

1 **Growth performance, feed utilisation and body composition of**  
2 **advanced nursing Nile tilapia (*Oreochromis niloticus*) fed diets**  
3 **containing Black Soldier Fly (*Hermetia illucens*) larvae meal**

4

5 Emilie Devic<sup>1</sup>, William Leschen<sup>1</sup>, Francis Murray<sup>1</sup>, and David C. Little<sup>1</sup>

6 <sup>1</sup>*Institute of Aquaculture, School of Natural Sciences, University of Stirling, UK*

7

8

9 Correspondence:

10 Emilie Devic, Institute of Aquaculture, University of Stirling, Stirling, FK9 4LA, UK.

11 Email: e.d.devic@stir.ac.uk

12 Phone: +44 (0) 1786 467874; Fax: +44 (0) 1786 472133

13

14 Accepted for publication in *Aquaculture Nutrition* by Wiley-Blackwell.

15

16 **Running title:** BSF larvae meal in advanced nursing tilapia diets

17

18 **Keywords:** Insect meal, strategic, fingerlings, alternative, nutrient utilisation, feed

19

20 **Abstract**

21 A 32-day experiment was conducted to evaluate the effects on the performance, feed  
22 utilisation efficiency and body composition of a strategic inclusion of Black Soldier Fly  
23 larvae meal (MM) in a commercially formulated diet for advance nursing Nile tilapia  
24 (*Oreochromis niloticus*). Four isonitrogenous and isoenergetic diets were commercially  
25 formulated and manufactured as a control and 3 test diets with strategic inclusions of MM  
26 inclusions (0, 30, 50 and 80 g kg<sup>-1</sup>) and poultry byproduct meal substituting gradually  
27 three conventional expensive feedstuffs: fish meal, fish oil and soybean meal. Fish  
28 (5.7±0.5 g fish<sup>-1</sup>) were nursed in a cage-in-lake system (Volta Lake, Ghana), under  
29 conditions similar to commercial farming practices. Control and experimental diets were  
30 fed to triplicate cages by hand to visual satiety, 6 times day<sup>-1</sup>. Growth performance (final  
31 weight; weight gain and SGR); feed utilisation efficiency indices (FCR and PER) and  
32 feed intake were not significantly different ( $P \geq 0.05$ ) between treatments. Survival was  
33 significantly different ( $P < 0.05$ ) but more likely explained by the stress related to frequent  
34 handling on the smaller fish. Fish whole body composition (dry matter, crude protein,  
35 lipid, ash and fibre) was unaffected by the treatment ( $P \geq 0.05$ ), except for the fatty acid  
36 compositions which mirrored that of the diets.

37

## 38 **Introduction**

39 Farmed fish contribute to food security and represent a rich source of dietary animal  
40 protein, micronutrients and fatty acids (FA) in low-income countries (Beveridge *et al.*  
41 2013). In Ghana, for instance, most aquaculture production (around 80%) consists of Nile  
42 tilapia (*Oreochromis niloticus*) (FAO 2005), but local fish farmers struggle to compete  
43 with cheaper imports from China and are constrained by both availability, quality and  
44 cost of pelleted fish feeds and feed ingredients (Hecht 2007; Rurangwa *et al.* 2015).  
45 Conventional feed ingredients such as fish meal (FM), fish oil (FO) and plants protein  
46 sources (oilseed plants, grain legumes, etc.), for which there is an increasing demand due  
47 to the intensification of farming methods relying on complete fish feeds (Tacon & Metian  
48 2008), are available in low income countries such as Ghana, consisting either of poor  
49 quality local products or high-cost imported items (Gabriel *et al.* 2007; Obirikorang *et al.*  
50 2015). Importance of quality ingredients and artificial feeds, even for herbivorous species  
51 such as tilapia, makes perfect sense at critical stages (juveniles or broodstock) when fish  
52 are maintained under intensive clear-water farming conditions and depend entirely on  
53 nutritionally complete diets (Tacon 1988). Global research for the identification of cost-  
54 effective substitutes to conventional materials continues (El-Sayed & Tacon 1997; El-  
55 Sayed 2004; Karalazos 2007; Hasan *et al.* 2007; Ayoola 2010; Obirikorang *et al.* 2015).  
56 Insect meals such as fly larvae or maggots meals (MM) have been identified as high  
57 protein and valuable feed ingredient for livestock in general (Veldkamp *et al.* 2012; van  
58 Huis *et al.* 2013; Makkar *et al.* 2014) and freshwater fish specifically, given their natural  
59 feeding habits (Bailey & Harrison 1948; Randall 1967; Odesanya *et al.* 2011; Barroso *et*  
60 *al.* 2014; Henry *et al.* 2015). Previous research on tilapia juveniles have shown that both  
61 meals from housefly larvae (*Musca domestica*) and blowfly larvae (*Chrysomya*

62 *megacephala*) can replace up to 100% of the FM in practical diets for tilapia fingerlings  
63 without affecting fish performance compared to FM-based control diets (Ogunji *et al.*  
64 2008a, b, c; Sing *et al.* 2014). On the other hand, fresh Black Soldier Fly (BSF, *Hermetia*  
65 *illucens*) larvae fed whole or chopped to blue tilapia (*Oreochromis aureus*) reduced  
66 significantly the fish growth (Bondari & Sheppard 1987). BSF larvae meal has been used  
67 as a substitute to FM in several fish species diets except tilapia (Makkar *et al.* 2014; Henry  
68 *et al.* 2015). In complete diets, BSF meal, which can be produced locally (Devic *et al.*  
69 2013), may be blended with other good protein sources such as poultry byproduct meal  
70 (PBM) to substitute high-priced FM, FO and soybean meal (SBM). This study  
71 investigates the effects on the performance, feed utilisation efficiency and body  
72 composition of the strategic inclusions BSF larvae meal (MM) in commercially  
73 formulated diets for juvenile Nile tilapia (*O. niloticus*).

74

## 75 **Material and Methods**

### 76 *Diets*

77 BSF larvae (*H. illucens*) meal (MM) was produced within a pilot system located in  
78 Greater Accra (Ghana) described by Charlton *et al.* (2015). Larvae were fed on a substrate  
79 mix composed of 35% spent grain (brewery solid waste) or wheat bran (depending on  
80 availability), 22% processing wastes from a local fish feed factory, 12% yeast slurry  
81 (brewery waste water) and 31% water (bringing the moisture content to approximately  
82 50%) and were harvested after 13 days of development (prior reaching the prepupae  
83 stage). Oven-dried larvae (60-80°C, 2 hours) were subsequently ground into a fine and  
84 homogeneous meal using a flour mill machine. Nutritional composition of the MM was

85 analysed (Table 1) according to the methods described below in order to assist in diet  
86 formulation.

