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

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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 (HIM0), 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

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 anti-

94 nutritional factors (Spranghers et al., 2017). One of the most promising insect species to partially
95 replace FM is the Black Soldier Fly (*Hermetia illucens* L. - HI; Stratiomyidae family). HI is able to
96 convert low value by-products and wastes into protein and fat sources (Spranghers et al., 2017;
97 Meneguz et al., 2018) representing a sustainable way to produce edible proteins for livestock
98 feeding. The partial substitution of FM with HI larvae meal (HIM) has been successfully tested in
99 various fish species including rainbow trout (*Oncorhynchus mykiss* Walbaum) (Renna et al., 2017;
100 Elia et al., 2018; Huyben et al., 2018), Atlantic salmon (*Salmo salar* L.) (Lock et al., 2016; Belghit et
101 al., 2018a), European seabass (*Dicentrarchus labrax* L.) (Magalhães et al., 2017), yellow catfish
102 (*Pelreobagrus fulvidraco* Richardson) fry (Xiao et al., 2018), Jian carp (*Cyprinus carpio* var. Jian) (Li
103 et al., 2017; Zhou et al., 2018), and Nile tilapia (*Oreochromis niloticus* L.) (Devic et al., 2018).
104 The production of caviar and sturgeon's meat is a financially relevant aquaculture sector.
105 Compared to other sturgeon's species, Siberian sturgeon (*Acipenser baerii* Brandt) has a short
106 reproductive cycle (7 to 8 years) and can be reared in fresh water (Bronzi et al., 2011). For these
107 reasons, Siberian sturgeon is one of the most commonly farmed sturgeons (Şener et al., 2006;
108 Bronzi et al., 2011). Compared to other species (e.g., Atlantic salmon, rainbow trout, and European
109 sea bass), less attention has been paid to the nutrition requirements of sturgeons. However, the
110 research of FM substitutes is a crucial point for the future of sturgeon rearing and production
111 (Sicuro et al., 2015). In the last ten years, several authors have evaluated the use of vegetable
112 ingredients other than soybean meal (Liu et al., 2009), like spirulina microalgae (Palmegiano et al.,
113 2008), rice concentrate (Sicuro et al., 2015) and sesame oil cake or corn gluten (Jahanbakhshi et
114 al., 2013) or animal proteins such as meat and bone, poultry by-products or feather meals (Liu et
115 al., 2009; Zhu et al., 2011) as FM substitutes in sturgeon's diets concluding that a partial FM
116 replacement with these alternative protein sources is feasible without adverse effects.

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 (HIM0), 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 HIM0 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 pellet preparation. The pellets were obtained using a 3.0 mm die meat grinder and were dried at 50°C for 48 h. The diets were stored in dark bags at -20°C until utilization. The ingredients of the 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 ± 1°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 ± 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.

165

166 *2.3 Sampling*

167 At the end of the growth trial, after 24 h of fasting, 6 fish per tank (24 fish per treatment) were
168 sacrificed by over anaesthesia (MS-222, 300 mg L^{-1}), individually weighed (KERN PLE-N v. 2.2; KERN
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
173 Institute of Health, USA) for the calculation of the Fulton's condition factor (K). Subsequently, 3 of
174 the 6 fish sampled (12 fish per treatment) were frozen, finely ground with a knife mill (Grindomix
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
177 eviscerated; the liver and viscera were weighed to calculate the hepatosomatic (HSI) and
178 viscerosomatic (VSI) indices, respectively.

179

180 *2.4 Data calculation*

181 The growth performance indices were calculated as follows:

182 • Survival rate (SR, %) = (number of final fish / number of fish at start) $\times$ 100;

183 • Weight gain (WG, g) = iFBW (g) – iIBW (g);

184 • Specific growth rate (SGR, % day^{-1}) = [(lnFBW - lnIBW) / number of days] $\times$ 100;

185 • Feed conversion ratio (FCR) = total feed supplied (g, dry matter (DM)) / tank WG (g);

186 • 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;

191 • HSI (%) = [liver weight (g) / fish weight (g)] $\times$ 100;

192 • VSI (%) = [viscera weight (g) / fish weight (g)] $\times$ 100.

