight}$
 201 $(\text{kg}) / \text{Number of feeding days}$ (Guerreiro et al., 2020)

202 Feeding rate (FR, %/day) = $[\text{Total feed supplied (g, DM)} \times 100 / \text{Number of feeding}$
 203 $\text{days}] / [e^{(\ln \text{FBW} + \ln \text{IBW}) \times 0.5}]$ (Lock et al., 2018)

204 Protein efficiency ratio (PER) = $\text{WG (g)} / \text{Total protein fed (g, DM)}$

205 Where ABW is average body weight and calculated as $(\text{Initial body weight} + \text{Final body}$
 206 $\text{weight}) / 2$; SD is the standard deviation of the fish subsample.

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

212 Fulton's condition factor (K) = $(\text{FBW}/\text{FBL}^3) \times 100$

213 Hepatosomatic index (HSI, %) = $100 \times \text{Liver weight (g)}/\text{Fish weight (g)}$

214 Viscerosomatic index (VSI, %) = $100 \times \text{Viscera weight (g)}/\text{Fish weight (g)}$

215 Perivisceral fat index (PFI, %) = $100 \times \text{Perivisceral fat weight (g)}/\text{Fish weight (g)}$

216 Fillet yield (FY, %) = $100 \times \text{Fillet weight (g)}/\text{BW}$.

217 Where FBL is final total body length (mm). The right and left fillets of 5 fish per tank (15
 218 fish/treatment) were stored at -20 °C for subsequent proximate composition analyses.

219 Moreover, 3 fish per tank (9 fish/treatment) were sampled and stored at -20 °C for a whole-
 220 body composition (WBC) assessment.

221

222 *2.5 Digestibility trial*

223 Seventy-five day after the start of the trial, faeces were collected daily for 7 d using
 224 settling columns placed at the bottom of the tanks. After each meal, any uneaten feed was
 225 collected, as reported in section 2.3. One hour after each feeding, the faeces accumulated in
 226 each settling column were collected, centrifuged ($3,000 \times g$), pooled for each tank and stored
 227 at -20 °C until they were freeze dried for analyses. The apparent digestibility coefficients of the
 228 dry matter (ADC_{DM}), crude protein (ADC_{CP}) and ether extract (ADC_{EE}) of the 4 experimental
 229 diets were measured using the indirect acid-insoluble ash (AIA) method, with 1% celite (Fluka,
 230 Switzerland) added to the diets as an inert marker, and then calculated according to Renna et
 231 al. (2017). Celite is a common and reliable indigestible marker used to assess nutrient

232 digestibility in fish (Da et al., 2013; Chemello et al., 2020; Caimi et al., 2021). This marker was
233 found to not leak from faeces throughout a 24 h cycle and therefore feasible to recover in
234 adequate quantities in the faeces (Sales et al., 2001).

235

236 *2.6 Proximate composition of the HIM, diets, fish and fillets*

237 The HIM and feed samples were analysed as reported in Renna et al. (2017). The diets
238 were ground finely using a cutting mill (MLI 204; Bühler AG, Uzwil, Switzerland) and the
239 analyses were performed according to AOAC International 2000. Samples were dried in the
240 oven at 105 °C to reach constant weight for dry matter (AOAC no.934.01), then crude protein
241 was estimated using the Kjeldahl method (AOAC no.984.13), ash content measured (AOAC
242 no.942.05) by incinerating the samples in a muffle furnace at 550 °C, and crude fat determined
243 by the Soxhlet extraction method following the procedure AOAC no. 2003.05 (AOAC, 2003).
244 The gross energy content was determined using an adiabatic calorimetric bomb (C7000; IKA,
245 Staufen, Germany). Chitin was estimated according to Finke (2007). All the feed analyses were
246 performed in duplicate. Fatty acid profile was determined as described in detail by Sampels et
247 al. (2014) by methylating lipid with boron trifluoride-methanol complex (BF₃), dissolving in
248 0.5 mL of hexane and storing under normal atmosphere at -80 °C until gas chromatography
249 analysis. Fatty acid methyl esters were determined using a gas chromatograph. Analysis of the
250 amino acid composition of the experimental diets was performed in triplicate, using an
251 automatic amino acid analyzer AAA 400 (INGOS Prague) based on dye-forming reaction of
252 amino acids using ninhydrin as an oxidizing agent (Stejskal et al. 2019).