87 Raanan Fish Feed West Africa (Prampram Fishfeed Factory, Ghana) supplied the other  
88 feed ingredients, formulated and prepared the diets. Raanan PG40 commercial diet (370  
89 g kg<sup>-1</sup> crude protein and 95 g kg<sup>-1</sup> total lipid) was used as control for the experiment  
90 (PG40). Three test diets (MM30, MM50 and MM80) were formulated to be comparable  
91 to PG40 (isonitrogenous and isoenergetic) by gradually replacing high-priced imported  
92 FM (20; 50 and 70% substitution, respectively) and SBM (10, 20 and 35% substitution,  
93 respectively) with locally available MM (30, 50 and 80 g kg<sup>-1</sup>, respectively) and cheaper  
94 PBM (80, 100 130 g kg<sup>-1</sup>, respectively). The substitution levels of FM and SBM were  
95 driven by the limited quantity of MM available for the experiment. Then, nutritional  
96 composition (protein levels) was adjusted by addition of PBM as part as a least-cost  
97 strategy. Furthermore, FO was not included in the 3 test diets due to the high lipid content  
98 of the MM (244.5 g kg<sup>-1</sup>). Chemical compositions of the diets was analysed as described  
99 below (Table 1). Commercially packaged diets were kept on-farm under cool and shaded  
100 conditions (25°C, 50-60% relative humidity) and used within two months after  
101 manufacture.

102 [Insert Table 1]

### 103 *Experimental Design*

104 In order to demonstrate the relevance of the results, the experiment was conducted on-  
105 farm (commercial tilapia producer, Volta Lake, Ghana) under conditions similar to  
106 commercial husbandry practices. All-male, hormonally sex-reversed Nile tilapia  
107 fingerlings (*O. niloticus*) were obtained from a local commercial hatchery. Prior the start  
108 of the experiment, twenty-five thousand (25,000) fish were transferred into a single

109 floating cage (3x3 m) suspended in Volta Lake where they were fed six times a day with  
110 a standard diet (480 g kg<sup>-1</sup> crude protein and 50 g kg<sup>-1</sup> total lipid) for 3 weeks as an  
111 acclimation period to the lake conditions. Twelve floating cages (1 m<sup>2</sup> cage<sup>-1</sup>), set up in  
112 the outermost part of the grow-out and nursery site of the farm (500 m from the shore,  
113 water column of 30-35 m depth), were then stocked at random with one thousand five  
114 hundred (1,500) acclimated fingerlings (5.7±0.5 g fish<sup>-1</sup>) each. The experiment was  
115 conducted between the months of September and October 2014, for 32 days which was  
116 equivalent to the commercial advanced nursing period and allowed a body increase of at  
117 least 300% recommended for juvenile fish studies (NRC, 2011). Control and test diets  
118 were distributed daily by hand to triplicates cages; fish were fed to visual satiety, over 6  
119 feeding sessions day<sup>-1</sup> (at regular intervals of 2 hours) and amount of feed distributed was  
120 determined by difference with pre-weighed feed containers prepared daily. Water  
121 temperature (°C), pH and dissolved oxygen (DO; mg L<sup>-1</sup>) were recorded daily at 07:00  
122 hrs and 16:00 hrs using OxyGuard® Handy digital probes (Polaris and pH) immersed at  
123 50 cm under the water surface within cages.

124 At the start and on termination of the experiment, all the fish in each cage were counted  
125 and bulk weighed (Tanita KD 200 digital scale, precision: 0-1000gx1g). Growth was  
126 monitored through intermediate samplings carried out every 10 days, by counting and  
127 recording bulk weights of 3 separate sub-samples from each cage (representing  
128 approximately 20% of the population), using a scoop net of fish concentrated in the corner  
129 of the cage. Fish were starved for 24 hours prior samplings in order to limit stress and  
130 mortalities related to handling. Whole fish samples were collected at the start (n=20 fish  
131 from initial population) and on termination (n=5 fish cage<sup>-1</sup>) of the experiment, following

132 an overdose of metacaine sulfonate (MS-222) anaesthetic. Samples were systematically  
133 pooled and homogenized (on a cage basis) and stored at -20°C until further analyses.

134 *Chemical analyses*

135 MM, diets and whole fish samples were analysed at the University of Stirling (Stirling,  
136 UK) using standard methods to determine dry matter by drying at 110°C until constant  
137 weight (AOAC 1990); ash content by combustion in a muffle furnace at 600°C (AOAC  
138 1990); crude protein using the Kjeldahl method (calculated as N×6.25; Persson 2008);  
139 crude fibre using Foss FiberCap 2021/2023 system (Foss Application Note ASN3801;  
140 Foss Analytical, Hillerød, Denmark) and energy by bomb calorimetry (Gallenkamp  
141 autobomb, calibrated with benzoic acid). Lipid content in MM and diets was determined  
142 by Soxhlet extraction (Soxtec auto extraction unit; Foss Analytical, Hillerød, Denmark;  
143 Christie 2003) following acid hydrolysis (Tecator Soxtec method, Foss Analytical,  
144 Hillerød, Denmark). Total lipid from samples was extracted according to Folch *et al.*  
145 (1957) and determined gravimetrically. Fatty Acid Methyl Esters (FAME) were then  
146 prepared from total lipid by acid-catalysed transesterification (Christie 1993). Extraction  
147 and purification of FAME were performed as described by Tocher & Harvie (1988) and  
148 separated and quantified by gas-liquid chromatography using a Fisons GC-8160 (Thermo  
149 Scientific, Milan, Italy) equipped with a 30 m x 0.32 mm i.d. x 0.25 µm ZB-Wax column  
150 (Phenomenex, Cheshire, UK), ‘on column’ injection and flame ionisation detection.  
151 Hydrogen was used as carrier gas with initial oven thermal gradient of 50°C to 150°C at  
152 40°C.min<sup>-1</sup> to a final temperature of 230°C at 2°C.min<sup>-1</sup>. Individual FAME were  
153 identified by comparison to known standards (Supelco™ 37-FAME mix; Sigma-Aldrich  
154 Ltd., Poole, UK) and published data (Tocher and Harvie, 1988). Data were collected and  
155 processed using Chromcard for Windows (Version 1.19; Thermoquest Italia S.p.A.,

156 Milan, Italy). Fatty acid content ( $\text{g kg}^{-1}$  of sample) was calculated using heptadecanoic  
157 acid (17:0) as internal standard. Amino acid composition of the MM and diets ( $\text{g kg}^{-1}$  of  
158 sample) was determined by ALS Food and Pharmaceutical (Chatteris, UK) using HPLC  
159 method.

