

Alma Mater Studiorum Università di Bologna Archivio istituzionale della ricerca

First insights on Black Soldier Fly (Hermetia illucens L.) larvae meal dietary administration in Siberian sturgeon (Acipenser baerii Brandt) juveniles

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:

First insights on Black Soldier Fly (Hermetia illucens L.) larvae meal dietary administration in Siberian sturgeon (Acipenser baerii Brandt) juveniles / Caimi C.; Renna M.; Lussiana C.; Bonaldo A.; Gariglio M.; Meneguz M.; Dabbou S.; Schiavone A.; Gai F.; Elia A.C.; Prearo M.; Gasco L.. - In: AQUACULTURE. - ISSN 0044-8486. - STAMPA. - 515:(2020), pp. 734539.1-734539.10. [10.1016/j.aquaculture.2019.734539]

Availability:

This version is available at: https://hdl.handle.net/11585/741322 since: 2020-02-29

Published:

DOI: http://doi.org/10.1016/j.aquaculture.2019.734539

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/). When citing, please refer to the published version.

(Article begins on next page)

This is the final peer-reviewed accepted manuscript of:

Caimi, Christian, et al. «First Insights on Black Soldier Fly (Hermetia Illucens L.) Larvae Meal Dietary Administration in Siberian Sturgeon (Acipenser Baerii Brandt) Juveniles». Aquaculture, vol. 515, gennaio 2020, p. 734539.

The final published version is available online at: https://doi.org/10.1016/j.aquaculture.2019.734539

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/)

When citing, please refer to the published version.

- 1 First insights on Black Soldier Fly (Hermetia illucens L.) larvae meal
- 2 dietary administration in Siberian sturgeon (Acipenser baerii Brandt)
- **juveniles**

- 5 Christian Caimi^a, Manuela Renna^b, Carola Lussiana^a, Alessio Bonaldo^c, Marta Gariglio^b, Marco
- 6 Meneguz^a, Sihem Dabbou^b, Achille Schiavone^{b,d}, Francesco Gai^{d*}, Antonia Concetta Elia^e, Marino
- 7 Prearo^f, Laura Gasco^a
- 9 ^aDepartment of Agricultural, Forest and Food Sciences, University of Turin, Largo P. Braccini 2,
- 10 10095 Grugliasco (TO), Italy.
- bDepartment of Veterinary Science, University of Turin, Largo P. Braccini 2, 10095 Grugliasco (TO),
- 12 Italy.
- ^cDepartment of Veterinary Medical Sciences, University of Bologna, Via Tolara di Sopra 50, 40064,
- 14 Ozzano Emilia (BO), Italy.
- dinstitute of Science of Food Production, National Research Council, Largo P. Braccini 2, 10095
- 16 Grugliasco (TO), Italy.
- 17 eDepartment of Chemistry, Biology and Biotechnology, University of Perugia, Via Elce di Sotto 8,
- 18 06123 Perugia, Italy
- 19 ^fVeterinary Medical Research Institute for Piedmont, Liguria and Aosta Valley, Via Bologna 148,
- 20 10154 Torino, Italy

- * Corresponding author: Dr. Francesco Gai, Institute of Science of Food Production, National
- Research Council, Largo P. Braccini 2, 10095 Grugliasco (TO), Italy Tel. +39.011.6709232 Fax:
- 24 +39.011.6709297 Email: francesco.gai@ispa.cnr.it

- 26 E-mail addresses: christian.caimi@unito.it; manuela.renna@unito.it; carola.lussiana@unito.it;
- 27 alessio.bonaldo@unibo.it; marta.gariglio@unito.it; marco.meneguz@unito.it;
- 28 sihem.dabbou@unito.it; achille.schiavone@unito.it; francesco.gai@ispa.cnr.it;
- antonia.elia@unipg.it; marino.prearo@izsto.it; laura.gasco@unito.it.

Abstract

The *Hermetia illucens* (L.) larvae meal (HIM) has been tested on different fish species but its use on Siberian sturgeon has not been investigated yet. The aim of this study was to evaluate the effects of fish meal (FM) substitution with a highly defatted HIM on growth performance, biometric and morphometric indices, apparent digestibility of diets, whole body proximate and fatty acid compositions of *Acipenser baerii* (Brandt) juveniles. Five experimental groups were fed with a FM-based diet without HIM (HIMO), three diets with 25% (HIM25), 50% (HIM50) and 100% (HIM100) of FM substitution with HIM, or a vegetable protein based diet (VEG) without HIM. The feeding trial lasted 118 days and 4 replicates per diet were used. The HIM100 diet was refused by the fish and therefore this experimental group was excluded from statistical evaluation. Moreover, a decrease in feed consumption was recorded with the increase of HIM inclusion. The HIM50 diet negatively affected the growth performance of the fish. The somatic indices were not affected by treatment. Increasing levels of HIM showed increases of dry matter and ether extract contents in the whole body. Compared to HIM0, HIM diets caused modification in lauric acid (up to 65-fold increase) and total saturated fatty acids (up to 1.4-fold increase) contents in the fish whole body.

The apparent digestibility coefficients of dry matter, crude protein and gross energy were the highest for the VEG diet (77.0%, 90.4% and 85.8%, respectively). HIM25 and HIM50 showed lower apparent digestibility coefficients of crude protein (86.5% and 86.6%, respectively) when compared to HIM0 (88.5%). Overall, this study showed that it is possible to replace up to 25% of FM with HIM in the diet of Siberian sturgeons (equal to 18.5% HIM inclusion level) without affecting the growth performance, condition factor, biometric and morphometric indices, and whole body proximate composition of the fish. The fatty acid composition of the sturgeons' whole body was significantly modified already at 18.5% dietary HIM inclusion level.

Keywords: Insect meal; Fishmeal substitution; Animal performance; Fatty acid profile; Apparent digestibility coefficient.

Abbreviations: AA, amino acid; ADC, apparent digestibility coefficient; ADF, acid detergent fibre; ADL, acid detergent lignin; ANFs, antinutritional factors; BCFA, branched chain fatty acids; *c, cis*; CP, crude protein; DM, dry matter; EE, ether extract; FA, fatty acid; FAME, fatty acid methyl ester; FBW, final body weight; FC, feed consumption; FCR, feed conversion ratio; FM, fish meal; FR, feeding rate; GE, gross energy; HI, Hermetia illucens; HIM, Hermetia illucens larvae meal; HSI, hepatosomatic index; IBW, initial body weight; K, Fulton's condition factor; MCFA, medium chain fatty acids; MUFA, monounsaturated fatty acids; nd, not detected; NFE, Nitrogen free extracts; PAPs, processed animal proteins; PER, protein efficiency ratio; PUFA, polyunsaturated fatty acids; SEM, standard error of the mean; SFA, saturated fatty acids; SGR, specific growth rate; *t, trans*; TFA, total fatty acids; VEG, vegetable protein based diet; VSI, viscerosomatic index; WG, weight gain.

1. Introduction

70

71

72

73

74

75

76

77

78

79

80

81

82

83

84

85

86

87

88

89

90

91

92

93

By 2050, the demographic growth will result in an increase of 60% in the consumption of products of animal origin and aquaculture is considered as one of the livestock sectors able to support the global demand of animal products (Engle et al., 2017). Between 2001 and 2016, aquaculture production grew about 5.8% per year and such trend is set to grow (FAO, 2018). The intensification of production has led to an increase in the demand for raw materials, especially protein sources, to produce aquafeeds. For many years, fishmeal (FM) represented the main protein source in the production of feed for carnivorous farmed fish. However, in the next few years FM production will not be able to support aquaculture growth anymore (Gasco et al., 2018). To reduce both ecological impact and feed costs, at commercial level FM is partially replaced by vegetable protein meals (Soliman et al., 2017). However, plant feedstuffs raised some nutritional issues that reduce their potential in fish feed formulation (Soliman et al., 2017). The technological advances in plant ingredient processing and the feed extrusion process have solved most of these problems (Merrifield et al., 2011; Gai et al., 2016), but the formulation of diets containing 100% vegetable proteins still causes performance and health issues in carnivorous species where FM can hardly be totally replaced by vegetable proteins (Gai et al., 2012). Globally, terrestrial processed animal proteins (PAPs) are largely used in aquaculture. However, under European regulations only PAPs from non-ruminant animals (poultry and pigs - category 3) are allowed in fish feed formulation (EC No 56/2013). Alternative protein ingredients of animal origins, such as insect meals, may be an alternative solution to overcome this problem and replace, at least partly, the amount of FM in aquafeeds (Henry et al., 2015). Insect meals are rich in essential aminoacids (in particular lysine, methionine and leucine), have a fatty acid (FA) profile that can be manipulated choosing appropriate rearing substrates (Liland et al., 2017; Meneguz et al., 2018) for insects, and do not have any antinutritional factors (Spranghers et al., 2017). One of the most promising insect species to partially replace FM is the Black Soldier Fly (Hermetia illucens L. - HI; Stratiomyidae family). HI is able to convert low value by-products and wastes into protein and fat sources (Spranghers et al., 2017; Meneguz et al., 2018) representing a sustainable way to produce edible proteins for livestock feeding. The partial substitution of FM with HI larvae meal (HIM) has been successfully tested in various fish species including rainbow trout (Oncorhynchus mykiss Walbaum) (Renna et al., 2017; Elia et al., 2018; Huyben et al., 2018), Atlantic salmon (Salmo salar L.) (Lock et al., 2016; Belghit et al., 2018a), European seabass (Dicentrarchus labrax L.) (Magalhães et al., 2017), yellow catfish (Pelreobagrus fulvidraco Richardson) fry (Xiao et al., 2018), Jian carp (Cyprinus carpio var. Jian) (Li et al., 2017; Zhou et al., 2018), and Nile tilapia (Oreochromis niloticus L.) (Devic et al., 2018). The production of caviar and sturgeon's meat is a financially relevant aquaculture sector. Compared to other sturgeon's species, Siberian sturgeon (Acipenser baerii Brandt) has a short reproductive cycle (7 to 8 years) and can be reared in fresh water (Bronzi et al., 2011). For these reasons, Siberian sturgeon is one of the most commonly farmed sturgeons (Sener et al., 2006; Bronzi et al., 2011). Compared to other species (e.g., Atlantic salmon, rainbow trout, and European sea bass), less attention has been paid to the nutrition requirements of sturgeons. However, the research of FM substitutes is a crucial point for the future of sturgeon rearing and production (Sicuro et al., 2015). In the last ten years, several authors have evaluated the use of vegetable ingredients other than soybean meal (Liu et al., 2009), like spirulina microalgae (Palmegiano et al., 2008), rice concentrate (Sicuro et al., 2015) and sesame oil cake or corn gluten (Jahanbakhshi et al., 2013) or animal proteins such as meat and bone, poultry by-products or feather meals (Liu et al., 2009; Zhu et al., 2011) as FM substitutes in sturgeon's diets concluding that a partial FM replacement with these alternative protein sources is feasible without adverse effects.