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

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

258 Atherogenicity index (AI) = $[C12:0 + (4 \times C14:0) + C16:0]/\Sigma UFA$

259 Thrombogenicity index (TI) = $(C14:0 + C16:0 + C18:0)/[(0.5 \times \Sigma MUFA) + (0.5 \times \Sigma n6 PUFA)$

260 $+ (3 \times \Sigma n3 PUFA) + (n3/n6)]$

261 Unsaturation index (UI) = $1 \times (\% \text{ monoenoics}) + 2 \times (\% \text{ dienoics}) + 3 \times (\% \text{ trienoics}) + 4 \times$

262 $(\% \text{ tetraenoics}) + 5 \times (\% \text{ pentaenoics}) + 6 \times (\% \text{ hexaenoics})$

263

264 *2.7 Economic analyses and environmental sustainability of the experimental diets*

265 An economic conversion ratio (ECR) and an economic profit index (EPI) were calculated for

266 each tested group to determine the relative efficacy of the tested diets and their subsequent

267 benefits, using the following formulas [Moutinho et al., (2017)]:

268 $ECR (\text{€}/\text{kg of fish}) = \text{Feed conversion ratio} \times D_P$

269 $EPI (\text{€}/\text{fish}) = (WG \times S_P) - (WG \times D_P)$

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

271 The per kilogram cost (in €), excluding labour and taxes, of all the used components bought

272 from commercial retailers was as follows: FM = €1.48; HIM = €3.50; wheat meal = €0.60; fish

273 oil = €1.33; mineral mixture = €0.51; vitamin mixture = €3.90; soy concentrate = €1.50; corn

274 gluten meal = €0.37; soybean meal = €0.33; merigel = €0.75; fish oil = €1.33; soybean oil =

275 €0.58; vitamin premix = €3.90; mineral premix = €0.51; L-methionine = €6.00; L-lysine =

276 €1.50. The followed prices of the diets were calculated: H0 = €0.97; H9 = €1.17; H18 = €1.36

277 and H36 = €1.75. The sales price of pikeperch was calculated as €7.58/kg based on published

278 price report (FAO 2020b) and personal communication with 2 European fish farms who

279 produce pikeperch in RAS systems. The fish-in-to-fish-out ratio (FIFO) was used as a practical

280 measure of the quantity of live fish from capture fisheries required for each kilogram of farmed

281 pikeperch. This indicator was calculated as follows (Tacon and Metian, 2008):

282 $FIFO = (L_{FM} + L_{FO}) / (Y_{FMw} + Y_{FOw}) \times \text{Feed conversion ratio}$

283 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
284 yield from wild fish; Y_{FOw} is the fish oil yield from wild fish.

285 The simulated environmental impacts associated with 1 kg farmed pikeperch production were
286 calculated according to Tran et al. (2022a) as a multiplication between environmental impacts
287 of the diet and respective Feed Conversion Ratio. Six environmental impact categories of
288 experimental diets, including global warming potential (GWP, kg CO₂ equivalent [eq.]),
289 energy use (EU, kg oil eq.), acidification (kg SO₂ eq.), eutrophication (kg P eq.), land use
290 (m² arable land [a.]) and water use (WU, m³), were calculated based on the life cycle assessment
291 database for animal feed ingredients (GFLI, 2022). These categories for black soldier fly (*H.*
292 *illucens*) were retrieved from Smetana et al. (2019). Environmental impacts were calculated as
293 follows:

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

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

299

300 2.8 Statistical analysis

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

305 The effects of the diet on composition of the pikeperch fillets were tested, by means of Kruskal–
306 Wallis non-parametric analysis, using the median test and multiple pair wise comparisons by

307 ranks. Differences were considered significant at $P < 0.05$. The data were expressed as the mean
308 \pm SD, and statistical analyses were performed using STATISTICA 12.0.