#### 160 *Calculations and statistical methods*

161 Fish performance and feed utilisation were evaluated according the following indices:

- 162 ▪ Live Weight Gain (WG, g) = Final live weight (Wf, g) – Initial live weight (Wi, g)
- 163 ▪ Specific Growth Rate (SGR, %  $\text{day}^{-1}$ ) =  $[\text{Ln}(Wf) - \text{Ln}(Wi)] / \text{days} * 100$
- 164 ▪ Food Conversion Ratio (FCR) = Feed distributed (g DM) / WG
- 165 ▪ Daily feeding rate (% biomass  $\text{day}^{-1}$ ) =  $[(\text{Feed distributed (g DM)} / \text{number of feeding}$   
166  $\text{days}) / \text{Biomass (g)}] * 100$
- 167 ▪ Protein Efficiency Ratio (PER) = WG / Total protein fed (g DM)
- 168 ▪ Survival Rate (%) =  $[(\text{Fish stocked initially} - \text{Mortality}) / \text{Fish stocked initially}] * 100$

169 Statistical analyses were carried out using IBM SPSS Statistics software (version 21).  
170 Data were subjected to one-way analysis of variance (ANOVA) followed by Tukey's  
171 HSD test for unplanned multiple comparisons. Correlations between the dietary  
172 inclusions of MM + PBM and the performance or nutritional results were analysed using  
173 Pearson's coefficient. A significance of  $P < 0.05$  was considered for all analyses  
174 performed.

175

#### 176 **Results**

177 Water temperature and dissolved oxygen varied slightly during the course of the  
178 experiment and the diurnal periods with values ranging from 26.8 to 30.5°C and 5.1 to  
179 8.1  $\text{g L}^{-1}$ , respectively. All the values were within tolerance limits for tilapia (Beveridge



180 & McAndrew 2000; El-Sayed 2006). Growth and feed utilisation of the fish fed the  
181 control and experimental diets were not affected by the treatments (Table 2). During the  
182 32-day experimental period, fish grew from an initial weight of  $5.7 \pm 0.5$  g fish<sup>-1</sup> to a final  
183 weight  $16.6 \pm 0.5$  g fish<sup>-1</sup>. Live weight gain and SGR of the fish fed the control and the  
184 MM-based diets were not significantly different ( $P \geq 0.05$ ). Overall feeding response was  
185 good with total amounts of feed distributed ( $26.0 \pm 0.3$  kg cage<sup>-1</sup>) and feeding rates  
186 ( $4.2 \pm 0.2$  % biomass day<sup>-1</sup>) not significantly different between treatments ( $P \geq 0.05$ ),  
187 indicating similar feed intake. Feed utilisation efficiency (FCR and PER) was not  
188 compromised by the dietary treatments ( $P \geq 0.05$ ). However, MM30 diet led to a  
189 significantly lower survival ( $81.7 \pm 1.9$  %) compared to others ( $P < 0.05$ ) and MM80  
190 survival rate ( $90.1 \pm 0.5$  %) was found significantly higher than PG40 ( $86.1 \pm 0.3$  %).

191 [Insert Table 2]

192 Analysed fish body compositions compared between treatments indicated no significant  
193 differences ( $P \geq 0.05$ ) for dry matter, crude protein, crude lipid, ash and crude fibre (Table  
194 3). However, whole carcass FA composition varied significantly between dietary  
195 treatments. Strong linear relationships were found between the dietary inclusion of MM  
196 and selected FA; in particular, MM dietary inclusion was positively correlated to the total  
197 saturated FA ( $r 0.672$ ;  $P < 0.05$ ) and a negatively to the n-3 PUFA ( $r -0.725$ ;  $P < 0.05$ ).

198 [Insert Table 3]

199

## 200 **Discussion**

201 Nowadays, feed formulation strategies account for the feed ingredients nutritional quality  
202 but also cost, availability and sustainability. Local constrains identified in Ghana (cost of  
203 importation, poor quality of local materials, etc.) increase the pressure on feed

204 manufacturers to find cost-efficient alternative feed ingredients to replace always more  
205 costly feedstuffs such as marine ingredients (Gabriel *et al.* 2007; Obirikorang *et al.* 2015).  
206 According to recent publications (Barroso *et al.* 2014; Henry *et al.* 2015), insect meals  
207 from dipterans such as the common housefly (*M. domestica*) or the black soldier fly (*H.*  
208 *illucens*) feature nutritional characteristics similar to FM suggesting that they could be  
209 suitable substitutes for this conventional feed ingredient. Nevertheless, compared to high-  
210 quality FM such as anchovies, herring or menhaden (NRC, 2011), the BSF meal produced  
211 in Ghana for the purpose of the experiment, displayed a lower protein content and higher  
212 lipid content. Similar observation was made by Barroso *et al.* (2014) for a BSF meal  
213 which presented even lower levels of crude protein, lipid and ash (362 g kg<sup>-1</sup>; 180 g kg<sup>-1</sup>  
214 and 93 g kg<sup>-1</sup>, respectively) compared to the BSF meal produced in Ghana. Insect life  
215 stage, feeding substrate and processing methods influence their nutritional composition  
216 (Aniebo & Owen 2010; van Huis *et al.* 2013) which explains the differences reported  
217 here. Also similar to that previously found by Barroso *et al.* (2014), the amino acid profile  
218 of the MM was comparable to conventional FM including 9 out of 10 of the essential  
219 amino acids (BSF meal is known to be low in tryptophan; Newton *et al.* 1977; Henry *et*  
220 *al.* 2015). It is therefore a great source of protein. MM used in the current experiment was  
221 also a rich source of FA, in particular saturated and monounsaturated and it was slightly  
222 richer in EPA and DHA compared to MM used in other studies (St-Hilaire *et al.* 2007b;  
223 Kroeckel *et al.* 2012; Barroso *et al.* 2014) owing to the substrate mix on which the larvae  
224 fed (St-Hilaire *et al.* 2007a). Although MM is locally available (Devic *et al.* 2013) and a  
225 suitable source of nutrients for fish as demonstrated in several other studies (St-Hilaire *et*  
226 *al.* 2007b; Kroeckel *et al.* 2012, Lock *et al.* 2015), inclusion in a complete diet in  
227 substitution of conventional ingredients such as FM, FO and SBM requires adjustments

228 in the formulation. Lower protein content of the MM was therefore corrected by  
229 increasing the PBM inclusion in the test diets, which is considered as a good source of  
230 protein although lacking of some essential amino acids (NRC 2011). In addition,  
231 according to El-Sayed (1998), PBM can replace totally FM in practical diets for tilapia  
232 and that inclusion up to 470 g kg<sup>-1</sup> did not compromise the fish performance. Total  
233 substitution of FO related to the high lipid content of the MM resulted in MM30 and  
234 MM50 dietary lipid contents being about 18% lower than PG40 and MM80. Low-fat diets  
235 are preferred for warmwater omnivorous fish such as tilapia (El-Sayed 2006) and  
236 recommended dietary lipid content for tilapia fingerlings vary between 80 and 120 g kg<sup>-1</sup>  
237 (Jauncey 1998). Greater inclusion of MM in such a formulation would have certainly  
238 led to lipid contents exceeding the recommended range. Nevertheless, a possible solution  
239 could be to use defatted MM instead of crude, which would enable higher inclusion levels  
240 as suggested by Fasakin *et al.* (2003). The FA composition of the MM-based diets was  
241 also affected by the substitution of FM and FO, nonetheless, essential FA requirements  
242 for optimal growth of tilapia fingerlings (C<sub>18</sub> PUFA such as 18:2n-6 and 18:3n-3) were  
243 satisfied (NRC 2011). Finally the formulation strategy applied in the current allowed the  
244 production of comparable diets in terms of macronutrients and meeting the requirements  
245 for tilapia fingerlings (Jauncey 1998; El-Sayed 2006; NRC 2011).