94

95

96

97

98

99

100

101

102

103

104

105

106

107

108

109

110

111

112

113

114

115

So far, the use of HIM in sturgeon's feeds has not been investigated. Therefore, the purpose of the present study is to assess the effects of the dietary inclusion of *H. illucens* larvae meal as FM replacer on growth performance, biometric and morphometric indices, whole body composition (proximate constituents and FA profile) and apparent digestibility of diets of *A. baerii* juveniles.

2. Materials and methods

A growth trial and a digestibility trial were conducted at the experimental facility of the Department of Agricultural, Forest and Food Sciences (DISAFA) of the University of Torino (Italy). The experiment was designed according to the guidelines of the current European Directive (2010/63/EU) on the protection of animals used for scientific purposes. The experimental protocol was approved by the Ethical Committee of the University of Turin (Italy) (protocol N° 143811).

2.1 Experimental diets

A highly defatted HIM (ether extract – EE: 4.03 g 100g⁻¹, as fed) purchased from Hermetia Deutschland GmbH & Co. KG (Baruth/Mark, Germany) was used in the trials. The HIM was defatted with a mechanical method; no solvent was used for this purpose. Five experimental diets were formulated to be isonitrogenous (crude protein – CP: on average 50.5 g 100g⁻¹, on as fed basis), isolipidic (EE: on average 12.4 g 100g⁻¹) and isoenergetic (gross energy – GE: on average 20.84 MJ kg⁻¹). The five experimental diets were the following: (i) a FM based diet (HIMO), without HIM inclusion; (ii, iii, iv) three diets with an inclusion, on as fed basis, of 18.5% (HIM25), 37.5% (HIM50) and 75.0% (HIM100) of HIM, with the purpose to replace the 25%, 50% and 100% of FM in the HIMO diet, respectively; and (v) a vegetable protein based diet (VEG), without HIM inclusion, formulated to replicate currently available commercial feeds. Due to the different chemical composition of HIM compared to FM, and in order to maintain diets isonitrogenous, isolipidic and

isoenergetic, the amounts of some other dietary ingredients (i.e. wheat meal and fish oil) were modified with the increase of HIM inclusion in the diets.

The experimental diets were prepared at the experimental facility of DISAFA. The ground ingredients were individually weighed and subsequently mixed with fish oil. From 250 to 500 mL kg⁻¹ of water was added to promote greater malleability and to obtain a suitable compound for

50°C for 48 h. The diets were stored in dark bags at -20°C until utilization. The ingredients of the

pellet preparation. The pellets were obtained using a 3.0 mm die meat grinder and were dried at

experimental diets are listed in Table 1.

2.2 Fish feeding and management

A 118 day growth trial was carried out using 20 square fibreglass tanks of 400 L supplied by artesian well water (constant temperature of $13 \pm 1^{\circ}$ C) in an open system (flow-through) with each tank having a water inflow of 8 L min⁻¹. Dissolved oxygen was measured every week and ranged between 8.5 and 9.3 mg L⁻¹ while water pH was equal to 7.6. The fish were exposed to natural photoperiod (April to July 2016). *A. baerii* juveniles were purchased from a private sturgeon farm (Cisliano (MI), Italy), transported to the experimental facility of DISAFA and acclimated to the rearing conditions for 2 weeks. After the acclimatization period, 440 fish were lightly anesthetised (MS-222 100 mg L⁻¹; PHARMAQ Ltd, Fordingbridge, UK), individually weighed (mean individual initial body weight - iIBW: 24.2 \pm 7.59 g) and randomly distributed to each tank (22 fish per tank). The experimental diets were randomly assigned to the tanks (four replicate tanks per diet). The fish were fed by hand to apparent visual satiation, three times a day (8:00, 14:00 and 20:00 h), six days per week. Not ingested feed was siphoned, dried and weighed to record the actual feed consumption (FC) per tank. The fish were weighed individually at the end of the trial (individual final body weight – iFBW). Mortality was checked every day.

2.3 Sampling

At the end of the growth trial, after 24 h of fasting, 6 fish per tank (24 fish per treatment) were 167 sacrificed by over anaesthesia (MS-222, 300 mg L⁻¹), individually weighed (KERN PLE-N v. 2.2; KERN 168 169 & Sohn GmbH, Balingen-Frommern, Germany; d: 0.001), photographed orthogonally (Lumix G1; 170 Panasonic Corp., Kadoma, Osaka, Japan) with a metric scale (mm) and measured to record fish 171 length (i.e. from mouthpart to the bottom of the caudal fin) with 1 mm accuracy. Data were 172 elaborated using ImageJ software (ImageJ 1.50b, Wayne Rasband, Public domain, National Institute of Health, USA) for the calculation of the Fulton's condition factor (K). Subsequently, 3 of 173 the 6 fish sampled (12 fish per treatment) were frozen, finely ground with a knife mill (Grindomix 174 175 GM200; Retsch GmbH, Haan, Germany), freeze-dried and stored (-80°C) for final whole body 176 proximate composition and FA profile analyses. The remaining 3 fish (12 fish per treatment) were eviscerated; the liver and viscera were weighed to calculate the hepatosomatic (HSI) and 177 178 viscerosomatic (VSI) indices, respectively.

179

- 2.4 Data calculation
- 181 The growth performance indices were calculated as follows:
- Survival rate (SR, %) = (number of final fish / number of fish at start) × 100;
- Weight gain (WG, g) = iFBW (g) − iIBW (g);
- Specific growth rate (SGR, % day⁻¹) = [(InFBW InIBW) / number of days] \times 100;
- Feed conversion ratio (FCR) = total feed supplied (g, dry matter (DM)) / tank WG (g);
- Protein efficiency ratio (PER) = tank WG (g) / total protein fed (g, DM).
- 187 Individual initial (iIBW) and final body (iFBW) weight were used to calculate the WG while SGR,
- 188 FCR and PER were calculated per tank.

- 189 The Fulton's condition factor and somatic indices were calculated as follows:
- 190 $K = [fish weight (g) / (body length)^3 (cm)] \times 100;$
- HSI (%) = [liver weight (g) / fish weight (g)] × 100;
- VSI (%) = [viscera weight (g) / fish weight (g)] \times 100.

194

195

196

197

198

199

200

201

202

203

204

205

206

207

208

209

2.5 Diets digestibility

At the end of the growth trial, the remaining fish (on average 16 per tank) were used for the digestibility trial. The fish were maintained in their respective tanks and fed the same experimental diets added with 1% celite® (Fluka, St. Gallen, Switzerland) as inert marker. The diets were prepared as described in section 2.1, and the inert marker was added at the expense of 1% of starch gelatinized. Feed was administered by hand to apparent visual satiation three times per day (8:00, 14:00 and 20:00 h), six days per week. After the first feed administration, all the fish were placed in a metal cage that was perforated on the bottom side, and placed on a rigid plastic tray. In this way, the faeces were settled in the plastic tray, allowing their sampling. As described in Guo et al. (2012), four hours after the meal, the faeces were collected using a suction pipe, placed on blotting paper to remove the excess water and immediately frozen (-80°C) until analysed. After the first faeces collection, the fish were released in their tanks and the same procedure was performed after the second feed administration. To avoid digestibility overestimation, only intact faeces were collected, and faeces collection lasted 20 days. The apparent digestibility coefficients (ADC) of DM, CP and GE were calculated as reported by Renna et al. (2017) and expressed as a percentage.