309

310 **3. Results**

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

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

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

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

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

334 The chemical composition and fatty acid profiles of the fillets of the fish fed the experimental
335 diets is reported in Table 8. Although EE remained unaffected by the treatments, the inclusion
336 of HIM significantly altered the DM, CP and ash content ($P < 0.05$). In details, DM was lower
337 in H36 than in H9 ($P < 0.05$). The CP of the fillets was improved in H9, compared to H0
338 (+2.5%) and H36 (+5.2%) ($P < 0.05$). The total replacement of FM by HIM decreased the ash
339 content, while H0, H9 and H18 did not show any correlation with this parameter.

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

343 Palmitic acid (C16:0) was the predominant SFA, with a significantly higher content in the H9
344 group than in the H36 group (Table 8). Stearic acid (C18:0) was also present at high levels, but
345 dietary insect meal inclusion showed no effect. Other SFAs made up less than 3% of the total
346 fatty acids. The total monounsaturated fatty acid (MUFA) level was not influenced by the feeds
347 with different insect meal inclusion levels. Oleic acid (C18:1n9) was the predominant MUFA
348 in all the experimental groups, but the insect meal inclusion level showed no effect. Moreover,
349 no difference was found for the total polyunsaturated fatty acids (PUFA) between the
350 experimental groups. Docosahexaenoic acid (DHA, C22:6n3) was the predominant PUFA, with
351 similar levels in the H0, H9 and H18 groups. The H36 group showed a significantly lower
352 relative content than H0 and H9 ($P = 0.001$). The second most abundant PUFA was linoleic
353 acid (C18:2n6), which showed a higher level in H36 than in the other diets. A significant
354 difference also emerged between groups for the n3:n6 ratio ($P < 0.001$) as well as for UI ($P =$
355 0.003), AI ($P = 0.002$) and TI ($P = 0.003$). On the contrary, the C18:2n6, C18:3n3, MUFA,

356 PUFA+MUFA and n6 values for the fillets were numerically lower than those of the
357 experimental insect-based feeds.

358 The effects of the insect meal inclusion level on the pikeperch diets, as observed for some
359 environmental parameters and economic aspects, are shown in Table 9. The increased inclusion
360 level of HIM increased the cost of the diet and had an adverse effect on ECR and EPI. However,
361 the inclusion of HIM progressively improved the fish-in-fish-out ratio ($P < 0.001$).
362 Environmental impacts associated with one kg pikeperch production were HIM-dose
363 dependent. Dietary HIM significantly elevated eutrophication and energy use ($P < 0.001$), while
364 acidification and land use remained comparable among the control, H9, and H18 groups ($P >$
365 0.05). At an inclusion level as low as 9%, dietary insect meal entailed similar GWP as the
366 control diet, while increasing HIM levels caused a significant burden on GWP ($P < 0.001$). It
367 is worth noting that low to moderate inclusion levels of HIM (9% and 18%) required a similar
368 amount of water to produce one kg pikeperch compared to HIM-free diet ($P > 0.05$), but the
369 higher inclusion (36%) created a higher water demand ($P < 0.001$).

370

371 **4. Discussion**

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

379

380 *4.1 Growth performance, condition factor, somatic indices, and digestibility of the diets*

381 The growth performance of juvenile pikeperch in the present study, measured as
382 specific growth rate (SGR) (range 0.76% to 0.95%/d), was comparable to the 0.77%/d in earlier
383 findings (Zakęś et al., 2008) but slightly lower than that reported previously [1.14% to 1.24 %/d
384 (Jarmołowicz et al., 2012)] and [(1.1% to 2.1%/day (Wang et al., 2009)]. The discrepancy could
385 be attributed to the different fish sizes utilised in these studies; in fact larger fish, such as those
386 utilised in our study, usually have lower SGR compared to fingerlings utilised in the other trials
387 (Wang et al., 2009; Jarmołowicz et al., 2012). A meta-analysis concerning the effects of FM
388 replacement by insect meal on the growth performance of fish conducted by Hua (2021)
389 revealed that possible inclusions up to 33% and 25% full and defatted HIM, respectively,
390 ensured a similar growth response to that of fish fed FM-based diets. Our results are consistent
391 with that finding and have confirmed that an 18% inclusion threshold (which, in our research,
392 led to 50% FM substitution) was possible for pikeperch. Previous studies that included HIM
393 also reported a threshold over the 13.2% to 40% range (or 25% to 50% FM substitution) (St-
394 Hilaire et al., 2007; Sealey et al., 2011; Renna et al., 2017; Dumas et al., 2018; Terova et al.,
395 2019) for rainbow trout (*Oncorhynchus mykiss*), whilst 14.8% to –25%, or a 100% substitution
396 level, was applied, with no adverse effects, to SGR in Atlantic salmon (Lock et al., 2016;
397 Belghit et al., 2019). Similarly, 10.6% to 14% levels, or 100% FM substitution, were found to
398 be possible for omnivorous common carp (*Cyprinus carpio*), without any negative effects on
399 SGR (Li et al., 2017; Zhou et al., 2018). Feeding Nile tilapia (*Oreochromis niloticus*) with a
400 dietary HM of 8% (Devic et al., 2018) or 30% (Muin et al., 2017) was also found to be
401 successful.