246 Fish performance were acceptable for tilapia farmed in cages (El-Sayed 2013) and not  
247 significantly different among treatments, indicating that dietary treatments did not  
248 compromise the growth. This result is comparable to those of other studies where MM or  
249 PBM were used as alternative ingredients in practical diets for tilapia in substitution of  
250 FM (Ogunji *et al.* 2008a, b; El-Sayed 2013; Sing *et al.* 2014). Also, similar to that  
251 previously reported in other studies (Fasakin *et al.* 2003; Ogunji *et al.* 2008a;

252 Karapanagiotidis *et al.* 2014; Sing *et al.* 2014), overall survival was good during the 32-  
253 day experimental period. Significant differences in survival were more likely explained  
254 by the stress related to frequent sampling and handling which would have more deeply  
255 affected the smaller fish (Bolivar *et al.* 2004; MacNiven & Little 2001); indeed, although  
256 initial weights were not significantly different between treatments, the fish stocked at a  
257 slightly smaller size had significantly lower survival rates than the larger fish. In addition,  
258 in comparison with other treatments, the significantly lower survival of MM30-fed fish  
259 could explain the slightly (but not significant) better weight gain and SGR ( $11.8 \pm 1.9$  g  
260 fish<sup>-1</sup> and  $3.7 \pm 0.4$  % day<sup>-1</sup>, respectively) probably owing to a reduction of the competition  
261 for the resources.

262 The feeding method applied in the experiment (manual distribution), which is common  
263 practice in countries where labor costs are low, limits feed wastage and prevents  
264 starvation as it is based on the fish feeding response (El-Sayed 2013). Multiple feeding  
265 can also improve growth and feed efficiency in species such as tilapia with relatively  
266 small stomachs and a continuous foraging behaviour (Shiau 2002; NRC 2011). Feed  
267 utilisation efficiency, measured through feeding rates, FCR and PER, was comparable  
268 between treatments. Feed intake was not affected by the MM dietary inclusions and the  
269 retroactively calculated feeding rates indicated that the fish were appropriately fed.  
270 Indeed, at 28°C, it is recommended to feed 5 to 20 g tilapia fingerlings at 6-4 % biomass  
271 day<sup>-1</sup> (Shiau 2002; Ng & Romano 2013). Palatability of feeds containing insect meal  
272 seems to be related to various factors such as the fish species and its feeding response but  
273 also the insect meal characteristics (species, farming and processing methods) (Henry *et*  
274 *al.* 2015). For instance, a diet containing defatted BSF meal seemed to be poorly palatable  
275 for juvenile turbot, *Psetta maxima* (Kroeckel *et al.* 2012), whereas inclusion of blowfly

276 meal in juvenile red tilapia feed did not affect the feed intake (Sing *et al.* 2014). Consistent  
277 with other studies (Ogunji *et al.* 2008; Sing *et al.* 2014), PER values were comparable  
278 between dietary treatments indicating that dietary proteins were similarly and efficiently  
279 used by the fish fed the different diets (Steffens 1989; De Silva & Anderson 1994).

280 The proximate composition of the fish was also not affected by the dietary treatments.  
281 However, the FA profile of the fish carcasses mirrored that of the diets and strong  
282 correlations indicated that dietary inclusions of MM, in particular its FA composition,  
283 influenced the FA composition of the whole fish body. The total substitution of the FO  
284 in the 3 experimental diets explained the n-6 and n-3 PUFA levels (respectively  
285 increasing and decreasing with increasing MM inclusions). Sánchez-Muros *et al.* (2015)  
286 made similar observations while replacing 50 % FM and 100 % FO with a *Tenebrio*  
287 *molitor* larvae meal in a diet for Nile tilapia fingerlings. At the juvenile stages, the farmers  
288 prioritize optimal growth and survival, using cost-effective and sustainable feeds and  
289 ingredients, and FA composition of the fish carcass is less concerning than for a market-  
290 size fish (Turchini *et al.* 2009). To restore the n-3 PUFA levels, which have beneficial  
291 effects on human health (Ruxton *et al.* 2004), finishing diets containing essential PUFA  
292 could be used during the last weeks of farming (fattening stage), improving, therefore,  
293 the nutritional quality of the marketable size fish (Karapanagiotidis *et al.* 2007).

294 Commercial aquafeed manufacturers continue to produce feeds for tilapia including 20  
295 to 250 g kg<sup>-1</sup> FM due to its high nutritional quality (FAO 2012). In the current study, the  
296 absence of differences between the fish growth performance, feed utilisation and body  
297 composition under the different dietary treatments lead to the conclusion that inclusions  
298 of up to 80 g kg<sup>-1</sup> MM and 130 g kg<sup>-1</sup> PBM, substituting FO totally, up to 70% FM and  
299 35% SBM do not affect the feed quality for advanced nursing tilapia. Providing that the

300 market price of the MM is competitive, feed production costs would be alleviated by the  
301 reduction of FM, FO and SBM and the strategic use of quality ingredients such as MM  
302 and PBM to balance the diet. More broadly, inclusions of cheaper, sustainable and locally  
303 available feedstuffs in juvenile tilapia commercial feed could support the sustainable  
304 intensification of aquaculture and contribute more widely to food security.  
305

306 **Acknowledgements**

307 This study and findings are part of the research carried out through the EU FP7  
308 PROteINSECT project (Grant Agreement n°312084). Authors would like to  
309 acknowledge the help and support of Raanan Fish Feed, the tilapia farm owner and its  
310 professional staff and to Nicole Pelusio (intern).