193

194 *2.5 Diets digestibility*

195 At the end of the growth trial, the remaining fish (on average 16 per tank) were used for the
196 digestibility trial. The fish were maintained in their respective tanks and fed the same
197 experimental diets added with 1% celite® (Fluka, St. Gallen, Switzerland) as inert marker. The diets
198 were prepared as described in section 2.1, and the inert marker was added at the expense of 1%
199 of starch gelatinized. Feed was administered by hand to apparent visual satiation three times per
200 day (8:00, 14:00 and 20:00 h), six days per week. After the first feed administration, all the fish
201 were placed in a metal cage that was perforated on the bottom side, and placed on a rigid plastic
202 tray. In this way, the faeces were settled in the plastic tray, allowing their sampling. As described
203 in Guo et al. (2012), four hours after the meal, the faeces were collected using a suction pipe,
204 placed on blotting paper to remove the excess water and immediately frozen (-80°C) until
205 analysed. After the first faeces collection, the fish were released in their tanks and the same
206 procedure was performed after the second feed administration. To avoid digestibility
207 overestimation, only intact faeces were collected, and faeces collection lasted 20 days. The
208 apparent digestibility coefficients (ADC) of DM, CP and GE were calculated as reported by Renna et
209 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 mg 100 g^{-1} ww. All chemical analyses were performed in duplicate.

2.7 Statistical analyses

Data were analysed by one-way ANOVA using IBM SPSS Statistics v. 25.0 for Windows. The following model was used: $Y_{ij} = \mu + D_i + \varepsilon_{ij}$

where Y_{ij} = observation; μ = overall mean; D_i = effect of diet (HIM0, HIM25, HIM50, VEG); ε_{ij} = 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 $p \leq 0.05$.

3. Results

3.1 Chemical composition of insect meal and experimental diets

The proximate composition of HIM and of the experimental diets are reported in Table 1. The DM, 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 HIM0 and HIM100, respectively), while the chitin content increased at increasing inclusion levels of HIM.

The FA composition of HIM and of the experimental diets is shown in Table 2. The most represented FA in HIM was lauric acid (C12:0), followed by palmitic (C16:0), myristic (C14:0) and oleic (C18:1 *c*9) acids (2020.65, 435.36, 325.51 and 303.20 mg $100g^{-1}$ DM, respectively). In the experimental diets containing HIM, lauric acid increased proportionally at the inclusion of insect meal. The concentrations of C12:0 in the diets containing HIM were noticeably higher than those observed in HIM0 and VEG. When compared to the other diets, the HIM0 diet showed a higher amount of total n3 FA (1348.15 mg $100g^{-1}$ DM), with C22:6 n3 as the most represented individual FA in this group (666.59 mg $100g^{-1}$ DM). The total n3 polyunsaturated fatty acids (PUFA) decreased proportionally at increasing levels of HIM. The VEG diet showed a lower concentration of total saturated fatty acids (SFA) and was richer in total n6 PUFA (in particular linoleic acid, C18:2 n6)

when compared to the other diets. HIM0 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 HIM0, 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 HIM0. 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 considered dependent variables.

3.3 Biometric and morphometric indices

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

284 those fed with HIM0 and VEG, and a significantly higher EE (+38.4%) content when compared to
285 those fed with HIM0. The DM and EE contents were comparable among HIM0, HIM25 and VEG.
286 The CP and ash contents were unaffected by the treatment.
287 As far as the FA composition is concerned, the concentration of C12:0 significantly increased (from
288 2.70 to 61.23 and 151.61 mg 100 g^{-1} ww in HIM0, HIM25 and HIM50, respectively) as the level of
289 HIM increased in the diet, while VEG and HIM0 showed comparable values. The fish fed with
290 HIM50 showed a higher concentration of C14:0 when compared to the fish fed with HIM0 and VEG
291 (+47.9% in both cases), while the fish fed with HIM25 showed intermediate values. Various
292 branched chain fatty acids (BCFA) were detected, but only a few of them (C17 *anteiso* and C18 *iso*)
293 were affected by the diet. The concentration of total BCFA in the fish whole body did not
294 significantly differ among the treatments. The concentration of C18:1 *c*9 was significantly higher in
295 the fish fed with HIM50 than in those fed with HIM0 (869.27 and 600.58 mg $100g^{-1}$ww,
296 respectively), while the fish fed with HIM25 and VEG showed intermediate values. The
297 concentrations of C18:2 n6, C18:3 n6, and total n6 PUFA were the highest in the fish fed with VEG,
298 followed by the fish fed with HIM50 and HIM25, and finally by those fed with HIM0. As regard to
299 individual long-chain n3 PUFA, the absolute lowest values were recorded in the fish fed with VEG
300 (C20:5 n3) and HIM0 (C22:5 n3 and C22:6 n3), while the absolute highest values were observed in
301 the fish fed with HIM25. The Σ n3 / Σ n6 FA ratio in fish whole body ranked in the order HIM0 =
302 HIM25 > HIM50 > VEG.
303
304 *3.5 Digestibility trial*
305 The ADCs of the experimental diets are reported in Table 6. The VEG diet showed significantly
306 higher values than all the other diets for all the considered ADCs. ADC_{DM} and ADC_{GE} did not differ