402 Increasing the dietary HIM inclusion to 36% (100% FM substitution) depressed the growth
403 performance of pikeperch, as shown by the significantly lower WG, FW and SGR in H36 than
404 in the control diet. Hua (2021) reported that the negative effect on fish growth, caused by
405 increasing levels of insect meal, could refer to a nutritional imbalance. Such a worsening of the

406 performance parameters was supported by the general decrease in the digestibility coefficients
407 recorded as the HIM inclusion increased. In addition, an increasing dietary inclusion of HIM
408 reduced essential fatty acid components, PUFA and MUFA (Table 3), which play important
409 roles in the growth and health-promoting effects of aquatic animals (Turchini et al., 2009). The
410 presence of chitin, a non-protein nitrogen, in the cuticle of insects (Henry et al., 2015), could
411 be a factor that impairs the growth rate of pikeperch fed H36. An analysis of chitin revealed a
412 content in the HIM of 5.34% as it is, leading to dietary inclusions of 0.47%, 0.97% and 1.93%
413 for H9, H18 and H36, respectively. These values are similar to the ones reported in the study
414 of Stejskal et al. (2020). Previous studies pointed out a reduction in the SGR of turbot (Kroeckel
415 et al., 2012) fed 17% HIM as a replacement of 20% FM. In contrast, feeding increasing levels
416 of HIM did not affect the SGR of European perch (Stejskal et al., 2020) or Atlantic salmon
417 (Belghit et al., 2018) fed diets containing 40% and 60% of HIM, respectively. The detrimental
418 effect of chitin on the growth performance of fed organisms could be due to the compromise of
419 protein digestibility related to its capacity to reduce the activity of proteolytic enzymes that
420 break down peptides into aminoacids or bind proteins (Henry et al., 2015; Weththasinghe et al.,
421 2021) and the induction of stress in fish (Gopalakannan and Arul, 2006). This is illustrated by
422 a decreasing condition factor (K), which is known to reflect the growth rate of fish (Mahadevan
423 et al., 2020). K is an index of the health and metabolic status of fish; the lower K value in
424 pikeperch fed H36 could possibly be the result of a synergic effect, considering that fish in this
425 group were smaller and less fatty in respect the other groups. Conversely, fish in the H18 group
426 showed a higher K value due to the different metabolism of fat as shown by the HSI an VSI
427 indices.

428 One criterion that should be considered concerning the possibility of introducing alternative
429 ingredients to FM in aquafeeds is palatability, which can influence the feed intake and other
430 physiological characteristics of fed organisms (Galkanda-Arachchige et al., 2020). HIM

431 appeared to be palatable to pikeperch as a higher feed intake was recorded for the H36 group
432 compared with HIM inclusion levels up to 18%, where a similar feed intake was recorded.
433 These results are in contrast to those observed for Jian carp (*Cyprinus carpio*) (Li et al., 2017),
434 rainbow trout (Renna et al., 2017), Japanese seabass (*Lateolabrax japonicus*) (Wang et al.,
435 2019), and European perch (Stejskal et al., 2020) where a decreased palatability was observed
436 with increasing HIM inclusion level. Interestingly, our results indicated that HIM inclusions of
437 9% and 36% did not affect the somatic indices (VSI, HSI and PFI), while HIM inclusion of
438 18% significantly reduced these parameters. In fish metabolism, the liver plays a key role and
439 HSI is often used to assess the effect of diet on liver functionality (Dernekbaşı, 2012; Chemello
440 et al., 2020). In salmonids, values between 1% and 2% are considered standard for HSI while
441 lower or higher values could indicate issues such as oxidized feed, disorders in lipid and glucose
442 metabolism, or vitamin deficiency (Pearce et al., 2003). In our study, all the fish groups
443 recorded HSI values in the range considered normal for salmonids, therefore an HIM inclusion
444 level up to 36% in pikeperch feeds could be tolerated without negative impacts on lipid and
445 glucose metabolism.