210

211

2.6 Chemical analyses of dietary ingredients, diets and fish whole body

The chemical analyses of the dietary ingredients were performed before diet formulation. Feed samples were ground using a cutting mill (MLI 204; Bühler AG, Uzwil, Switzerland) and analysed for DM (#934.01), CP (#984.13) and ash (#942.05) contents according to AOAC International (2000); EE (#2003.05) was analysed according to AOAC International (2003). The GE content was determined using an adiabatic calorimetric bomb (C7000; IKA, Staufen, Germany). Chitin was estimated according to Finke (2007) by correction considering the amino acid (AA) content of the acid detergent fibre (ADF) fraction and assuming the remainder of the ADF fraction is chitin. The FA composition of HIM and of the experimental diets was assessed as described by Renna et al. (2017). Fatty acid methyl esters (FAME) were separated, identified and quantified on the basis of the chromatographic conditions reported by Renna et al. (2014). The results were expressed as $mg 100 g^{-1} DM (Table 2).$ The proximate composition of the fish whole body was determined according to the same procedures implemented for feed analyses (AOAC International, 2000; 2003). The freeze-dried and ground samples of the fish whole bodies were also used to assess their FA composition, as reported by Renna et al. (2017). FAME were separated using the same analytical instruments and temperature program previously described for the FA analysis of feeds. Peaks were identified by injecting pure FAME standards as detailed by Renna et al. (2012). The results were expressed as

230

231

232

229

212

213

214

215

216

217

218

219

220

221

222

223

224

225

226

227

228

2.7 Statistical analyses

Data were analysed by one-way ANOVA using IBM SPSS Statistics v. 25.0 for Windows. The

mg 100 g⁻¹ ww. All chemical analyses were performed in duplicate.

- following model was used: $Y_{ij} = \mu + D_i + \epsilon_{ij}$
- where Y_{ij} = observation; μ = overall mean; D_i = effect of diet (HIMO, HIM25, HIM50, VEG); ϵ_{ij} =
- 235 residual error.

The Kolmogorov–Smirnov test was used to check the assumption of normality. The assumption of equal variances was assessed by Levene's homogeneity of variance test. If such an assumption did not hold, the Brown-Forsythe statistic was applied to test the equality of group means instead of the F one. Pairwise multiple comparisons were performed to test the difference between each pair of means (Tukey's test and Tamhane's T2 in the cases of equal variances assumed or not assumed, respectively). The results were expressed as mean and pooled standard error of the mean (SEM). Significance was declared at $p \le 0.05$.

243

244

245

236

237

238

239

240

241

242

3. Results

3.1 Chemical composition of insect meal and experimental diets

246 The proximate composition of HIM and of the experimental diets are reported in Table 1. The DM, 247 CP and EE contents were comparable among the diets. The ash content showed a reduction at the increase of HIM inclusion in the diet (13.15 to 7.90 g 100g⁻¹ in HIMO and HIM100, respectively), 248 while the chitin content increased at increasing inclusion levels of HIM. 249 The FA composition of HIM and of the experimental diets is shown in Table 2. The most 250 represented FA in HIM was lauric acid (C12:0), followed by palmitic (C16:0), myristic (C14:0) and 251 oleic (C18:1 c9) acids (2020.65, 435.36, 325.51 and 303.20 mg 100g-1 DM, respectively). In the 252 experimental diets containing HIM, lauric acid increased proportionally at the inclusion of insect 253 254 meal. The concentrations of C12:0 in the diets containing HIM were noticeably higher than those 255 observed in HIMO and VEG. When compared to the other diets, the HIMO diet showed a higher amount of total n3 FA (1348.15 mg 100g⁻¹ DM), with C22:6 n3 as the most represented individual 256 257 FA in this group (666.59 mg 100g⁻¹ DM). The total n3 polyunsaturated fatty acids (PUFA) decreased 258 proportionally at increasing levels of HIM. The VEG diet showed a lower concentration of total 259 saturated fatty acids (SFA) and was richer in total n6 PUFA (in particular linoleic acid, C18:2 n6)

when compared to the other diets. HIMO showed the highest Σ n3 / Σ n6 PUFA ratio among the considered experimental diets.

3.2 Growth trial

The fish fed HIM100 showed a low diet acceptance, rapidly decreased the voluntary ingestion of feed and, few weeks after the beginning of the trial, they refused the diet. Therefore, to avoid stress or suffering in the animals, we decided to stop this dietary treatment. These fish were fed again with a commercial diet and excluded from further investigations.

The survival and growth performance of the fish are reported in Table 3. Siberian sturgeons fed

The survival and growth performance of the fish are reported in Table 3. Siberian sturgeons fed the HIMO, HIM25, HIM50, and VEG diets easily accepted the experimental diets. Nevertheless, a decrease in feed consumption (FC) was recorded in diets added with HIM (-5.3% for HIM25 and -6.0% for HIM50). This led to a worsening of FBW (-10.9%), WG (-12.9%) and SGR (-6.9%) in the HIM50 group compared to HIMO. Nevertheless, FCR and PER were similar among the treatments. The fish fed the VEG diet reported values comparable to the fish fed the control diet for all the

3.3 Biometric and morphometric indices

considered dependent variables.

The Fulton's condition factor, HSI and VSI are reported in Table 4. All the biometric and morphometric indices were not significantly affected by treatment.

3.4 Proximate composition and fatty acid profile of fish whole body

The proximate composition and FA profile of the fish whole body are reported in Table 5. The substitution of FM by HIM progressively increased the DM and EE contents of the fish. Particularly, the fish fed with HIM50 showed a significantly higher DM (+10.0%) content when compared to

those fed with HIMO and VEG, and a significantly higher EE (+38.4%) content when compared to those fed with HIMO. The DM and EE contents were comparable among HIMO, HIM25 and VEG.

The CP and ash contents were unaffected by the treatment.

As far as the FA composition is concerned, the concentration of C12:0 significantly increased (from 2.70 to 61.23 and 151.61 mg 100 g⁻¹ ww in HIM0, HIM25 and HIM50, respectively) as the level of HIM increased in the diet, while VEG and HIMO showed comparable values. The fish fed with HIM50 showed a higher concentration of C14:0 when compared to the fish fed with HIM0 and VEG (+47.9% in both cases), while the fish fed with HIM25 showed intermediate values. Various branched chain fatty acids (BCFA) were detected, but only a few of them (C17 anteiso and C18 iso) were affected by the diet. The concentration of total BCFA in the fish whole body did not significantly differ among the treatments. The concentration of C18:1 c9 was significantly higher in the fish fed with HIM50 than in those fed with HIM0 (869.27 and 600.58 mg 100g⁻¹ww, respectively), while the fish fed with HIM25 and VEG showed intermediate values. The concentrations of C18:2 n6, C18:3 n6, and total n6 PUFA were the highest in the fish fed with VEG, followed by the fish fed with HIM50 and HIM25, and finally by those fed with HIM0. As regard to individual long-chain n3 PUFA, the absolute lowest values were recorded in the fish fed with VEG (C20:5 n3) and HIMO (C22:5 n3 and C22:6 n3), while the absolute highest values were observed in the fish fed with HIM25. The Σ n3 / Σ n6 FA ratio in fish whole body ranked in the order HIM0 = HIM25 > HIM50 > VEG.

303

304

302

284

285

286

287

288

289

290

291

292

293

294

295

296

297

298

299

300

301

3.5 Digestibility trial

The ADCs of the experimental diets are reported in Table 6. The VEG diet showed significantly higher values than all the other diets for all the considered ADCs. ADC_{DM} and ADC_{GE} did not differ

significantly among HIM0, HIM25 and HIM50, while ADC $_{CP}$ was significantly lower in HIM25 (-2.3%) and HIM50 (-2.1%) when compared to HIM0.

309

310

311

312

313

314

315

316

317

318

319

320

321

322

323

324

325

326

327

328

329

330

308

307

4. Discussion

Insects, such as Diptera and Coleoptera, are part of the natural diet of Siberian sturgeons (Pyka and Kolman, 2003). To our knowledge no studies using HIM as protein source have been carried out with sturgeons yet, and our results indicate that up to 18.5% of highly defatted HIM can be included without impairing survival rate and growth performance in this species. Nevertheless, WG and SGR decreased with the increase of HIM in the diets as a consequence of a decrease in feed consumption. At 75% of HIM inclusion (total FM substitution - HI100), we observed a noticeable decrease in feed ingestion by the sturgeons. The decrease in feed consumption recorded with the increase of HIM could be attributable to different causes. One factor could be the chitin content of the diets. It has been reported how even low levels of chitin could decrease feed consumption in fish (Gopalakannan and Arul, 2006; Olsen et al., 2006; Kroeckel et al., 2012). In particular, when feeding turbot juveniles (Psetta maxima) with diets containing insect meals, Kroeckel et al. (2012) faced a decrease of feed acceptance at increasing levels of insect meal, and the diet with 75.6% of inclusion of HI prepupae meal showed the lowest daily feed intake and performances. These authors argued that the dietary level of chitin could have been one of the reasons due to the fact that turbot is a fish without gut chitinase activity. Chitin, an indigestible polysaccharide, is a primary component of the exoskeleton of arthropods (such as insects and shrimps). In insects, the amount of chitin varies according to the species and the development stage (Finke, 2007). In the current trial, following the method proposed by Finke (2007), the chitin contents of the experimental diets were equal to 0 (HIMO and VEG), 0.72 (HIM25), 1.92 (HIM50) and 3.75 (HIM100) g 100g-1 as fed. Not all studies performed using insect meals reported

palatability problems (Lock et al., 2016; Magalhães et al., 2017; Renna et al., 2017; Belghit et al., 2018a; Devic et al., 2018). It could be argued that these inconsistent results are due to differences in meal composition and processing (as already reported for other PAPs) or as a consequence of the presence/absence of endogenous chitinases in the investigated fish species such as Atlantic salmon, European seabass, rainbow trout and Nile tilapia. Another cause could be the excessive hardness of the pellet observed in the production phase, particularly in the experimental diet where HIM completely substituted FM (HIM100). In white sturgeons (A. transmontanus Richardson) larvae, Gawlica et al. (2002) reported that the physical texture of the diet significantly affected feed intake, with negative effects being associated with dry and hard diets. The absolute lowest FC recorded in the fish fed with HIM50 (37.5% of HIM inclusion) reduced the nutrients intake and thus the final fish weight and SGR (1.48). The fish fed HIM25 (18.5% of HIM inclusion) showed comparable FC values than those fed HIM50. They also suffered a reduction of iFBW and SGR when compared to the fish fed HIMO, but such difference was not statistically significant, as it was instead for the fish fed HIM50. On the contrary, Renna et al. (2017) showed that an inclusion of up to 40% of a partially defatted HIM did not affect the FBW and SGR of rainbow trout. Moreover, Xiao et al. (2018) reported better weight gain rate and SGR than a control diet (containing fish and soybean meals as primary protein sources) when yellow catfish were fed with diets containing up to 22.3% of HIM inclusion while, when the inclusion level was higher than 34.3%, a decrease of growth performance was observed. The inconsistency among available literature may be also due to the different fish species considered in the trials. The overall reduction of the growth performance observed in this study can also be related to the reduction of ADC_{CP}, which occurred already at 18.5% level of HIM inclusion in the diet. The observed decrease of protein digestibility could be determined by the presence of chitin in the insect meal (Renna et al., 2017; Zhou et al., 2018). In European seabass, the inclusion up to 19.5%

