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

Emilie Devic[1], William Leschen[1], Francis Murray[1], and David C. Little[1]

[1]*Institute of Aquaculture, School of Natural Sciences, University of Stirling, UK*

Correspondence:

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

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

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

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

Running title: BSF larvae meal in advanced nursing tilapia diets

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

Abstract

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

Introduction

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

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

Material and Methods

Diets

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

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

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

[Insert Table 1]

Experimental Design

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

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

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

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

Chemical analyses

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

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

Calculations and statistical methods

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

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

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

Results

Water temperature and dissolved oxygen varied slightly during the course of the experiment and the diurnal periods with values ranging from 26.8 to 30.5°C and 5.1 to 8.1 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±0.5 g fish^{-1} to a final
183 weight 16.6±0.5 g fish^{-1}. Live weight gain and SGR of the fish fed the control and the
184 MM-based diets were not significantly different (P≥0.05). Overall feeding response was
185 good with total amounts of feed distributed (26.0±0.3 kg cage^{-1}) and feeding rates
186 (4.2±0.2 % biomass day^{-1}) not significantly different between treatments (P≥0.05),
187 indicating similar feed intake. Feed utilisation efficiency (FCR and PER) was not
188 compromised by the dietary treatments (P≥0.05). However, MM30 diet led to a
189 significantly lower survival (81.7±1.9 %) compared to others (P<0.05) and MM80
190 survival rate (90.1±0.5 %) was found significantly higher than PG40 (86.1±0.3 %).
191 [Insert Table 2]
192 Analysed fish body compositions compared between treatments indicated no significant
193 differences (P≥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

manufacturers to find cost-efficient alternative feed ingredients to replace always more costly feedstuffs such as marine ingredients (Gabriel *et al*. 2007; Obirikorang *et al*. 2015). According to recent publications (Barroso *et al.* 2014; Henry *et al.* 2015), insect meals from dipterans such as the common housefly (*M. domestica*) or the black soldier fly (*H. illucens*) feature nutritional characteristics similar to FM suggesting that they could be suitable substitutes for this conventional feed ingredient. Nevertheless, compared to high-quality FM such as anchovies, herring or menhaden (NRC, 2011), the BSF meal produced in Ghana for the purpose of the experiment, displayed a lower protein content and higher lipid content. Similar observation was made by Barroso *et al.* (2014) for a BSF meal which presented even lower levels of crude protein, lipid and ash (362 g kg^{-1}; 180 g kg^{-1} and 93 g kg^{-1}, respectively) compared to the BSF meal produced in Ghana. Insect life stage, feeding substrate and processing methods influence their nutritional composition (Aniebo & Owen 2010; van Huis *et al.* 2013) which explains the differences reported here. Also similar to that previously found by Barroso *et al.* (2014), the amino acid profile of the MM was comparable to conventional FM including 9 out of 10 of the essential amino acids (BSF meal is known to be low in tryptophan; Newton *et al.* 1977; Henry *et al.* 2015). It is therefore a great source of protein. MM used in the current experiment was also a rich source of FA, in particular saturated and monounsaturated and it was slightly richer in EPA and DHA compared to MM used in other studies (St-Hilaire *et al.* 2007b; Kroeckel *et al.* 2012; Barroso *et al.* 2014) owing to the substrate mix on which the larvae fed (St-Hilaire *et al.* 2007a). Although MM is locally available (Devic *et al.* 2013) and a suitable source of nutrients for fish as demonstrated in several other studies (St-Hilaire *et al.* 2007b; Kroeckel *et al.* 2012, Lock *et al.* 2015), inclusion in a complete diet in 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^{-1} 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^{-1}
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_{18} 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±1.9 g
260 $fish^{-1}$ and 3.7±0.4 % day^{-1}, 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^{-1} (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

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

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

Commercial aquafeed manufacturers continue to produce feeds for tilapia including 20 to 250 g kg^{-1} FM due to its high nutritional quality (FAO 2012). In the current study, the absence of differences between the fish growth performance, feed utilisation and body composition under the different dietary treatments lead to the conclusion that inclusions of up to 80 g kg^{-1} MM and 130 g kg^{-1} PBM, substituting FO totally, up to 70% FM and 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

Acknowledgements

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

References

Aniebo, A.O. & Owen, O.J. (2010) Effects of age and method of drying on the proximate composition of housefly larvae (*Musca domestica* Linnaeus) meal (HFLM). Pakistan J. Nutr., 9, 485–487.