446

447 *4.2 Whole body and fillet composition*

448 No consistent trends were observed with the composition of the body of pikeperch fed
449 graded levels of HIM among the low and medium inclusion levels. However the pikeperch fed
450 the H36 diet, except for the ash content, showed a significantly different composition than other
451 groups. This pattern could be explained by considering feed nutrient digestibility, as the lower
452 body nutrient content recorded in the pikeperch fed H36, compared to the other groups, could
453 be attributed to a decline in nutrient digestibility as reported in other fish trials carried out in
454 several species fed increasing content of insect meal (Coutinho et al. 2021). Furthermore, the

455 detrimental effect of chitin on protein digestibility is well known (Henry et al., 2015; Gasco et
456 al., 2016).

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

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

469 Another pronounced trend was observed for the fish muscle saturated fatty acids, lauric and
470 myristic acids, which increased significantly with insect meal dietary inclusion. A similar
471 pattern was also reported for rainbow trout fed increasing levels of defatted HIM (Renna et al.,
472 2017). However, these differences in lauric and myristic acids seem to be too mild to alter the
473 total SFA across the fed groups. Interestingly, the considerably lower lauric acid content in the
474 fish fillets than in the feed may attributed to a prioritised energy utilisation of this FA (Renna
475 et al., 2017) in pikeperch. PUFAs are significant components of muscle lipids in pikeperch, and
476 they were found to range from 50.2% to 57.0% (Guler et al., 2007). These fatty acids were
477 found to be high in our study (55% to 57% total detectable fatty acids) and independent of the
478 administered diets. Compared to data reported for sander farmed in a different system (PUFAs,
479 34% to 44%) (Polıcar et al., 2016), the present study has shown relatively higher percentages

480 of these fatty acids. DHA and EPA are important fatty acids that play vital roles in human
481 health. DHA was found to be predominant in our study, ranging from 28% to 32% of the total
482 detected fatty acids, and was affected by dietary HIM. An HIM inclusion of 18% maintained
483 the DHA content relative similar to the FM group. The percentage of EPA instead varied by
484 4.5% to 4.9%, regardless of the dietary HIM. The DHA values are higher than those previously
485 published for pikeperch (Polcar et al., 2016; Kowalska et al., 2010). Therefore, using HIM at
486 moderate inclusion levels, in combination with a marine oil source, could be a good way of
487 enhancing the beneficial fatty acids of pikeperch for human nutrition.

488

489 *4.3 Economic analysis and environmental sustainability*

490 There is a general lack of economic analysis on insect meal inclusion in aquafeeds (Arru et al.,
491 2019; Stejskal et al., 2020). The current study has revealed that increasing inclusion levels of
492 HIM resulted in elevated ECR and reduced EPI, which is consistent with recent findings for
493 European perch (Stejskal et al., 2020). Arru et al. (2019) revealed low profitability as a result
494 of insect meal (*T. molitor*) inclusion in farmed seabass aquafeeds. This economic insufficiency
495 could mainly be due to the uncompetitive price of insect meal vs. FM (IPIFF, 2018; Arru et al.,
496 2019). Fortunately, insect meal production is increasing globally (IPIFF, 2018; Gasco et al.,
497 2020) and the price of insect meal is thus expected to be comparative with that of FM in the
498 near future (Arru et al., 2019; Hua et al., 2019). In the meantime, the marketing of seafood
499 products with socially and environmentally sustainable feed ingredients, such as insect meal,
500 could improve consumers' perceptions and their willingness to pay (Zander and Feucht, 2018).
501 Together with the economic aspects, the environmental impacts associated with aquafeeds are
502 of critical concern (Ghamkhar and Hicks, 2020). Our study has shown that dietary HIM has
503 negative impacts on the environment associated with eutrophication and energy use. On the
504 other hand, an inclusion level of up to 18% resulted in comparable acidification and land use