331

332

333

334

335

336

337

338

339

340

341

342

343

344

345

346

347

348

349

350

351

352

353

of HI prepupae meal did not negatively affect the performances or the ADCs of the diets (Magalhães et al., 2017). Renna et al. (2017) reported significant differences for ADC_{DM} and ADC_{CP} between diets with 25 and 50% inclusion of HIM, with the lowest values found in the diet with 50% of HIM inclusion. Chitin interferes with the digestibility of proteins (Marono et al., 2015), but it has also been observed that several fish species are able to synthesize endogenous chitinases, probably due to differences in their gut microbiota. This may explain the apparently contrasting results obtained by different authors while working with different fish species (Henry et al., 2015). To our knowledge, no chitinase activity has been reported in sturgeons. The fish fed with the VEG diet reported similar values than those fed with HIMO in terms of performance while, as far as digestibility is concerned, the fish fed with the VEG diet showed the highest ADC values. Our results indicate that Siberian sturgeons have good capability to use raw plant materials. A comparative study between rainbow trout and Acipenser naccarii Bonaparte showed that sturgeons digest proteins and lipids like carnivorous fish and carbohydrates like omnivorous fish (Furnè et al., 2009). Such a good capability to digest raw plant materials by sturgeons has already been observed by other authors (Liu et al., 2009). Kaushik et al. (1989) showed how Siberian sturgeon juveniles are not able to use complex carbohydrates (such as crude starch) fully, but these authors suggested that the inclusion of pre-treated starch or cereals in diets for sturgeons improve the growth rate and nutrient utilization. It is well known that extrusion can strongly improve the availability of complex carbohydrates. In our trial, the diets were not extruded and, to balance diets, the level of wheat meal inclusion was reduced from 140 (HIM0) to 100 (HIM50) g kg⁻¹ ¹ of wet feed. Considering average starch values found in literature for the individual feed ingredients used in our experimental diets (ARRAINA 2015; Xie et al., 2017), we were able to estimate the starch content of the diets that were equal to 111.6, 95.6, 79.7 and 30.8 (g kg⁻¹, as fed) for HIM0, HIM25, HIM50 and VEG respectively. Compared to the VEG diet, the other diets

355

356

357

358

359

360

361

362

363

364

365

366

367

368

369

370

371

372

373

374

375

376

377

contained a considerable amount of complex carbohydrates that could have contributed to the decrease of ADCs. Even if the VEG diet contained high levels of vegetable meals, no wheat meal was used, obtaining a diet with an overall lower content of complex carbohydrates and then likely more digestible by the sturgeons. Moreover, about 30% of the vegetable feed ingredients used to produce the VEG diet was soybean meal, and it has been reported how sturgeons are able to digest soybean meal efficiently (Degani, 2002; Liu et al., 2009). In particular, Liu et al. (2009) demonstrated that, in sturgeons, the ADC_{DM} and ADC_{CP} of soybean meal are higher than those of FM and PAPs, such as meat and bone meal or poultry by-product meal. Considering the Fulton's condition factor, rainbow trout and yellow catfish fed with HIM showed K ranging from 1.18 to 1.21 (Renna et al., 2017) and from 1.00 to 1.09 (Xiao et al., 2018), respectively. K is an index of the health status of fish and, in some species, values less than 1 are considered indicators of bad health status. In the present trial, K values averaged 0.25 - 0.26, being lower than those found in other fish species. In agreement with our results, in other studies conducted on sturgeons, K values were always less than 1. Indeed, in a growth trial performed by Zhu et al. (2011) on Siberian sturgeons fed with a blend of rendered animal protein, K values equal to 0.7-0.8 were reported. In a study conducted on lake sturgeons (Acipenser fulvescens Rafinesque), K measured at fork length or total length was equal to 0.58 and 0.73 respectively (Jackson et al., 2002). In sturgeons, K values lower than 1 seem then to be a direct consequence of fish morphology (narrow and elongated body) and not a symptom of bad health status. This assumption is confirmed by the high survival rate and overall good performance observed in all the considered treatments. The lack of significant differences in the morphometric indices among dietary treatments confirms the findings of other authors in other fish species fed with insect meals (Renna al., 2017; Xiao et

379

380

381

382

383

384

385

386

387

388

389

390

391

392

393

394

395

396

397

398

399

400

401

402

al., 2018).

Results on the effects of the dietary inclusion of insect meals on the proximate composition of fish lack consistency in the available literature. Similarly to what previously found by Renna et al. (2017) in rainbow trout fillets, in the current trial the increase of HIM in the diets caused a progressive increase of the DM and EE contents of the sturgeons whole body. In European sea bass juveniles, no significant differences were observed in DM and EE contents of whole body at different inclusion levels of a full-fat Tenebrio molitor larvae meal (Gasco et al., 2016). Kroeckel et al. (2012) found decreasing DM and EE contents in the whole body of turbot juveniles while increasing the inclusion level of HI prepupae meal in the experimental diets. As far as HIMO and diets containing HIM are concerned, the results obtained in our trial are somehow ambiguous. Even if not statistically significant, from Table 4 we can observe an increase in HSI and VSI values from HIMO to HIM50. Contemporarily, a general decrease of the growth performance of the fish was observed (Table 3). An increase in the TFA content of the whole body was observed in the sturgeons fed with insect meal when compared to the sturgeons fed with the control diet, supporting the recent findings of Belghit et al. (2018b) on freshwater Atlantic salmon. The observed sharp increase (from 0.11% to 4.56% of TFA) in the content of lauric acid at the increase of HIM inclusion in the diet was expected. In fact, as outlined in Table 2, the HIM used in this trial was very rich in lauric acid. This confirms the recent findings of other authors who investigated the chemical composition of HI larvae and prepupae when fed different rearing substrates (Liland et al., 2017; Spranghers et al., 2017; Meneguz et al., 2018). Concerning SFA, a significant increase in the fish whole body was also found for the contents of myristic and arachidic (C20:0) acids, even if to a lesser extent. Particularly because of the substantial increase in C12:0, the total SFA concentration of the sturgeon whole body was found to increase while increasing the level of dietary HIM inclusion. Similar findings were reported by other authors on rainbow trout (Mancini et al., 2017; Renna et al., 2017), Jian carp (Zhou et al., 2018) and Atlantic salmon (Belghit et al.,

403

404

405

406

407

408

409

410

411

412

413

414

415

416

417

418

419

420

421

422

423

424

425

2018b, 2019) fed H. illucens larvae or prepupae meal. The chromatographic conditions applied in our trail allowed us detecting various BCFA with a number of carbon atoms ranging between 15 and 18 (both iso and anteiso forms) in the sturgeons whole body. Such findings are in line with the individual BCFA found by Wang et al. (2016) in other common freshwater fish species. Other authors previously reported the FA composition of different sturgeon species, such as white sturgeon (Xu et al., 1993; 1996; Gawlicka et al., 2002), Russian sturgeon (A. gueldenstaedtii Brandt) (Şener et al., 2005; Zhu et al., 2017), Gulf sturgeon (A. oxyrinchus desotoi Vladykov) (Chen et al., 1995), and the hybrid A. naccarii × A. baerii (Vaccaro et al., 2005). To the best of our knowledge, only two studies previously investigated the FA composition of Siberian sturgeons (Nieminen et al., 2014; Aidos et al., 2019). Despite the available above-mentioned literature on the FA composition of sturgeons, information on BCFA is very scant. Until now, only Chen et al. (1995) reported BCFA in the muscle samples of cultured and wild Gulf sturgeons. The overall amount of BCFA found in the Siberian sturgeons in our trial (up to 2.30% of TFA) was comparable to that found in ruminant-derived food products (usually considered the most important source of BCFA in the human diet), most probably because we analysed the fish whole body. Indeed Wang et al. (2016) found that fish skin contains on average higher amounts of BCFA compared to different muscle types. Some BCFA were also detected in the insect meal (Table 2). Also Spranghers et al. (2017) found variable amounts of total BCFA in H. illucens prepupae reared on different organic waste substrates. However, these authors reported no details regarding the detected individual iso and anteiso forms, and therefore no comparisons at this regard can be made with our findings. The dietary treatment did not affect the total amount of BCFA, nor the majority of the detected individual iso and anteiso forms, in the Siberian sturgeons in our trial. In the current trial, the amount of C17 anteiso slightly differed in the experimental diets, ranking in the order HIM50 > VEG = HIM25 > HIM0 (Table 2), thus mostly mirroring the concentration of this