311

312 **References**

- 313 Aniebo, A.O. & Owen, O.J. (2010) Effects of age and method of drying on the  
314 proximate composition of housefly larvae (*Musca domestica* Linnaeus) meal  
315 (HFLM). *Pakistan J. Nutr.*, 9, 485–487.
- 316 AOAC (1990) Official Methods of Analysis, 15<sup>th</sup> ed. Association of Official Analytical  
317 Chemists, Inc., Washington, D.C., USA.
- 318 Ayoola, A.A. (2010) Replacement of Fishmeal with Alternative Protein Sources in  
319 Aquaculture Diets. North Carolina State University.
- 320 Bailey, R.M. & Harrison, H.M.J. (1948) Food Habits of the Southern Channel Catfish  
321 (*Ictalurus Lacustris Punctatus*) in the Des Moines River, Iowa. *Trans. Am. Fish.*  
322 *Soc.*, 75, 110–138.
- 323 Barroso, F.G., de Haro, C., Sánchez-Muros, M.-J., Venegas, E., Martínez-Sánchez, A.  
324 & Pérez-Bañón, C. (2014) The potential of various insect species for use as food  
325 for fish. *Aquaculture*, 422-423, 193–201.
- 326 Beveridge, M.C.M. & McAndrew, B.J. (2000) *Tilapias: Biology and Exploitation.*  
327 Kluwer Academic Publishers.
- 328 Beveridge, M.C.M., Thilsted, S.H., Phillips, M.J., Metian, M., Troell, M. & Hall, S.J.  
329 (2013) Meeting the food and nutrition needs of the poor: the role of fish and the  
330 opportunities and challenges emerging from the rise of aquaculture. *J. Fish Biol.*,  
331 83, 1067–1084.
- 332 Bolivar, R.B., Jimenez, E.B.T., Sugue, J.R.A. & Brown, C.L. (2004) Effect of stocking  
333 sizes on the yield and survival of Nile tilapia (*Oreochromis niloticus* L.) on-grown  
334 in ponds. In: 6th International Symposium on Tilapia in Aquaculture Philippine



335 International Convention Center Roxas Boulevard, Manila, Philippines September  
336 12-16, 2004. pp. 574–583.

337 Bondari, K. & Sheppard, D.C. (1987) Soldier fly, *Hermetia illucens* L., larvae as feed  
338 for channel catfish, *Ictalurus punctatus* (Rafinesque), and blue tilapia,  
339 *Oreochromis aureus* (Steindachner). *Aquac. Fish. Manag.*, 18, 209–220.

340 Charlton, A.J., Dickinson, M., Wakefield, M.E., Fitches, E., Kenis, M., Han, R., Zhu,  
341 F., Kone, N., Grant, M., Devic, E., Bruggeman, G., Prior, R. & Smith, R. (2015)  
342 Exploring the chemical safety of fly larvae as a source of protein for animal feed. *J.*  
343 *Insects as Food Feed*, 1, 7–16.

344 Christie, W.W. (1993) Preparation of Ester Derivatives of Fatty Acids for  
345 Chromatographic Analysis. In: *Advances in Lipid Methodology -Two* (Christie,  
346 W.W. ed.), pp. 69–111. The Oily Press, Dundee, UK.

347 Christie, W.W. (2003) *Lipid Analysis - Isolation, Separation, Identification and*  
348 *Structural Analysis of Lipids*, 3<sup>rd</sup> ed. The Oily Press, Dundee, UK.

349 De Silva, S.S. & Anderson, T.A. (1994) *Fish nutrition in aquaculture*, 1<sup>st</sup> ed. Chapman  
350 & Hall, London, UK.

351 Devic, E., Little, D.C., Leschen, W. & Jauncey, K. (2013) A Model for Substitution of  
352 Fishmeal with Maggot- Meal in Tilapia Feeds - A Commercial Production Farm in  
353 West Africa. *Isr. J. Aquac. - BAMIGDEH*, ISTA 10 special Issue.

354 El-Sayed, A.-F.M. (1998) Total replacement of fish meal with animal protein sources in  
355 Nile tilapia, *Oreochromis niloticus* (L.), feeds. *Aquac. Res.*, 29, 275–280.

356 El-Sayed, A.-F.M. (2004) Protein nutrition of farmed tilapia: searching for  
357 unconventional sources. In: *New Dimensions in Farmed Tilapia: Proceedings of*

358 the Sixth International Symposium on Tilapia Aquaculture. pp. 364–378.

359 El-Sayed, A.-F.M. (2006) Tilapia Culture. CABI Publishing, Wallingford, UK.

360 El-Sayed, A.-F.M. (2013) Tilapia feed management practices in sub-Saharan Africa. In:

361 On-farm feeding and feed management in aquaculture (M.R. Hasan M.B. New

362 eds.), FAO Fish. Aquac. Tech. Pap. No. 583, pp. 377–405. FAO, Rome, Italy.

363 El-Sayed, A.-F.M. & Tacon, A.G.J. (1997) Fishmeal replacers for tilapia : A review. In:

364 Feeding Tomorrow’s Fish (Tacon, A.G.J. & Basurco, B. eds.). Cahiers Options

365 Méditerranéennes, 22. pp. 205–224.

366 FAO (2005) National Aquaculture Sector Overview. Ghana. National Aquaculture

367 Sector Overview Fact Sheets. In: National Aquaculture Sector Overview Fact

368 Sheets. FAO Fisheries and Aquaculture Department [online].

369 FAO (2012) The State of World Fisheries and Aquaculture 2012. FAO, Rome, Italy.

370 Fasakin, E.A., Balogun, A.M. & Ajayi, O.O. (2003) Evaluation of full-fat and defatted

371 maggot meals in the feeding of clariid catfish *Clarias gariepinus* fingerlings.

372 Aquac. Res., 34, 733–738.

373 Folch, J., Lees, M. & Sloane Stanley, G.H. (1957). A simple method for isolation and

374 purification of total lipides from animal tissues. J. Biol. Chem., 226, 497–509.

375 Gabriel, U.U., Akinrotimi, O. a, Bekibele, D.O., Onunkwo, D.N., Anyanwu, P.E. &

376 Harcourt, P. (2007). Locally produced fish feed : potentials for aquaculture

377 development in subsaharan Africa. African J. Agric. Res., 2, 287–295.

378 Hasan, M.R., Hecht, T., De Silva, S.S. & Tacon, A.G.J. (2007) Study and analysis of

379 feeds and fertilizers for sustainable aquaculture development. FAO Fisheries

380 Technical Paper No. 497, pp.510. FAO, Rome, Italy.

381 Hecht, T. (2007) Review of feeds and fertilizers for sustainable aquaculture  
382 development in sub-Saharan Africa. In Study and analysis of feeds and fertilizers  
383 for sustainable aquaculture development (Hasan M.R., Hecht T., De Silva S.S. &  
384 Tacon A.G.J. eds). FAO Fish. Tech. Pap. No. 497, pp. 77–109. FAO, Rome, Italy.

385 Henry, M., Gasco, L., Piccolo, G. & Fountoulaki, E. (2015) Review on the use of  
386 insects in the diet of farmed fish: Past and future. *Anim. Feed Sci., Technol.* 203,  
387 1–22.

388 Jauncey, K. (1998) *Tilapia Feeds and Feeding*. Pisces Press Ltd., Stirling, UK.

389 Karalazos, V. (2007) Sustainable alternatives to fish meal: Effects on growth, tissue  
390 fatty acid composition and lipid metabolism. University of Stirling.