505 with the control diet. Our study also highlighted the benefits of using insect meal HIM in the
506 diet for pikeperch at a moderate inclusion level (18%) in terms of water resource use relative
507 to an HIM-free diet. The high variability in environmental impact indices following
508 replacement of FM by HIM could be attributed to the percentage of HIM vs. FM ingredients
509 and slight modification of wheat meal across experimental diets. Indeed, the larger impact of
510 HIM production, associated with energy use, GWP, eutrophication, and land use, than those of
511 FM, has been confirmed (Salomone et al., 2017; Smetana et al., 2019, Tran et al., 2022b).
512 Recent studies employing life cycle assessment have demonstrated that feeding arctic char
513 (*Salvelinus alpinus*) with dietary HIM also entailed a heavier environmental burden of EU than
514 insect-free diets, while multiple benefits were reported for abiotic depletion, acidification,
515 eutrophication, the global warming potential, the human toxicity potential and the marine
516 aquatic ecotoxicity potential (Smárason et al., 2017). Similar findings were reported for
517 rainbow trout fed dietary *T. molitor* (Le Feon et al., 2019).
518 Although insect meal inclusion entails more environmental impacts than improvements, Le
519 Feon et al. (2019) found a positive effect on the use of biotic resources and water. In addition
520 to water use, we also found similarities in land use among H0, H9, and H18. In other words,
521 the low to moderate inclusion level of HIM did not negatively affect the environmental impact
522 indices associated with the most limited natural resources – water and land. This phenomenon
523 could be associated with the change in wheat meal inclusion levels across experimental diets.
524 It is well acknowledged that the production of wheat meal among plant ingredients requires a
525 significantly higher amount of water and arable land than FM (GFLI, 2022; Silva et al., 2018;
526 Smetana et al., 2019). Therefore, a substantial decrease in wheat meal following FM
527 replacement by HIM to ensure nutrient balance, in combination with slightly higher water use
528 and land use from production of HIM over FM (Samuel-Fitwi et al., 2013; Smetana et al., 2019),
529 could result in comparable impacts on these natural resources among the control, H9, and H18

530 groups. Additionally, feed conversion ratio was reported to be responsible for the
531 environmental impacts of the aquaculture system (Bohnes et al., 2019) and for that associated
532 with one kg pikeperch production in the present study. As illustrated by the comparable feed
533 conversion ratio, 3 diets, H0, H9, and H18, were efficiently utilized by pikeperch (Tran et al.,
534 2021). However, despite a gradual decrease in wheat meal, a significantly higher feed
535 conversion ratio following 100% replacement FM with HIM did not improve environmental
536 impacts on pikeperch production. It is apparent that although an FM-free diet with the addition
537 of HIM did not benefit pikeperch aquaculture in terms of either production performance or
538 environmental consequences, elimination of FM originated from marine resources in aquafeed
539 could be beneficial for the marine ecosystem as indicated by FIFO. In the present study,
540 replacement of FM by HIM significantly improved the FIFO as less marine fish forage was
541 required to produce the live weight of farmed fish (Tacon and Metian, 2008; Naylor et al.,
542 2009). The same result has been reported for European perch (Stejskal et al., 2020) and for
543 Siberian sturgeon (Rawski et al., 2021). We found that FIFO could be decreased by 40.1% in
544 pikeperch fed an insect-based diet, without affecting the growth performance (group H18).
545 From a global perspective, an increasing use of fish by-products and other FM alternatives
546 could be a strategic way of ensuring the environmental sustainability of the aquaculture industry
547 (Hua et al., 2019; Cottrell et al., 2020; Gasco et al., 2020), thereby reducing FM, and the fish
548 oil proportion in aquafeeds. Consequently, the global FIFO is expected to reduce considerably
549 in the coming decades (Kok et al., 2020). Since aquaculture is increasingly dependent on
550 terrestrial crops and forage fish as feed inputs, and thereby damaging to aquatic ecosystems and
551 fisheries (Smith et al., 2011; Troell et al., 2014), the use of insect meal could provide a
552 promising alternative to tackle the growth of aquaculture in an era that has limited natural and
553 marine fishery resources.