427

428

429

430

431

432

433

434

435

436

437

438

439

440

441

442

443

444

445

446

447

448

449

FA found in the whole body of the fish (Table 5). Regarding MUFA, from a quantitative point of view the most interesting result found in the sturgeon whole body was the proportional increase in the concentration of oleic acid at the increase of HIM inclusion in the diet. Such increase reflected the oleic acid concentration in the experimental diets (Table 2). Oleic acid is by far the most abundant individual MUFA in H. illucens larvae and prepupae, and the amount detected in the HIM used in our trial (about 8% of TFA) was very similar to that found in the available literature (Spranghers et al., 2017; Meneguz et al., 2018). As far as PUFA are concerned, the highest total n6 PUFA concentration found in the whole body of the sturgeons fed with the VEG diet was expected. The VEG diet used in this trial contained corn and soybean as plant materials, linoleic acid being the most abundant FA in both corn and soybean. It is also interesting to notice that the concentrations of C18:2 n6 and total n6 PUFA were higher in the whole body of the sturgeons fed HIM25 and HIM50 when compared to HIM0, most probably as the consequence of the C18:2 n6 content (195.96 mg 100g⁻¹ DM) found in HIM. Regarding the n3 PUFA, in HIM C18:3 n3 was detected in very low amounts, while EPA, DPA and DHA were not detected (Table 2). The total n3 PUFA in HIM only reached the 0.7% of TFA and this has to be considered a big issue from the point of view of both fish dietary requirements of essential fatty acids and human health related outcomes, when trying to replace fishmeal with insect meal in aquafeed. In the whole body of the sturgeons used in our trial, the total n3 PUFA concentration, as well as the concentrations of EPA, DPA, and DHA did not decrease while increasing HIM in the diet because, with the aim of maintaining the diets isolipidic and isoenergetic, we contemporarily increased the amount of fish oil (Table 2). Adding even low amounts of fish oil while replacing fishmeal with insect meal in commercial aquafeeds may be a strategy to meet the lipid nutritional requirements of the fish and to prevent the lowering of the quality of the lipid fraction of the fish destined to

451

452

453

454

455

456

457

458

459

460

461

462

463

464

465

466

467

468

469

470

471

472

human consumption. Another very promising strategy may be the modulation of the n3 FA concentration in the insects using appropriate growth media (Liland et al., 2017).

5. Conclusion

In this first study performed on Siberian sturgeons, results showed that it is possible to replace up to 25% of FM with a highly defatted HIM without impairing the growth performance, condition factor, biometric and morphometric indices, and whole body proximate composition of the fish. Only few variations were observed in the fatty acid composition of the sturgeon whole body already at 25% of fishmeal substitution with the insect meal. For future practical applications, it will be necessary to evaluate different insect species and lower levels of insect meal inclusion in the diets for Siberian sturgeons.

Funding

This study was supported by the University of Torino (Italy) founding (CORP_RILO_17_01) and Cassa di Risparmio di Torino project BioSin (GASL RIC N COMP 18 02).

References

Aidos, L., Vasconi, M., Abbate, F., Valente, L.M.P., Lanfranchi, M., Di Giancamillo, A., 2019. Effects of stocking density on reared Siberian sturgeon (*Acipenser baerii*) larval growth, muscle development and fatty acids composition in a recirculating aquaculture system. Aquacult. Res. 50, 588-598. https://doi.org/10.1111/are.13936.

- 497 AOAC International, 2000. Official methods of analysis of AOAC International. 16th ed.
- 498 Gaithersburg: Association of Official Analytical Chemists.

- 500 AOAC International, 2003. Official methods of analysis of AOAC International. 17th ed.
- 501 Gaithersburg: Association of Official Analytical Chemists.

502

- ARRAINA, 2015. Arraina 1st Technical booklet. A database of Aquaculture feed ingredients.
- 504 http://arraina.eu/images/ARRAINA/Media_Center/ARRAINA_1st_Technical_Booklet_web.pdf
- 505 (accessed 25 February 2019).

506

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

511

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

515

- Belghit, I., Waagbø, R., Lock, E.-J., Liland, N.S., 2018b. Insect-based diets high in lauric acid reduce
- 517 liver lipids in freshwater Atlantic salmon. Aquacult. Nutr. 1-15 https://doi.org/10.1111/anu.12860

- Bronzi, P., Rosenthal, H., Gessner, J., 2011. Global sturgeon aquaculture production: an overview.
- 520 J. Appl. Ichthyol. 27, 169-175. https://doi.org/10.1111/j.1439-0426.2011.01757.x.

- 522 Chen, I.-C., Chapman, F.A., Wei, C.-I., Portier, K.M., O'Keefe, S.F., 1995. Differentiation of cultured
- and wild sturgeon (Acipenser oxyrinchus desotoi) based on fatty acid composition. J. Food Sci. 60,
- 524 631-635. https://doi.org/10.1111/j.1365-2621.1995.tb09844.x.

- 526 Degani, G., 2002. Availability of protein and energy from three protein sources in hybrid sturgeon
- 527 Acipenser gueldenstadtii x A. bester. Aquac. Res. 33 (9), 725-727. https://doi.org/10.1046/j.1365-
- 528 2109.2002.00709.x.

529

- Devic, E., Leschen, W., Murray, F., Little, D.C., 2018. Growth performance, feed utilization and
- 531 body composition of advanced nursing Nile tilapia (Oreochromis niloticus) fed diets containing
- 532 Black Soldier Fly (Hermetia illucens) larvae meal. Aquac. Nutr. 24, 416–423.
- 533 https://doi.org/10.1016/j.anifeedsci.2015.08.006.

534

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

540

- Engle, R.C., D'Abramo, L., Ponniah, A.G., Slater, M., 2017. Global Aquaculture 2050. J. World.
- 542 Aquac. Soc. 48 (1), 3-6. https://doi: 10.1111/jwas.12400.

- 544 FAO, 2018. The State of World Fisheries and Aquaculture 2018 Meeting the sustainable
- 545 development goals. Rome.

- 547 FEEDIPEDIA. Feedipedia: an on-line encyclopedia of animal feeds; 2019.
- 548 http://www.feedipedia.org/ Accessed 17 September 2019.

549

- Figueiredo-Silva, A.C., Kaushik, S., Terrier, F., Schrama, J.W., Médale, F., Geurden, I. 2012. Link
- 551 between lipid metabolism and voluntary food intake in rainbow trout fed coconut oil rich in
- medium-chain TAG. Br J Nutr. 107(11), 1714-25. https://doi: 10.1017/S0007114511004739

553

- Finke, M.D., 2007. Estimate of chitin in raw whole insects. Zoo Biol. 26 (2), 105–115.
- 555 https://doi.org/10.1002/zoo.20123.

556

- 557 Furnè, M., Sanz, A., Garcìa-Gallego, M., Hidalgo, M.C., Domezain, A., Domezain, J., Morales, A.E.,
- 558 2009. Metabolic organization of the sturgeon Acipenser naccarii A comparative study with
- 559 rainbow trout *Oncorhynchus mykiss*. Aquaculture 289 (1-2), 161–166.
- 560 https://doi.org/10.1016/j.aquaculture.2008.12.028.

561

- 562 Gai, F., Gasco, L., Daprà, F., Palmegiano, G.B., Sicuro, B., 2012. Enzymatic and histological
- 563 evaluations of gut and liver in rainbow trout *Oncorhynchus mykiss*, fed with rice protein
- concentrate based diets. J. World. Aquac. Soc. 43 (2), 218–229. https://doi.org/10.1111/j.1749-
- 565 7345.2012.00557.x.

Gai, F., Peiretti, P.G., Brugiapaglia, A., Gasco, L., 2016. Effects of dietary protein source and feeding regime on growth performance, nutrient digestibility, fatty acids, and quality characteristics of rainbow trout, *Oncorhynchus mykiss*, fillets. J. World. Aquac. Soc. 47 (4), 496-507. https://doi.org/10.1111/jwas.12294.

, ,,

- Gasco, L., Henry, M., Piccolo, G., Marono, S., Gai, F., Renna, M., Lussiana, C., Antonopoulou, E.,
- 573 Mola, P., Chatzifotis, S., 2016. *Tenebrio molitor* meal in diets for European sea bass (*Dicentrarchus*
- 574 labrax L.) juveniles: growth performance, whole body composition and in vivo apparent
- 575 digestibility. Anim. Feed Sci. Technol. 220, 34-45.
- 576 https://doi.org/10.1016/j.anifeedsci.2016.07.003.

577

571

- Gasco L., Gai F., Maricchiolo G., Genovese L., Ragonese S., Bottari T., Caruso G., 2018. Fish meal
- alternative protein sources for aquaculture feeds: current situation and alternative sources. In:
- Feeds for the Aquaculture Sector. Springer International Publishing, Cham, pp. 1-28.

581

- Gawlicka, A., Herold, M.A., Barrows, F.T., De La Noüe, J., Hung, S.S.O., 2002. Effects of dietary
- 583 lipids on growth, fatty acid composition, intestinal absorption and hepatic storage in white
- 584 sturgeon (Acipenser transmontanus R.) larvae. J. Appl. Ichthyol. 18, 673-681.
- 585 https://doi.org/10.1046/j.1439-0426.2002.00371.x.