391 Karapanagiotidis, I.T., Bell, M. V., Little, D.C. & Yakupitiyage, A. (2007) Replacement  
392 of dietary fish oils by alpha-linolenic acid-rich oils lowers omega 3 content in  
393 tilapia flesh. *Lipids*, 42, 547–559.

394 Karapanagiotidis, I.T., Daskalopoulou, E., Vogiatzis, I., Rumbos, C., Mente, E. &  
395 Athanassiou, C.G. (2014) Substitution of Fishmeal by Fly *Hermetia illucens*  
396 Prepupae Meal in the Diet of Gilthead Seabream (*Sparus aurata*). *HydroMedit*  
397 2014, 110–114.

398 Kroeckel, S., Harjes, A.-G.E., Roth, I., Katz, H., Wuertz, S., Susenbeth, A. & Schulz, C.  
399 (2012) When a turbot catches a fly: Evaluation of a pre-pupae meal of the Black  
400 Soldier Fly (*Hermetia illucens*) as fish meal substitute - Growth performance and  
401 chitin degradation in juvenile turbot (*Psetta maxima*). *Aquaculture*, 364-365, 345–  
402 352.

403 Lock, E.-J., Arsiwalla, T. & Waagbø, R. (2015) Insect larvae meal as an alternative

404 source of nutrients in the diet of Atlantic salmon (*Salmo salar*) postsmolt. Aquac.  
405 Nutr. Doi:10.1111/anu.12343

406 MacNiven, A.M. & Little, D.C. (2001) Development and evaluation of a stress  
407 challenge testing methodology for assessment of Nile tilapia (*Oreochromis*  
408 *niloticus*, Linn.) fry quality. Aquac. Res., 32, 671–679.

409 Makkar, H.P.S., Tran, G., Heuzé, V. & Ankers, P. (2014) State-of-the-art on use of  
410 insects as animal feed. Anim. Feed Sci. Technol., 197, 1–33.

411 Newton, G.L., Booram, C.V., Barker, R.W. & Hale, O.M. (1977) Dried *Hermetia*  
412 *illucens* Larvae Meal as a supplement for swine. J. Anim. Ecol., 44, 395–400.

413 Ng, W.-K.K. & Romano, N. (2013) A review of the nutrition and feeding management  
414 of farmed tilapia throughout the culture cycle. Rev. Aquac., 5, 220–254.

415 NRC (2011) Nutrient requirements of fish and shrimp. National Research Council,  
416 National Academy Press, Washington DC, USA.

417 Obirikorang, K.A., Amisah, S., Fialor, S.C. & Skov, P.V. (2015) Local agro-industrial  
418 by-products with potential use in Ghanaian aquaculture: a review. Aquac. Int., 23,  
419 403–425.

420 Odesanya, B.O., Ajayi, S.O., Agbaogun, B.K.O. & Okuneye, B. (2011) Comparative  
421 evaluation of nutritive value of maggots. Int. J. Sci. Eng. Res., 2, 1–5.

422 Ogunji, J.O., Kloas, W., Wirth, M., Neumann, N. & Pietsch, C. (2008a) Effect of  
423 housefly maggot meal (magmeal) diets on the performance, concentration of  
424 plasma glucose, cortisol and blood characteristics of *Oreochromis niloticus*  
425 fingerlings. J. Anim. Physiol. Anim. Nutr. (Berl), 92, 511–518.

- 426 Ogunji, J.O., Kloas, W., Wirth, M., Schulz, C. & Rennert, B. (2008b) Housefly Maggot  
427 Meal (Magmeal) as a Protein Source for *Oreochromis niloticus* (Linn.). Asian  
428 Fish. Sci. 21, 319–331.
- 429 Ogunji, J., Toor, R.U.A.S., Schulz, C. & Kloas, W. (2008c) Growth performance,  
430 nutrient utilization of Nile tilapia *Oreochromis niloticus* fed housefly maggot meal  
431 (magmeal) diets. Turkish J. Fish. Aquat. Sci., 147, 141–147.
- 432 Persson, J.A. (2008) Handbook for Kjeldahl Digestion, 4<sup>th</sup> ed. FOSS, DK-3400,  
433 Hilleroed, Denmark.
- 434 Randall, J.E. (1967) Food Habits of Reef Fishes of the West Indies. Stud. Trop.  
435 Oceanogr., 5, 665–847.
- 436 Rurangwa, E., Agyakwah, S.K., Boon, H. & Bolman, B.C. (2015). Development of  
437 Aquaculture in Ghana Analysis of the fish value chain and potential business cases.
- 438 Ruxton, C.H.S., Reed, S.C., Simpson, M.J.A. & Millington, K.J. (2004) The health  
439 benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. J. Hum.  
440 Nutr. Diet., 17, 449–459.
- 441 Sánchez-Muros, M.J., de Haro, C., Sanz, A., Trenzado, C.E., Villareces, S. & Barroso,  
442 F.G. (2015). Nutritional evaluation of *Tenebrio molitor* meal as fishmeal substitute  
443 for tilapia (*Oreochromis niloticus*) diet. Aquac. Nutr. 22: 943–955.
- 444 Shiau, S.Y. (2002) Tilapia, *Oreochromis* spp. In: Nutrient requirements and feeding of  
445 finfish for aquaculture (Webster, C.D. & Lim, C.E. eds), pp. 273–292. CABI  
446 Publishing, Wallingford, UK.
- 447 Sing, K.-W., Kamarudin, M.S., Wilson, J.-J. & Sofian-Azirun, M. (2014) Evaluation of  
448 Blowfly (*Chrysomya megacephala*) Maggot Meal as an Effective, Sustainable

- 449 Replacement for Fishmeal in the Diet of Farmed Juvenile Red Tilapia  
450 (*Oreochromis* sp.). Pak. Vet., J. 8318, 85–92.
- 451 Steffens, W. (1989) Principles of fish nutrition (Ellis Horwood Series in Aquaculture  
452 and Fisheries Support, Series ed.). Ellis Horwood Ltd, Chichester, UK
- 453 St-Hilaire, S., Cranfill, K., McGuire, M.A., Mosley, E.E., Tomberlin, J.K., Newton, L.,  
454 Sealey, W., Sheppard, C. & Irving, S. (2007a) Fish offal recycling by the black  
455 soldier fly produces a foodstuff high in omega-3 fatty acids. J. World Aquac. Soc.,  
456 38, 309–313.
- 457 St-Hilaire, S., Sheppard, C., Tomberlin, J.K., Irving, S., Newton, L., McGuire, M.A.,  
458 Mosley, E.E., Hardy, R.W., Sealey, W. (2007b) Fly prepupae as a feedstuff for  
459 rainbow trout, *Oncorhynchus mykiss*. J. World Aquac. Soc., 38, 59–67.
- 460 Tacon, A.G.J. (1988). The nutrition and feeding of farmed fish and shrimp - a training  
461 manual. 3. Feeding methods, FAO Field Document, Project GCP/RLA/075/ITA  
462 “Support to the Regional Aquaculture Activities for Latin America and the  
463 Caribbean”, Field Document No. 7/E, Brasilia, Brazil, 208 pp.
- 464 Tacon, A.G.J. & Metian, M. (2008) Global overview on the use of fish meal and fish oil  
465 in industrially compounded aquafeeds: Trends and future prospects. Aquaculture,  
466 285, 146–158.
- 467 Tocher, D.R. & Harvie, D.G. (1988) Fatty acid compositions of the major  
468 phosphoglycerides from fish neural tissues; (n-3) and (n-6) polyunsaturated fatty  
469 acids in rainbow trout (*Salmo gairdneri*) and cod (*Gadus morhua*) brains and  
470 retinas. Fish Physiol. Biochem., 5, 229–39.
- 471 Turchini, G.M., Torstensen, B.E. & Ng, W.-K. (2009) Fish oil replacement in finfish