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

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

570 **5. Conclusion**

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

577 **Author contributions**

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

587 **Declaration of competing interests**

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

590 **Acknowledgements**

591 This study was supported financially by the Ministry of Agriculture of the Czech Republic and
592 by the NAZV project (grant number QK1810296). This JU-IAPW research paper is part of a
593 project that has received funding from the European Union's Horizon 2020 research and
594 innovation programme (grant agreement No 652831 [AQUAEXCEL²⁰²⁰]), the TNA project ID
595 number: AE070026. This output reflects only the author's view and the European Union cannot
596 be held responsible for any use that may be made of the information contained therein.

597

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- 920

921 **Table 1. Ingredients and proximate composition (% , as fed) of the HIM and of the**
 922 **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 |

923 HIM = defatted *Hermetia illucens* larva meal; DM = dry matter; CP = crude protein; EE = ether

924 extract; NFE = nitrogen free extracts.

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

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

927 ³Vitamin mixture (IU or mg/kg diet): DL- α tocopherol acetate, 60 IU; sodium menadione
 928 bisulphate, 5 mg; retinyl acetate, 15,000 IU; DL-cholecalciferol, 3,000 IU; thiamin, 15 mg;
 929 riboflavin, 30 mg; pyridoxine, 15 mg; B₁₂, 0.05 mg; nicotinic acid, 175 mg; folic acid, 500 mg;
 930 inositol, 1,000 mg; biotin, 2.5 mg; calcium panthotenatate, 50 mg (purchased from Granda
 931 Zootechnici S.r.l., Cuneo, Italy).

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

934 124 g; iron sulphate, 20 g; zinc sulphate, 4 g; copper sulphate, 3 g; potassium iodide, 4 mg;
935 cobalt sulphate, 20 mg; manganese sulphate, 3 g; sodium fluoride, 1 g (purchased from Granda
936 Zootecnici S.r.l., Cuneo, Italy).

937 ⁵Celite, a source of acid-insoluble ash.

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

939 ⁷Estimated as ADF – ADFN.

940 ⁸Calculated as $100 - (\text{CP} + \text{EE} + \text{Ash} + \text{Chitin})$.

941 ⁹Determined by means of a calorimetric bomb.

942

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943 **Table 2. Amino acid content (% of protein) of the fishmeal, defatted black soldier fly**
 944 ***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 |

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

946 ¹H0, H9, H18, H36 represent experimental diets where fishmeal was replaced by HIM at 0%,

947 25%, 50% and 100%, respectively.

948 **Table 3. Fatty acid composition (% of the total fatty acids) of fishmeal, defatted black**
 949 **soldier fly (*Hemeticia 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 |

950 FM = herring fish meal; HIM = defatted black soldier fly (*Hemeticia illucens*); nd = traces, <
 951 0.05%; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA =
 952 polyunsaturated fatty acids.

953 ¹H0, H9, H18, H36 represent experimental diets where fishmeal was replaced by HIM at 0%,
 954 25%, 50% and 100%, respectively.

955

956 **Table 4. Survival and growth performances of the pikeperch fed the experimental diets**
 957 **(mean \pm standard deviation).**

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

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

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

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

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

966

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

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

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

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

972 ²Fulton's condition factor (K) = [Final body weight (g)/Final body length (mm)³] \times 100.

973 ³Hepatosomatic index (HSI) = 100 \times Liver weight (g)/Fish weight (g).

974 ⁴Viscerosomatic index (VSI) = 100 \times Viscera weight (g)/Fish weight (g).

975 ⁵Perivisceral fat index (PFI) = 100 \times Perivisceral fat weight (g)/Fish weight (g).

976 ⁶Fillet yield (FY) = 100 \times Fillet weight (g)/BW.