586

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

- 591 Guo, Z., Zhu, X., Liu, J., Han, D., Yang, Y., Lan, Z., Xie, S., 2012. Effects of dietary protein level on
- 592 growth performance, nitrogen and energy budget of juvenile hybrid sturgeon, Acipenser baerii 🖁 🗴
- 593 A. gueldenstaedtii 🗗. Aquaculture 338, 89-95.
- 594 https://doi.org/10.1016/j.aquaculture.2012.01.008.

- Henry, M.A., Gasco, L., Chatzifotis, S., Piccolo, G., 2018. Does dietary insect meal affect the fish
- immune system? The case of mealworm, *Tenebrio molitor* on European sea bass, *Dicentrarchus*
- 598 *labrax*. Dev. Comp. Immunol. 81, 204-209. https://doi.org/10.1016/j.dci.2017.12.002.

599

- Henry, M., Gasco, L., Piccolo, G., Fountoulaki, E., 2015. Review on the use of insects in the diet of
- 601 farmed fish: past and future. Anim. Feed Sci. Technol. 203, 1–22.
- 602 https://doi.org/10.1016/j.anifeedsci.2015.03.001.

603

- Huyben, D., Vidaković, A., Hallgren, S.W, Langeland, M., 2018. High-throughput sequencing of gut
- 605 microbiota in rainbow trout (Oncorhynchus mykiss) fed larval and pre-pupae stages of black soldier
- 606 fly (Hermetia illucens). Aquaculture 500, 485-491.
- 607 https://doi.org/10.1016/j.aquaculture.2018.10.034.

608

- Jahanbakhshi, A., Imanpoor, M.R., Taghizadeh, V., Shabani, A., 2013. Hematological and serum
- 610 biochemical indices changes induced by replacing fish meal with plant protein (sesame oil cake
- and corn gluten) in the Great sturgeon (Huso huso). Comp. Clin. Pathol. 22, 1087–1092.
- 612 https://doi.org/10.1007/s00580-012-1532-4.

- Jackson, J.R., VanDeValk, A.J., Brooking, T.E., VanKeeken, O.A., Rudstam, L.G., 2002. Growth and
- feeding dynamics of lake sturgeon, Acipenser fulvescens, in Oneida Lake, New York: results from
- 616 the first five years of a restoration program. J. Appl. Ichthyol. 18 (4-6), 439-443.
- 617 https://doi.org/10.1046/j.1439-0426.2002.00394.x.

- Kaushik, S.J., Luquet, P., Blanc, D., Paba, A., 1989. Studies on the nutrition of Siberian sturgeon,
- 620 Acipenser baeri. I. Utilization of digestible carbohydrates by sturgeon. Aquaculture 76 (1-2), 97-
- 621 107. https://doi.org/10.1016/0044-8486(89)90254-8.

622

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

627

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

- 633 Liland, N.S., Biancarosa, I., Araujo, P., Biemans, D., Bruckner, C.G., Waagbø, R., Torstensen, B.E.,
- 634 Lock, E.-J., 2017. Modulation of nutrient composition of black soldier fly (Hermetia illucens) larvae
- 635 by feeding seaweed-enriched media. PLoS ONE 12(8): e0183188.
- 636 https://doi.org/10.1371/journal.pone.0183188.

- Liu, H., Wu, X., Zhao, W., Xue, M., Guo, L., Zheng, Y., Yu, Y., 2009. Nutrient apparent digestibility
- 639 coefficients of selected protein sources for juvenile Siberian sturgeon (Acipenser baerii Brandt),
- 640 compared by two chromic oxide analyses methods. Aquac. Nutr. 15 (6), 650-656.
- 641 https://doi.org/10.1111/j.1365-2095.2008.00634.x.

- Lock, E.J., Arsiwalla, T., Waagbø, R., 2016. Insect larvae meal as an alternative source of nutrients
- 644 in the diet of Atlantic salmon (Salmo salar) postsmolt. Aquac. Nutr. 22 (6), 1202-1213.
- 645 https://doi.org/10.1111/anu.12343.

646

- Magalhães, R., Sánchez-López, A., Leal, R.S., Martínez-Llorens, S., Oliva-Teles, A., Peres, H., 2017.
- 648 Black soldier fly (Hermetia illucens) pre-pupae meal as a fish meal replacement in diets for
- 649 European seabass (*Dicentrarchus labrax*). Aquaculture 476, 79-85.
- 650 https://doi.org/10.1016/j.aquaculture.2017.04.021.

651

- Mancini, S., Medina, I., Iaconisi, V., Gai, F., Basto, A., Parisi, G., 2017. Impact of black soldier fly
- larva meal on the chemical and nutritional characteristics of rainbow trout fillets. Animal 12 (8), 1–
- 654 10. https://doi.org/10.1017/S1751731117003421.

655

- Marono, S., Piccolo, G., Loponte, R., Di Meo, C., Attia, Y.A., Nizza, A., 2015. *In vitro* crude protein
- 657 digestibility of Tenebrio molitor and Hermetia illucens insect meals and its correlation with
- 658 chemical composition traits. Ital. J. Anim. Sci. 14 (3), 338-343.
- 659 https://doi.org/10.4081/ijas.2015.3889.

- Meneguz, M., Schiavone, A., Gai, F., Dama, A., Lussiana, C., Renna, M., Gasco, L., 2018. Effect of
- rearing substrate on growth performance, waste reduction efficiency and chemical composition of
- 663 black soldier fly (Hermetia illucens) larvae. J. Sci. Food Agric. 98, 776-5784.
- 664 https://doi.org/10.1002/jsfa.9127.

- Merrifield, D.L., Olsen, R.E., Myklebust, R., Ringø, E.,, 2011. Dietary effect of soybean (Glycine max)
- products on gut histology and microbiota of fish, in: El-Shemy, H.A. (Ed.), Soybean and Nutrition.
- 668 InTech, Rijeka, Croatia, pp. 231-250. doi: 10.5772/20101.

669

- Nieminen, P., Westenius, E., Halonen, T., Mustonen, A.-M., 2014. Fatty acid composition in tissues
- 671 of the farmed Siberian sturgeon (Acipenser baerii). Food Chem. 159, 80-
- 84.http://dx.doi.org/10.1016/j.foodchem.2014.02.148.

673

- Olsen, R., Suontama, J., Langmyhr, E., Mundheim, H., Ringo, E., Melle, E., Malde, M., Hmere, G.,
- 675 2006. The replacement of fish meal with Antarctic krill, Euphausia superba in diets for Atlantic
- 676 salmon, Salmo salar. Aquac. Nutr. 12, 280-290. https://doi.org/10.1111/j.1365-
- 677 2095.2006.00400.x

678

- Palmegiano, G.B., Gai, F., Daprà, F., Gasco, L., Pazzaglia, M., Peiretti, P.G., 2008. Effects of Spirulina
- 680 and plant oil on the growth and lipid traits of white sturgeon (Acipenser transmontanus)
- 681 fingerlings. Aquac. Res. 39 (6), 587-595. https://doi.org/10.1111/j.1365-2109.2008.01914.x.

- 683 Pyka, J., Kolman, R., 2003. Feeding intensity and growth of Siberian sturgeon Acipenser baerii
- Brandt in pond cultivation. Arch. Pol. Fish. 11 (2), 287-294.

- Renna, M., Cornale, P., Lussiana, C., Malfatto, V., Fortina, R., Mimosi, A., Battaglini, L.M., 2012. Use
- of Pisum sativum (L.) as alternative protein resource in diets for dairy sheep: effects on milk yield,
- 688 gross composition and fatty acid profile. Small Ruminant Res. 102, 142-150.
- 689 https://doi.org/10.1016/j.smallrumres.2011.07.007.

- Renna, M., Gasmi-Boubaker, A., Lussiana, C., Battaglini, L.M., Belfayez, K., Fortina, R., 2014. Fatty
- acid composition of the seed oils of selected Vicia L. taxa from Tunisia. Ital. J. Anim. Sci. 13 (2),
- 693 308–316. https://doi.org/10.4081/ijas.2014.3193.

694

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

700

- 701 Şener, E., Yildiz, M., Savaş, E., 2005. Effects of dietary lipids on growth and fatty acid composition
- in Russian sturgeon (*Acipenser gueldentaedtii*) juveniles. Turk. J. Vet. Anim. Sci. 29, 1101-1107.

703

- 504 Şener, E., Yildiz, M., Savaş, E., 2006. Effect of vegetable protein and oil supplementation on growth
- 705 performance and body composition of Russian sturgeon juveniles (Acipenser queldentaedtii
- 706 Brandt, 1833) at low temperatures. Turk. J. Fish. Aquat. Sci. 6, 23–27.

- Sicuro, B., Piccinno, M., Daprà, F., Gai, F., Vilella, S., 2015. Utilization of rice protein concentrate in
- 709 Siberian sturgeon (*Acipenser baerii* Brandt) nutrition. Turk. J. Fish. Aquat. Sci. 15, 313–319.

- 711 Soliman, N.F., Yacout, D., Hassaan, M., 2017. Responsible fishmeal consumption and alternatives
- 712 in the face of climate changes. Int. J. Mar. Sci. 7 (15), 130-140.
- 713 https://doi.org/10.5376/ijms.2017.07.0015.

714

- Spranghers, T., Ottoboni, M., Klootwiik, C., Ovyn, A., Deboosfere, S., De Meulenaer, B., Michiels, J.,
- 716 Eeckhout, M., De Clerg, P., De Smet, S., 2017. Nutritional composition of black soldier fly
- 717 (Hermetia illucens) prepupae reared on different organic waste substrate. J. Sci. Food Agric. 97,
- 718 2594–2600. https://doi.org/10.1002/jsfa.8081.