472 nutrition. *Rev. Aquac.*, 1, 10–57.

473 van Huis, A., Itterbeeck, J. Van, Klunder, H., Mertens, E., Halloran, A., Muir, G. &  
474 Vantomme, P. (2013) Edible insects: Future prospects for food and feed security.  
475 *FAO For. Pap.*, 171, 1–201.

476 Veldkamp, T., Duinkerken, G. van, van Huis, A., Lakemond, C.M.M., Ottevanger, E.,  
477 Bosch, G. & van Boekel, M.A.J.S. (2012) Insects as a sustainable feed ingredient  
478 in pig and poultry diets - a feasibility study, Wageningen UR Livestock Research.

479

480 **Tables**

481 **Table 1** Ingredient composition (g kg<sup>-1</sup>) of the control (PG40) and the 3 test diets (MM30,  
 482 MM50 and MM80) and proximate, amino acid, and fatty acid compositions (g kg<sup>-1</sup> of  
 483 meal) of the Black Soldier Fly (BSF) larvae meal and the diets. Values are presented ‘as  
 484 is’, based on duplicate analyses.

	BSF meal	Experimental diets			
		PG40	MM30	MM50	MM80
<b>Ingredient composition (g kg<sup>-1</sup>)</b>					
Fish meal	-	100.0	80.0	50.0	30.0
Soybean meal	-	200.0	180.0	160.0	130.0
BSF meal	-	-	30.0	50.0	80.0
Poultry byproduct meal	-	50.0	80.0	100.0	130.0
Fish oil	-	20.0	-	-	-
Corn meal	-	304.0	304.0	304.0	304.0
Wheat bran	-	130.0	130.0	140.0	130.0
Poultry blood meal	-	100.0	100.0	100.0	100.0
Feather meal	-	90.0	90.0	90.0	90.0
Vitamin premix	-	3.0	3.0	3.0	3.0
Anti-mold	-	1.5	1.5	1.5	1.5
Klinofeed ®	-	1.0	1.0	1.0	1.0
Methionine	-	0.5	0.5	0.5	0.5
<b>Proximate composition (g kg<sup>-1</sup>)</b>					
Dry matter	950.3	949.4	957.5	952.5	958.3
Crude protein	416.4	372.8	378.4	371.7	376.7
Crude lipid	232.4	94.8	78.3	77.6	93.4
Crude fibre	76.6	30.5	33.1	35.0	34.4
Ash	116.5	62.9	67.6	68.1	66.8
Nitrogen-Free Extract	108.4	388.4	400.1	400.1	387
Gross Energy (MJ/kg)	21.7	19.6	19.2	19.4	19.7
<b>Essential amino acids (g kg<sup>-1</sup>)</b>					
Arginine	20	13.6	13.7	13.1	13.3
Histidine	11.8	22.5	23.7	23.4	24.1
Lysine	27	21.8	21.8	21.5	21.8
Methionine	7.5	11.2	11.7	11.4	11.5
Phenylalanine	17.5	16.2	15.7	16.3	16.6
Leucine	29	6.6	5.8	5.1	5.9
Iso-Leucine	18.4	21.7	21.8	22.4	22.2
Valine	26.3	19.3	19.6	19.6	19
Threonine	17.2	34.1	34.3	34.4	34.4



---

**Fatty acid composition (g kg<sup>-1</sup>)**

14:00	10.2	1	1	1.3	1.6
16:00	33.3	14.4	9.7	10.3	10.7
18:00	4.7	3.6	3.2	3.5	3.6
20:00	0.1	0.3	0.2	0.2	0.2
Total saturated <sup>1</sup>	49.2	19.7	14.4	15.6	16.5
16:1n-7	6	1.4	1.2	1.3	1.4
18:1n-9	26.7	24.1	16.9	17.1	16.9
18:1n-7	5.5	1.5	1.3	1.3	1.4
22:1n-11	0.3	0.7	0.4	0.3	0.3
Total monounsaturated <sup>2</sup>	40.8	29.2	20.9	20.9	20.9
18:2n-6	18.6	15.1	12.9	13.3	12.5
20:2n-6	0.3	0.3	0.2	0.2	0.1
20:4n-6	0.2	0.2	0.2	0.1	0.1
Total n-6 <sup>3</sup>	19.2	15.8	13.4	13.7	12.9
18:3n-3	1.7	1.7	1.3	1.2	1.1
18:4n-3	1.9	0.2	0.2	0.1	0.1
20:5n-3 (EPA)	0.9	0.8	0.5	0.4	0.4
22:6n-3 (DHA)	0.1	1.8	1.2	0.8	0.7
Total n-3 <sup>4</sup>	4.6	5.1	3.5	2.7	2.5
Total Polyunsaturated <sup>5</sup>	23.8	21.1	17.1	16.6	15.5
Total fatty acids	113.9	70	52.5	53.1	52.9
n-3/n-6	0.2	0.3	0.3	0.2	0.2

485

486

487

<sup>1</sup>Includes 15:0; 22:0 and 24:0 ; <sup>2</sup>Includes 16:1n-9; 20:1n-11; 20:1n-7; 22:1n-9 and 24:1n-9 ; <sup>3</sup>Includes 18:3n-6; 20:3n-6 and 22:4n-6 ; <sup>4</sup>Includes 20:3n-3; 20:4n-3 and 22:5n-3 ; <sup>5</sup>Includes 16:2 and 16:3

488 **Table 2** Growth performance and feed utilisation indices determined for nursing tilapia  
 489 fingerlings fed control and experimental diets for 32 days