307 significantly among HIM0, HIM25 and HIM50, while ADC_{CP} was significantly lower in HIM25 (-
308 2.3%) and HIM50 (-2.1%) when compared to HIM0.
309
310 **4. Discussion**
311 Insects, such as Diptera and Coleoptera, are part of the natural diet of Siberian sturgeons (Pyka
312 and Kolman, 2003). To our knowledge no studies using HIM as protein source have been carried
313 out with sturgeons yet, and our results indicate that up to 18.5% of highly defatted HIM can be
314 included without impairing survival rate and growth performance in this species. Nevertheless,
315 WG and SGR decreased with the increase of HIM in the diets as a consequence of a decrease in
316 feed consumption. At 75% of HIM inclusion (total FM substitution – HI100), we observed a
317 noticeable decrease in feed ingestion by the sturgeons. The decrease in feed consumption
318 recorded with the increase of HIM could be attributable to different causes. One factor could be
319 the chitin content of the diets. It has been reported how even low levels of chitin could decrease
320 feed consumption in fish (Gopalakannan and Arul, 2006; Olsen et al., 2006; Kroeckel et al., 2012).
321 In particular, when feeding turbot juveniles (*Psetta maxima*) with diets containing insect meals,
322 Kroeckel et al. (2012) faced a decrease of feed acceptance at increasing levels of insect meal, and
323 the diet with 75.6% of inclusion of HI prepupae meal showed the lowest daily feed intake and
324 performances. These authors argued that the dietary level of chitin could have been one of the
325 reasons due to the fact that turbot is a fish without gut chitinase activity. Chitin, an indigestible
326 polysaccharide, is a primary component of the exoskeleton of arthropods (such as insects and
327 shrimps). In insects, the amount of chitin varies according to the species and the development
328 stage (Finke, 2007). In the current trial, following the method proposed by Finke (2007), the chitin
329 contents of the experimental diets were equal to 0 (HIM0 and VEG), 0.72 (HIM25), 1.92 (HIM50)
330 and 3.75 (HIM100) g $100g^{-1}$ as fed. Not all studies performed using insect meals reported

331 palatability problems (Lock et al., 2016; Magalhães et al., 2017; Renna et al., 2017; Belghit et al.,
332 2018a; Devic et al., 2018). It could be argued that these inconsistent results are due to differences
333 in meal composition and processing (as already reported for other PAPs) or as a consequence of
334 the presence/absence of endogenous chitinases in the investigated fish species such as Atlantic
335 salmon, European seabass, rainbow trout and Nile tilapia. Another cause could be the excessive
336 hardness of the pellet observed in the production phase, particularly in the experimental diet
337 where HIM completely substituted FM (HIM100). In white sturgeons (*A. transmontanus*
338 Richardson) larvae, Gawlica et al. (2002) reported that the physical texture of the diet significantly
339 affected feed intake, with negative effects being associated with dry and hard diets.
340 The absolute lowest FC recorded in the fish fed with HIM50 (37.5% of HIM inclusion) reduced the
341 nutrients intake and thus the final fish weight and SGR (1.48). The fish fed HIM25 (18.5% of HIM
342 inclusion) showed comparable FC values than those fed HIM50. They also suffered a reduction of
343 iFBW and SGR when compared to the fish fed HIM0, but such difference was not statistically
344 significant, as it was instead for the fish fed HIM50. On the contrary, Renna et al. (2017) showed
345 that an inclusion of up to 40% of a partially defatted HIM did not affect the FBW and SGR of
346 rainbow trout. Moreover, Xiao et al. (2018) reported better weight gain rate and SGR than a
347 control diet (containing fish and soybean meals as primary protein sources) when yellow catfish
348 were fed with diets containing up to 22.3% of HIM inclusion while, when the inclusion level was
349 higher than 34.3%, a decrease of growth performance was observed. The inconsistency among
350 available literature may be also due to the different fish species considered in the trials.
351 The overall reduction of the growth performance observed in this study can also be related to the
352 reduction of ADC_{CP}, which occurred already at 18.5% level of HIM inclusion in the diet. The
353 observed decrease of protein digestibility could be determined by the presence of chitin in the
354 insect meal (Renna et al., 2017; Zhou et al., 2018). In European seabass, the inclusion up to 19.5%