719

- 720 Vaccaro, A.M., Buffa, G., Messina, C.M., Santulli, A., Mazzola, A., 2005. Fatty acid composition of a
- 721 cultured sturgeon hybrid (Acipenser naccarii × A. baerii). Food Chem. 93, 627-631.
- 722 https://doi.org/10.1016/j.foodchem.2004.09.042.

723

- Wang, D.H., Jackson, J.R., Twining, C., Rudstam, L.G., Zollweg-Horan, E., Kraft, C., Lawrence, P.,
- 725 Kothapalli, K., Wang, Z., Brenna, J.T., 2016. Saturated branched chain, normal odd-carbon-
- numbered, and n-3 (Omega-3) polyunsaturated fatty acids in freshwater fish in the Northeastern
- 727 United States. J. Agric. Food Chem. 64, 7512–7519. https://doi.org/10.1021/acs.jafc.6b03491.

- 729 Xiao, X., Jin, P., Zheng, L., Cai, M., Yu, Z., Yu, J., Zhang, J., 2018. Effects of black soldier fly (Hermetia
- 730 illucens) larvae meal protein as a fishmeal replacement on the growth and immune index of yellow

- 731 catfish (*Pelteobagrus fulvidraco*). Aquacult. Res. 49 (4), 1569-1577.
- 732 https://doi.org/10.1111/are.13611.

- Xie, WX, Gong, YX, Yu, KX (2017). Quantitative analysis of total starch content in wheat flour by
- reaction headspace gas chromatography. Anal. Bioanal. Chem. 409 (22), 5195-5200.
- 736 https://doi.org/10.1007/s00216-017-0494-4.

737

- 738 Xu, R., Hung, S.S.O., German, J.B., 1993. White sturgeon tissue fatty acid compositions are affected
- 739 by dietary lipids. J Nutr. 123, 1685-1692. https://doi.org/10.1093/jn/123.10.1685.

740

- Xu, R., Hung, S.S.O., German, J.B., 1996. Effects of dietary lipids on the fatty acid composition of
- 742 triglycerides and phospholipids in tissues of white sturgeon. Aquacult. Nutr. 2, 101-109.
- 743 https://doi.org/10.1111/j.1365-2095.1996.tb00016.x.

744

- 745 Zhou, J.S., Liu, S.S., Ji, H., Yu, H.B., 2018. Effect of replacing dietary fish meal with black soldier fly
- larvae meal on growth and fatty acid composition of Jian carp (*Cyprinus carpio* var. Jian). Aquacult.
- 747 Nutr. 24 (1), 424–433. https://doi.org/10.1111/anu.12574.

748

- 749 Zhu, H., Gong, G., Wang, J., Wu, X., Xue, M., Niu, C., Guo, L., Yu, Y., 2011. Replacement of fish meal
- 750 with blend of rendered animal protein in diets for Siberian sturgeon (Acipenser baerii Brandt),
- 751 results in performance equal to fish meal fed fish. Aquacult. Nutr. 17 (2), 389–395.
- 752 https://doi.org/10.1111/j.1365-2095.2010.00773.x.

- 754 Zhu, H., Li, Q., Wang, H., Zhu, T., Qin, J., Li, E., Chen, L., 2017. Growth, fatty acid composition and
- 755 lipid deposition of Russian sturgeon (Acipenser gueldenstaedtii) fed different lipid sources.
- 756 Aquacult. Res. 48, 5126-5132. https://doi.org/10.1111/are.13148.

Table 1. Ingredients and proximate composition of *H. illucens* larvae meal and experimental diets.

	HIM	HIM0	HIM25	HIM50	HIM100	VEG
Ingredients (g kg ⁻¹)						
Fish meal (Chile, super	-	700.0	525.0	350.0	0	320.0
prime) ^a						
HI larvae meal ^b	-	0	185.0	375.0	750.0	0
Wheat meal	-	140.0	120.0	100.0	55.0	0
Corn gluten meal	-	0	0	0	0	150.0
Soybean protein	-	0	0	0	0	200.0
concentrate						
Soybean meal	-	0	0	0	0	140.0
Starch gelatinized, D500	-	80.0	80.0	80.0	80.0	80.0
Fish oil	-	60.0	70.0	75.0	95.0	90.0
Vitamine mixture ^c	-	10.0	10.0	10.0	10.0	10.0
Mineral mixture ^d	-	10.0	10.0	10.0	10.0	10.0
Proximate composition ^e						
DM (g 100g ⁻¹)	94.94	96.41	96.39	96.29	96.83	97.37
CP (g 100g ⁻¹ , as fed)	62.51	50.29	50.65	50.20	50.27	50.87
EE (g 100g ⁻¹ , as fed)	4.03	12.68	12.62	12.10	11.73	12.81
Ash (g 100g ⁻¹ , as fed)	8.20	13.15	11.71	10.24	7.90	9.91
CF (g 100g ⁻¹ , as fed) ^f	7.0	0.32	1.61	2.89	5.25	1.77
Chitin (g 100g ⁻¹ , as fed) ^g	4.97	nd	0.72	1.92	3.75	nd
NFE (g 100g ⁻¹ , as fed) ^h	18.26	23.56	23.41	24.57	24.85	24.65

GE (MJ kg⁻¹, as fed) 20.76 19.77 19.65 20.64

Abbreviations: HI, Hermetia illucens; HIM, Hermetia illucens larvae meal; VEG, vegetable protein

20.38

20.44

based diet; DM, dry matter; CP, crude protein; EE, ether extract; NFE, Nitrogen free extracts; GE,

- 762 gross energy.
- ^a Purchased from Corpesca S.A. (Santiago, Chile). Proximate composition (g 100g⁻¹, as fed basis):
- 764 88.7 DM; 63.8 CP; 8.4 EE; 14.9 ash.
- ^b Purchased from Hermetia Deutschland GmbH & Co. KG (Baruth/Mark, Germany).
- ^c Vitamin mixture (IU or mg kg⁻¹ diet): DL-α tocopherol acetate, 60 IU; sodium menadione
- bisulphate, 5 mg; retinyl acetate, 15,000 IU; DL-cholecalciferol, 3000 IU; thiamin, 15 mg; riboflavin,
- 30 mg; pyridoxine, 15 mg; B₁₂, 0.05 mg; nicotinic acid, 175 mg; folic acid, 500 mg; inositol, 1000
- mg; biotin, 2.5 mg; calcium panthotenate, 50 mg (purchased from Granda Zootecnici S.r.l., Cuneo,
- 770 Italy).
- d Mineral mixture (g or mg kg-1 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
- 774 sulphate, 20 mg; manganese sulphate, 3 g; sodium fluoride, 1 g (purchased from Granda
- 775 Zootecnici S.r.l., Cuneo, Italy).
- 776 ^e Values are reported as mean of duplicate analyses
- 777 Crude Fiber estimated according to Feedipedia and Arraina booklet ingredients database
- 778 g Estimated as ADF ADFN (Finke, 2007)
- ^h Nitrogen Free Extracts; Calculated as 100 (CP + EE + Ash + Crude Fiber)

rable 2. Fatty acid pro	HIM	HIM0	HIM25	HIM50	HIM100	VEG
	111141	1111110	1111123	11111130	111111100	V EG
C12:0	2020.65	22.17	388.43	673.58	1402.94	16.65
C14:0	325.51	452.72	488.41	506.04	471.53	375.93
C15 iso	nd	16.61	17.65	15.51	11.18	14.71
C15 anteiso	nd	5.40	2.55	3.33	1.27	3.34
C14:1 <i>c</i> 9 + C15:0	15.24	46.20	44.70	45.20	34.13	36.39
C16 iso	9.14	16.99	14.42	12.65	14.98	22.64
C16:0	435.36	1102.78	1113.22	1148.65	800.06	942.69
C17 iso	nd	29.35	31.33	27.57	13.90	25.57
C17 anteiso	3.32	17.07	23.34	29.11	27.27	24.47
C16:1 <i>c</i> 9	124.05	514.11	555.83	580.22	502.10	527.68
C17:0	3.33	38.92	43.13	44.51	26.63	31.82
C17:1 <i>c</i> 9	nd	18.05	16.48	18.87	12.12	19.02
C18:0	64.87	271.14	260.78	265.69	165.27	227.11
C18:1 t	nd	85.72	103.68	123.01	108.05	124.49
C18:1 <i>c</i> 9	303.20	930.75	1045.14	1203.62	984.07	1100.78
C18:1 <i>c</i> 11	9.34	329.89	313.57	325.14	228.54	313.62
C18:1 <i>c</i> 12	nd	nd	16.70	33.83	20.07	Nd
C18:1 <i>c</i> 14 + <i>t</i> 16	nd	nd	139.64	275.37	161.70	Nd
C18:2 n6	195.96	195.35	263.29	274.97	253.95	383.26
C18:3 n3	25.08	64.47	81.94	99.46	76.93	79.69
C18:3 n6	nd	10.77	6.89	7.79	3.67	14.07
C20:0	15.84	16.69	36.05	67.64	33.27	17.09

C20:1 <i>c</i> 9	nd	52.96	68.62	89.99	68.43	80.15
C20:1 <i>c</i> 11	nd	370.41	437.66	545.61	432.73	512.71
C20:2 n6	7.33	154.85	172.02	148.84	101.83	147.51
C20:4 n6	nd	31.44	9.24	7.68	7.40	27.97
C20:5 n3	nd	580.27	573.06	481.13	301.52	469.54
C22:1 n9	nd	197.72	256.58	339.27	245.21	295.80
C22:5 n3	nd	36.82	43.17	41.52	17.64	40.86
C22:6 n3	nd	666.59	584.14	590.03	248.17	512.89
∑SFA	2925.97	1989.84	2419.33	2794.26	2968.29	1702.01
Σ MUFA	451.82	2545.82	2998.59	3580.15	2797.14	3010.64
∑ PUFA	228.36	1740.57	1733.76	1651.42	1011.10	1675.79
∑ PUFA / ∑ SFA	0.08	0.87	0.72	0.59	0.34	0.98
∑ n3	25.08	1348.15	1282.31	1212.14	644.26	1102.99
∑ n6	203.29	392.42	534.63	439.29	366.84	572.80
∑ n3 / ∑ n6	0.12	3.44	2.40	2.76	1.76	1.93
TFA	3606.16	6276.23	7151.68	8025.84	6776.54	6388.44

Abbreviations: DM, dry matter; HIM, *Hermetia illucens* larvae meal; VEG, vegetable protein based diet; *c, cis*; *t, trans*; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; TFA, total fatty acids; nd, not detected.