977

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

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

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

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

983 ¹H0, H9, H18, H36 represent experimental diets where fishmeal was replaced by defatted black
 984 soldier fly (*Hemeticia illucens*) at 0%, 25%, 50% and 100%, respectively.
 985

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

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

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

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

990 ¹H0, H9, H18, H36 represent experimental diets where fishmeal was replaced by defatted black
 991 soldier fly (*Hemeticia illucens*) at 0%, 25%, 50% and 100%, respectively.
 992

993

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

| Item | Experimental diets ¹ | | | | P-value |
|-----------------------|---------------------------------|--------------------------|----------------------------|---------------------------|---------|
| | 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 |

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

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

999 ¹H0, H9, H18, H36 represent experimental diets where fishmeal was replaced by defatted black
 1000 soldier fly (*Hermetia illucens*) at 0%, 25%, 50% and 100%, respectively.

1001 ²Unsaturation index (UI) = 1 × (% monoenoics) + 2 × (% dienoics) + 3 × (% trienoics) + 4 ×
1002 (% tetraenoics) + 5 × (% pentaenoics) + 6 × (% hexaenoics).

1003 ³Atherogenicity index (AI) = [C12:0 + (4 × C14:0) + C16:0]/ΣUnsaturated fatty acids.

1004 ⁴Thrombogenicity index (TI) = (C14:0 + C16:0 + C18:0)/[(0.5×ΣMUFA) + (0.5 × Σn6 PUFA)
1005 + (3 × Σn3 PUFA) + (n3/n6)].

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1009 **Table 9 Economic and environmental sustainability parameters of pikeperch fed the**
 1010 **experimental diets (mean \pm 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 \pm 0.06 ^c | 1.50 \pm 0.08 ^{bc} | 1.75 \pm 0.04 ^b | 3.17 \pm 0.27 ^a | <0.001 |
| EPI ² , €/fish | 1.06 \pm 0.02 ^a | 1.03 \pm 0.02 ^a | 1.00 \pm 0.03 ^a | 0.81 \pm 0.03 ^b | <0.001 |
| FIFO ² | 1.66 \pm 0.08 ^a | 1.33 \pm 0.07 ^b | 0.98 \pm 0.02 ^c | 0.40 \pm 0.03 ^d | <0.001 |
| Environmental impacts associated with 1 kg pikeperch production | | | | | |
| GWP, kg CO ₂ eq. | 2.59 \pm 0.13 ^c | 3.1 \pm 0.17 ^{bc} | 3.6 \pm 0.09 ^b | 6.45 \pm 0.54 ^a | <0.001 |
| Acidification, kg SO ₂ eq. | 11.67 \pm 0.58 ^b | 12.96 \pm 0.71 ^b | 14.24 \pm 0.36 ^b | 23.42 \pm 1.96 ^a | <0.001 |
| Eutrophication, kg P eq. | 0.26 \pm 0.01 ^d | 0.98 \pm 0.05 ^c | 1.71 \pm 0.04 ^b | 4.44 \pm 0.37 ^a | <0.001 |
| Land use, m ² a | 2.11 \pm 0.11 ^b | 2.23 \pm 0.12 ^b | 2.35 \pm 0.06 ^b | 3.61 \pm 0.3 ^a | <0.001 |
| Energy use, kg oil eq. | 0.34 \pm 0.02 ^d | 0.53 \pm 0.03 ^c | 0.73 \pm 0.02 ^b | 1.58 \pm 0.13 ^a | <0.001 |
| Water use, m ³ | 0.036 \pm 0.002 ^b | 0.036 \pm 0.002 ^b | 0.036 \pm 0.001 ^b | 0.051 \pm 0.004 ^a | <0.001 |

1011 GWP = global warming potential; eq. = equivalent.

1012 ^{a-d}Different letters within a row indicate significant differences ($P < 0.05$).

1013 ¹H0, H9, H18, H36 represent experimental diets where fishmeal was replaced by defatted black
 1014 soldier fly (*Hemeta illucens*) at 0%, 25%, 50% and 100%, respectively.

1015 ²ECR = Feed conversion ratio \times D_P;

1016 EPI = (Weight gain \times S_P) – (Weight gain \times D_P);

1017 FIFO = (L_{FM} + L_{FO})/(Y_{FMw} + Y_{FOw}) \times Feed conversion ratio;

1018 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

1019 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

1020 fish; Y_{FOw} is the fish oil yield from wild fish.

1021