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 HIM0 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} 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

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

403 Results on the effects of the dietary inclusion of insect meals on the proximate composition of fish
404 lack consistency in the available literature. Similarly to what previously found by Renna et al.
405 (2017) in rainbow trout fillets, in the current trial the increase of HIM in the diets caused a
406 progressive increase of the DM and EE contents of the sturgeons whole body. In European sea
407 bass juveniles, no significant differences were observed in DM and EE contents of whole body at
408 different inclusion levels of a full-fat *Tenebrio molitor* larvae meal (Gasco et al., 2016). Kroeckel et
409 al. (2012) found decreasing DM and EE contents in the whole body of turbot juveniles while
410 increasing the inclusion level of HI prepupae meal in the experimental diets. As far as HIM0 and
411 diets containing HIM are concerned, the results obtained in our trial are somehow ambiguous.
412 Even if not statistically significant, from Table 4 we can observe an increase in HSI and VSI values
413 from HIM0 to HIM50. Contemporarily, a general decrease of the growth performance of the fish
414 was observed (Table 3). An increase in the TFA content of the whole body was observed in the
415 sturgeons fed with insect meal when compared to the sturgeons fed with the control diet,
416 supporting the recent findings of Belghit et al. (2018b) on freshwater Atlantic salmon. The
417 observed sharp increase (from 0.11% to 4.56% of TFA) in the content of lauric acid at the increase
418 of HIM inclusion in the diet was expected. In fact, as outlined in Table 2, the HIM used in this trial
419 was very rich in lauric acid. This confirms the recent findings of other authors who investigated the
420 chemical composition of HI larvae and prepupae when fed different rearing substrates (Liland et
421 al., 2017; Spranghers et al., 2017; Meneguz et al., 2018). Concerning SFA, a significant increase in
422 the fish whole body was also found for the contents of myristic and arachidic (C20:0) acids, even if
423 to a lesser extent. Particularly because of the substantial increase in C12:0, the total SFA
424 concentration of the sturgeon whole body was found to increase while increasing the level of
425 dietary HIM inclusion. Similar findings were reported by other authors on rainbow trout (Mancini
426 et al., 2017; Renna et al., 2017), Jian carp (Zhou et al., 2018) and Atlantic salmon (Belghit et al.,

427 2018b, 2019) fed *H. illucens* larvae or prepupae meal. The chromatographic conditions applied in
428 our trail allowed us detecting various BCFA with a number of carbon atoms ranging between 15
429 and 18 (both *iso* and *anteiso* forms) in the sturgeons whole body. Such findings are in line with the
430 individual BCFA found by Wang et al. (2016) in other common freshwater fish species. Other
431 authors previously reported the FA composition of different sturgeon species, such as white
432 sturgeon (Xu et al., 1993; 1996; Gawlicka et al., 2002), Russian sturgeon (*A. gueldenstaedtii*
433 Brandt) (Şener et al., 2005; Zhu et al., 2017), Gulf sturgeon (*A. oxyrinchus desotoi* Vladykov) (Chen
434 et al., 1995), and the hybrid *A. naccarii* × *A. baerii* (Vaccaro et al., 2005). To the best of our
435 knowledge, only two studies previously investigated the FA composition of Siberian sturgeons
436 (Nieminen et al., 2014; Aidos et al., 2019). Despite the available above-mentioned literature on
437 the FA composition of sturgeons, information on BCFA is very scant. Until now, only Chen et al.
438 (1995) reported BCFA in the muscle samples of cultured and wild Gulf sturgeons. The overall
439 amount of BCFA found in the Siberian sturgeons in our trial (up to 2.30% of TFA) was comparable
440 to that found in ruminant-derived food products (usually considered the most important source of
441 BCFA in the human diet), most probably because we analysed the fish whole body. Indeed Wang
442 et al. (2016) found that fish skin contains on average higher amounts of BCFA compared to
443 different muscle types. Some BCFA were also detected in the insect meal (Table 2). Also
444 Spranghers et al. (2017) found variable amounts of total BCFA in *H. illucens* prepupae reared on
445 different organic waste substrates. However, these authors reported no details regarding the
446 detected individual *iso* and *anteiso* forms, and therefore no comparisons at this regard can be
447 made with our findings. The dietary treatment did not affect the total amount of BCFA, nor the
448 majority of the detected individual *iso* and *anteiso* forms, in the Siberian sturgeons in our trial. In
449 the current trial, the amount of C17 *anteiso* slightly differed in the experimental diets, ranking in
450 the order HIM50 > VEG = HIM25 > HIM0 (Table 2), thus mostly mirroring the concentration of this