⁷⁸⁵ Values are reported as mean of duplicate analyses.

Table 3. Survival and growth performance of Siberian sturgeon juveniles fed the experimental diets (n = 4).

	HIM0	HIM25	HIM50	VEG	SEM	<i>p</i> -value
Survival rate (%)	98.81	97.50	97.62	100.00	0.739	0.528
iIBW (g)	24.20	24.26	24.21	24.19	0.017	0.494
iFBW (g)	159.32 a	148.12 ab	141.94 b	153.32 ab	2.198	0.015
WG	135.12 a	123.86 ab	117.73 b	129.13 ab	2.203	0.015
FC (g DM)	3003.04 a	2844.81 b	2823.15 b	3052.78 a	27.879	0.000
SGR (% d ⁻¹)	1.59 a	1.51 ab	1.48 b	1.58 a	0.015	0.008
FCR	1.03	1.08	1.12	1.05	0.016	0.213
PER	1.94	1.84	1.78	1.88	0.028	0.236

Abbreviations: HIM, *Hermetia illucens* larvae meal; VEG, vegetable protein based diet; SEM, standard error of the mean; *p*, probability; iIBW, individual initial body weight; iFBW, individual final body weight; FC, feed consumption; WG, weight gain; SGR, specific growth rate; FCR, feed conversion ratio; PER, protein efficiency ratio.

Different superscripts within a row indicate significant differences ($p \le 0.05$).

Table 4. Biometric (n = 24) and morphometric indices (n = 12) of Siberian sturgeon juveniles fed the experimental diets.

	HIM0	HIM25	HIM50	VEG	SEM	<i>p</i> -value
K	0.26	0.25	0.26	0.26	0.002	0.051
HSI	2.69	3.02	3.39	3.41	0.123	0.117
VSI	8.04	8.50	8.91	8.76	0.158	0.233

Abbreviations: HIM, *Hermetia illucens* larvae meal; VEG, vegetable protein based diet; SEM, standard error of the mean; *p*, probability; K, Fulton's condition factor; HSI, hepatosomatic index; VSI, viscerosomatic index.

Different superscripts within a row indicate significant differences ($p \le 0.05$).

796

797

801

Table 5. Whole body proximate (g 100 g $^{-1}$ ww) and fatty acid (mg 100g $^{-1}$ ww) compositions of Siberian sturgeon juveniles fed the experimental diets (n = 12).

	HIM0	HIM25	HIM50	VEG	SEM	<i>p</i> -value
Proximate composition						
DM	21.25 ^b	22.37 ^{ab}	23.37 ^a	21.30 ^b	0.239	0.002
СР	13.66	14.10	13.96	13.45	0.106	0.116
EE	4.50 ^b	5.13 ^{ab}	6.23ª	5.19 ^{ab}	0.198	0.016
Ash	2.33	2.39	2.40	2.14	0.041	0.095
Fatty acid composition						
C12:0	2.70 ^c	61.23 ^b	151.61ª	2.42 ^c	9.364	0.000
C14:0	140.46 ^b	178.88 ^{ab}	207.80ª	140.53 ^b	7.216	0.001
C15 <i>iso</i>	6.43	7.13	6.82	6.30	0.225	0.566
C15 anteiso	1.74	1.89	1.83	1.59	0.071	0.475
C14:1 <i>c</i> 9 + C15:0	20.13 ^{ab}	23.75 ^{ab}	24.10ª	19.36 ^b	0.705	0.024
C16 <i>iso</i>	2.82	3.52	3.60	3.67	0.133	0.080
C16:0	531.70	640.78	642.28	551.19	22.924	0.183
C17 <i>iso</i>	15.20	16.63	14.20	13.09	0.534	0.110
C17 anteiso	16.95 ^b	20.76 ^{ab}	23.68ª	21.18 ^{ab}	0.791	0.021
C16:1 <i>c</i> 9	209.56 ^b	264.07 ^{ab}	289.54ª	230.04 ^{ab}	10.732	0.036
C17:0	15.86	17.11	16.20	15.67	0.515	0.777
C18 iso	5.22 ^{ab}	5.47ª	4.08 ^b	4.12 ^{ab}	0.196	0.012
C17:1 <i>c</i> 9	12.16	14.81	14.41	12.14	0.521	0.127
C18:0	87.63	104.56	94.80	93.03	2.988	0.245
C18:1 <i>t</i> 9-11	4.45 ^b	6.10 ^{ab}	5.84 ^{ab}	7.15 ^a	0.258	0.001

C18:1 <i>c</i> 9	600.58 ^b	828.26 ^{ab}	869.27ª	747.45 ^{ab}	34.283	0.024
C18:1 <i>c</i> 11	142.82	180.69	180.24	168.59	5.705	0.059
C18:1 <i>c</i> 12	7.04 ^c	13.68 ^b	16.34 ^{ab}	18.50 ^a	0.840	0.000
C18:2 n6	71.37 ^c	130.18 ^b	138.15 ^b	180.38ª	7.438	0.000
C18:3 n6	3.16 ^c	7.71 ^b	8.64 ^b	11.90ª	0.557	0.000
C18:3 n3	19.10 ^b	30.46 ^a	32.04ª	30.39 ^a	1.392	0.001
C20:0	12.21 ^c	19.97 ^b	22.26 ^{ab}	27.04ª	1.172	0.000
C20:1 <i>c</i> 9	41.57 ^b	61.05 ^a	58.10ª	58.89 ^a	2.021	0.001
C20:1 <i>c</i> 11	151.74 ^b	221.46ª	216.53ª	222.42ª	7.966	0.001
C20:2 n6	8.25 ^b	12.79ª	13.17 ^a	13.69ª	0.532	0.000
C20:3 n6	2.79 ^b	4.80 ^a	4.92ª	5.96ª	0.240	0.000
C20:4 n6	13.85 ^b	19.38ª	15.41 ^b	14.82 ^b	0.587	0.003
C20:5 n3	101.20 ^b	151.76ª	117.81 ^{ab}	88.02 ^b	6.348	0.001
C22:5 n3	23.96 ^b	42.46 ^a	39.59ª	33.15 ^{ab}	1.849	0.001
C22:6 n3	107.59 ^b	164.70 ^a	154.99 ^{ab}	128.53 ^{ab}	7.065	0.013
∑ SFA	859.05 ^b	1101.69 ^{ab}	1213.25ª	899.19 ^b	41.428	0.005
∑ BCFA	54.80	62.54	61.03	56.25	1.988	0.467
∑ MUFA	1162.89 ^b	1576.44 ^{ab}	1633.93ª	1446.68 ^{ab}	60.278	0.028
∑ PUFA	351.28 ^b	564.24ª	524.71 ^a	506.84ª	21.537	0.002
∑ PUFA / ∑ SFA	0.42 ^c	0.53 ^{ab}	0.43 ^{bc}	0.57ª	0.015	0.000
∑ n3	251.86 ^b	389.38ª	344.42 ^{ab}	280.10 ^b	15.261	0.003
∑ n6	99.42°	174.87 ^b	180.29 ^b	226.74 ^a	8.865	0.000
∑ n3 / ∑ n6	2.51ª	2.29ª	1.87 ^b	1.24 ^c	0.084	0.000
TFA	2373.22 ^b	3242.37 ^a	3371.90ª	2852.71 ^{ab}	118.481	0.012

805	Abbreviations: HIM, Hermetia illucens larvae meal; VEG, vegetable protein based diet; SEM,
806	standard error of the mean; p , probability; ww, wet weight; DM, dry matter; CP, crude protein; EE,
807	ether extract; c, cis; t, trans; SFA, saturated fatty acids; BCFA, branched chain fatty acids; MUFA,
808	monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; TFA, total fatty acids.
809	Values are reported as mean of duplicate analyses.
810	Different superscripts within a row indicate significant differences ($p \le 0.05$).

Table 6. Apparent digestibility coefficients (ADC) of the experimental diets (n = 4).

814

	HIM0	HIM25	HIM50	VEG	SEM	<i>p</i> -value	
ADC _{DM}	71.5 ^b	70.6 ^b	72.4 ^b	77.0ª	0.713	0.000	
ADC_CP	88.5 ^b	86.5°	86.6°	90.4ª	0.436	0.000	
ADC_GE	83.3 ^b	81.7 ^b	81.4 ^b	85.8ª	0.511	0.001	

Abbreviations: HIM, Hermetia illucens larvae meal; VEG, vegetable protein based diet; SEM,

standard error of the mean; p, probability; DM, dry matter; CP, crude protein; GE, gross energy.

Different superscripts within a row indicate significant differences ($p \le 0.05$).