	<b>Dietary treatments</b>			
	<b>PG40</b>	<b>MM30</b>	<b>MM50</b>	<b>MM80</b>
Initial live weight (g fish <sup>-1</sup> )	5.5±0.2	5.1±0.2	6.1±0.7	6.1±0.3
Final live weight (g fish <sup>-1</sup> )	16.0±0.8	16.9±1.8	17.0±1.1	16.5±0.9
Live weight gain (g fish <sup>-1</sup> )	10.4±0.9	11.8±1.9	10.9±1.5	10.4±0.6
SGR (% day <sup>-1</sup> )	3.3±0.2	3.7±0.4	3.2±0.5	3.1±0.1
Total feed distributed (kg cage <sup>-1</sup> )	25.9±0.9	25.7±0.4	26.2±0.2	26.4±0.6
FCR	2.2±0.1	2.1±0.3	2.0±0.2	2.1±0.1
PER	1.2±0.1	1.2±0.2	1.3±0.1	1.2±0.0
Feeding rate (% biomass day <sup>-1</sup> )	4.4±0.1	4.3±0.4	4.0±0.1	4.1±0.1
Survival rate (%)	86.1±0.3 <sup>b</sup>	81.7±1.9 <sup>c</sup>	89.5±2.2 <sup>ab</sup>	90.1±0.5 <sup>a</sup>

490 Means± SD (n=3) bearing different superscripts within each row are significantly different (P<0.05)

491

492 **Table 3** Proximate composition (g kg<sup>-1</sup> of fish, wet weight basis) and fatty acid  
 493 composition (g kg<sup>-1</sup> of fish) of Nile tilapia fingerlings whole body at the start (Initial;  
 494 mean±SD; n=4) and on termination of the 32-day experimental period

	Initial	Dietary treatment			
		PG40	MM30	MM50	MM80
<b>Proximate composition (g kg<sup>-1</sup>)</b>					
Dry matter	238.1±3.4	286.0±5.1	278.5±2.5	282.0±2.5	285.2±1.1
Crude protein	148.8±1.5	153.6±3.0	152.7±1.3	152.9±0.9	154.3±0.5
Crude lipid	37.0±1.4	107.8±6.1	96.1±1.1	99.9±4.4	102.2±6.1
Ash	48.8±0.9	33.1±1.7	34.5±0.8	33.9±2.3	35.7±0.7
Crude fibre	0.7±0.2	0.8±0.1	0.8±0.1	0.8±0.1	0.8±0.1
<b>Fatty acid composition (g kg<sup>-1</sup> fish)</b>					
14:0	0.50±0.03	1.57±0.09 <sup>c</sup>	1.70±0.13 <sup>c</sup>	2.32±0.21 <sup>b</sup>	2.91±0.25 <sup>a</sup>
16:0	4.99±0.20	15.92±0.93 <sup>ab</sup>	14.57±0.57 <sup>b</sup>	16.65±1.99 <sup>ab</sup>	18.01±1.03 <sup>a</sup>
18:0	1.83±0.07	4.80±0.33	4.68±0.11	4.96±0.52	5.21±0.36
20:0	0.08±0.00	0.18±0.00	0.17±0.00	0.18±0.03	0.19±0.01
<b>Total saturated<sup>1</sup></b>	7.56±0.30	22.68±1.35 <sup>ab</sup>	21.34±0.84 <sup>b</sup>	24.35±2.78 <sup>ab</sup>	26.57±1.64 <sup>a</sup>
16:1n-7	0.93±0.05	2.71±0.16 <sup>b</sup>	2.58±0.12 <sup>b</sup>	2.94±0.37 <sup>ab</sup>	3.32±0.12 <sup>a</sup>
18:1n-9	7.04±0.33	25.42±1.83	22.11±0.80	24.72±2.94	25.70±1.38
18:1n-7	0.84±0.04	2.04±0.13 <sup>ab</sup>	1.96±0.06 <sup>b</sup>	2.28±0.32 <sup>ab</sup>	2.52±0.20 <sup>a</sup>
22:1n-11	0.08±0.00	0.36±0.02 <sup>a</sup>	0.18±0.02 <sup>b</sup>	0.20±0.02 <sup>b</sup>	0.18±0.01 <sup>b</sup>
<b>Total monounsaturat.<sup>2</sup></b>	9.72±0.46	32.98±2.28	28.92±0.97	32.55±3.89	34.26±1.80
18:2n-6	2.54±0.19	8.16±0.52	7.61±0.20	8.21±1.05	9.08±0.52
20:2n-6	0.22±0.01	0.61±0.05	0.58±0.01	0.63±0.07	0.67±0.03
20:4n-6	0.35±0.02	0.60±0.05 <sup>b</sup>	0.60±0.04 <sup>b</sup>	0.66±0.08 <sup>ab</sup>	0.76±0.05 <sup>a</sup>

<b>Total n-6<sup>3</sup></b>	3.69±0.27	10.91±0.77	10.24±0.36	11.10±1.44	12.33±0.72
18:3n-3	0.21±0.02	0.74±0.05	0.62±0.03	0.62±0.09	0.66±0.04
20:4n-3	0.03±0.00	0.11±0.01 <sup>a</sup>	0.07±0.00 <sup>b</sup>	0.07±0.01 <sup>b</sup>	0.07±0.00 <sup>b</sup>
20:5n-3 (EPA)	0.07±0.00	0.13±0.02 <sup>a</sup>	0.08±0.01 <sup>b</sup>	0.08±0.01 <sup>b</sup>	0.09±0.01 <sup>b</sup>
22:5n-3	0.16±0.01	0.47±0.04 <sup>a</sup>	0.32±0.03 <sup>b</sup>	0.29±0.04 <sup>b</sup>	0.32±0.02 <sup>b</sup>
22:6n-3 (DHA)	0.89±0.03	1.85±0.19 <sup>a</sup>	1.38±0.12 <sup>b</sup>	1.16±0.17 <sup>b</sup>	1.24±0.05 <sup>b</sup>
<b>Total n-3<sup>4</sup></b>	1.43±0.06	3.55±0.32 <sup>a</sup>	2.66±0.18 <sup>b</sup>	2.38±0.35 <sup>b</sup>	2.56±0.12 <sup>b</sup>
<b>Total polyunsat.<sup>5</sup></b>	5.30±0.34	14.77±1.09	13.19±0.53	13.79±1.82	15.21±0.82
<b>Total fatty acids</b>	22.58±1.08	70.42±4.71	63.46±2.17	70.68±8.42	76.04±4.22

495 Mean± SD (n=3) bearing different superscripts within each row are significantly different (P<0.05); comparisons  
496 were made between dietary treatments and excluded the initial values.

497 <sup>1</sup>Includes 15:0; 22:0 and 24:0

498 <sup>2</sup>Includes 16:1n-9; 17:1; 20:1n-11; 20:1n-9; 20:1n-7; 22:1n-9 and 24:1n-9

499 <sup>3</sup>Includes 18:3n-6; 20:3n-6; 22:4n-6 and 22:5n-6

500 <sup>4</sup>Includes 18:4n-3 and 20:3n-3

501 <sup>5</sup>Includes 16:2; 16:3 and 16:4

502