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

474 human consumption. Another very promising strategy may be the modulation of the n3 FA
475 concentration in the insects using appropriate growth media (Liland et al., 2017).
476
477 **5. Conclusion**
478 In this first study performed on Siberian sturgeons, results showed that it is possible to replace up
479 to 25% of FM with a highly defatted HIM without impairing the growth performance, condition
480 factor, biometric and morphometric indices, and whole body proximate composition of the fish.
481 Only few variations were observed in the fatty acid composition of the sturgeon whole body
482 already at 25% of fishmeal substitution with the insect meal. For future practical applications, it
483 will be necessary to evaluate different insect species and lower levels of insect meal inclusion in
484 the diets for Siberian sturgeons.
485
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758 **Table 1. Ingredients and proximate composition of *H. illucens* larvae meal and experimental**

759 **diets.**

	HIM	HIM0	HIM25	HIM50	HIM100	VEG
Ingredients (g kg^{-1})						
Fish meal (Chile, super prime)[a]	-	700.0	525.0	350.0	0	320.0
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 concentrate	-	0	0	0	0	200.0
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	20.38	20.44

Abbreviations: HI, *Hermetia illucens*; HIM, *Hermetia illucens* larvae meal; VEG, vegetable protein based diet; DM, dry matter; CP, crude protein; EE, ether extract; NFE, Nitrogen free extracts; GE, gross energy.

[a] Purchased from Corpesca S.A. (Santiago, Chile). Proximate composition (g $100g^{-1}$, as fed basis): 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; B_{12}, 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, 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 sulphate, 20 mg; manganese sulphate, 3 g; sodium fluoride, 1 g (purchased from Granda Zootecnici S.r.l., Cuneo, Italy).

[e] Values are reported as mean of duplicate analyses

[f] Crude Fiber estimated according to Feedipedia and Arraina booklet ingredients database

[g] Estimated as ADF – ADFN (Finke, 2007)

[h] Nitrogen Free Extracts; Calculated as 100 − (CP + EE + Ash + Crude Fiber)

781 **Table 2. Fatty acid profile (mg 100g^{-1} DM) of *H. illucens* larvae meal and experimental diets.**

	HIM	HIM0	HIM25	HIM50	HIM100	VEG
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 *c*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 *c*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 *c*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 *c*9	303.20	930.75	1045.14	1203.62	984.07	1100.78
C18:1 *c*11	9.34	329.89	313.57	325.14	228.54	313.62
C18:1 *c*12	nd	nd	16.70	33.83	20.07	Nd
C18:1 *c*14 + *t*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 *c*9	nd	52.96	68.62	89.99	68.43	80.15
C20:1 *c*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
∑ 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

782 Abbreviations: DM, dry matter; HIM, *Hermetia illucens* larvae meal; VEG, vegetable protein based
783 diet; *c*, *cis*; *t*, *trans*; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA,
784 polyunsaturated fatty acids; TFA, total fatty acids; nd, not detected.
785 Values are reported as mean of duplicate analyses.
786

787 **Table 3. Survival and growth performance of Siberian sturgeon juveniles fed the experimental**

788 **diets (n = 4).**

	HIM0	HIM25	HIM50	VEG	SEM	*p*-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

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

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

794

795

796 **Table 4. Biometric (n = 24) and morphometric indices (n = 12) of Siberian sturgeon juveniles fed**

797 **the experimental diets.**

	HIM0	HIM25	HIM50	VEG	SEM	*p*-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

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

799 standard error of the mean; *p*, probability; K, Fulton's condition factor; HSI, hepatosomatic index;

800 VSI, viscerosomatic index.

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

802

803 **Table 5. Whole body proximate (g 100 g^{-1} ww) and fatty acid (mg 100g^{-1} ww) compositions of**

804 **Siberian sturgeon juveniles fed the experimental diets (n = 12).**

	HIM0	HIM25	HIM50	VEG	SEM	*p*-value
Proximate composition						
DM	21.25^{b}	22.37^{ab}	23.37^{a}	21.30^{b}	0.239	0.002
CP	13.66	14.10	13.96	13.45	0.106	0.116
EE	4.50^{b}	5.13^{ab}	6.23^{a}	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}	151.61^{a}	2.42^{c}	9.364	0.000
C14:0	140.46^{b}	178.88^{ab}	207.80^{a}	140.53^{b}	7.216	0.001
C15 *iso*	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 *c*9 + C15:0	20.13^{ab}	23.75^{ab}	24.10^{a}	19.36^{b}	0.705	0.024
C16 *iso*	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 *iso*	15.20	16.63	14.20	13.09	0.534	0.110
C17 *anteiso*	16.95^{b}	20.76^{ab}	23.68^{a}	21.18^{ab}	0.791	0.021
C16:1 *c*9	209.56^{b}	264.07^{ab}	289.54^{a}	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}	5.47^{a}	4.08^{b}	4.12^{ab}	0.196	0.012
C17:1 *c*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 *t*9-11	4.45^{b}	6.10^{ab}	5.84^{ab}	7.15^{a}	0.258	0.001

C18:1 *c*9	600.58^{b}	828.26^{ab}	869.27^{a}	747.45^{ab}	34.283	0.024
C18:1 *c*11	142.82	180.69	180.24	168.59	5.705	0.059
C18:1 *c*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}	180.38^{a}	7.438	0.000
C18:3 n6	3.16^{c}	7.71^{b}	8.64^{b}	11.90^{a}	0.557	0.000
C18:3 n3	19.10^{b}	30.46^{a}	32.04^{a}	30.39^{a}	1.392	0.001
C20:0	12.21^{c}	19.97^{b}	22.26^{ab}	27.04^{a}	1.172	0.000
C20:1 *c*9	41.57^{b}	61.05^{a}	58.10^{a}	58.89^{a}	2.021	0.001
C20:1 *c*11	151.74^{b}	221.46^{a}	216.53^{a}	222.42^{a}	7.966	0.001
C20:2 n6	8.25^{b}	12.79^{a}	13.17^{a}	13.69^{a}	0.532	0.000
C20:3 n6	2.79^{b}	4.80^{a}	4.92^{a}	5.96^{a}	0.240	0.000
C20:4 n6	13.85^{b}	19.38^{a}	15.41^{b}	14.82^{b}	0.587	0.003
C20:5 n3	101.20^{b}	151.76^{a}	117.81^{ab}	88.02^{b}	6.348	0.001
C22:5 n3	23.96^{b}	42.46^{a}	39.59^{a}	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}	1213.25^{a}	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}	1633.93^{a}	1446.68^{ab}	60.278	0.028
∑ PUFA	351.28^{b}	564.24^{a}	524.71^{a}	506.84^{a}	21.537	0.002
∑ PUFA / ∑ SFA	0.42^{c}	0.53^{ab}	0.43^{bc}	0.57^{a}	0.015	0.000
∑ n3	251.86^{b}	389.38^{a}	344.42^{ab}	280.10^{b}	15.261	0.003
∑ n6	99.42^{c}	174.87^{b}	180.29^{b}	226.74^{a}	8.865	0.000
∑ n3 / ∑ n6	2.51^{a}	2.29^{a}	1.87^{b}	1.24^{c}	0.084	0.000
TFA	2373.22^{b}	3242.37^{a}	3371.90^{a}	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 ($p \leq 0.05$).
811

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

	HIM0	HIM25	HIM50	VEG	SEM	*p*-value
ADC_{DM}	71.5^{b}	70.6^{b}	72.4^{b}	77.0^{a}	0.713	0.000
ADC_{CP}	88.5^{b}	86.5^{c}	86.6^{c}	90.4^{a}	0.436	0.000
ADC_{GE}	83.3^{b}	81.7^{b}	81.4^{b}	85.8^{a}	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 ($p \leq 0.05$).