AOAC (1990) Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, Inc., Washington, D.C., USA.

Ayoola, A.A. (2010) Replacement of Fishmeal with Alternative Protein Sources in Aquaculture Diets. North Carolina State University.

Bailey, R.M. & Harrison, H.M.J. (1948) Food Habits of the Southern Channel Catfish (*Ictalurus Lacustris Punctatus*) in the Des Moines River, Iowa. Trans. Am. Fish. Soc., 75, 110–138.

Barroso, F.G., de Haro, C., Sánchez-Muros, M.-J., Venegas, E., Martínez-Sánchez, A. & Pérez-Bañón, C. (2014) The potential of various insect species for use as food for fish. Aquaculture, 422-423, 193–201.

Beveridge, M.C.M. & McAndrew, B.J. (2000) Tilapias: Biology and Exploitation. Kluwer Academic Publishers.

Beveridge, M.C.M., Thilsted, S.H., Phillips, M.J., Metian, M., Troell, M. & Hall, S.J. (2013) Meeting the food and nutrition needs of the poor: the role of fish and the opportunities and challenges emerging from the rise of aquaculture. J. Fish Biol., 83, 1067–1084.

Bolivar, R.B., Jimenez, E.B.T., Sugue, J.R.A. & Brown, C.L. (2004) Effect of stocking sizes on the yield and survival of Nile tilapia (*Oreochromis niloticus* L.) on-grown 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, 3rd ed. The Oily Press, Dundee, UK.

349 De Silva, S.S. & Anderson, T.A. (1994) Fish nutrition in aquaculture, 1st 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

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

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

El-Sayed, A.-F.M. (2013) Tilapia feed management practices in sub-Saharan Africa. In: On-farm feeding and feed management in aquaculture (M.R. Hasan M.B. New eds.), FAO Fish. Aquac. Tech. Pap. No. 583, pp. 377–405. FAO, Rome, Italy.

El-Sayed, A.-F.M. & Tacon, A.G.J. (1997) Fishmeal replacers for tilapia : A review. In: Feeding Tomorrow's Fish (Tacon, A.G.J. & Basurco, B. eds.). Cahiers Options Méditerranéennes, 22. pp. 205–224.

FAO (2005) National Aquaculture Sector Overview. Ghana. National Aquaculture Sector Overview Fact Sheets. In: National Aquaculture Sector Overview Fact Sheets. FAO Fisheries and Aquaculture Department [online].

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

Fasakin, E.A., Balogun, A.M. & Ajayi, O.O. (2003) Evaluation of full-fat and defatted maggot meals in the feeding of clariid catfish *Clarias gariepinus* fingerlings. Aquac. Res., 34, 733–738.

Folch, J., Lees, M. & Sloane Stanley, G.H. (1957). A simple method for isolation and purification of total lipides from animal tissues. J. Biol. Chem., 226, 497–509.

Gabriel, U.U., Akinrotimi, O. a, Bekibele, D.O., Onunkwo, D.N., Anyanwu, P.E. & Harcourt, P. (2007). Locally produced fish feed : potentials for aquaculture development in subsaharan Africa. African J. Agric. Res., 2, 287–295.

Hasan, M.R., Hecht, T., De Silva, S.S. & Tacon, A.G.J. (2007) Study and analysis of feeds and fertilizers for sustainable aquaculture development. FAO Fisheries 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 yo 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, 4th 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

nutrition. Rev. Aquac., 1, 10–57.

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

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

480 **Tables**

481 **Table 1** Ingredient composition (g kg^{-1}) of the control (PG40) and the 3 test diets (MM30,
482 MM50 and MM80) and proximate, amino acid, and fatty acid compositions (g kg^{-1} 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
Ingredient composition (g kg^{-1})					
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
Proximate composition (g $kg^{-1)}$					
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
Essential amino acids (g kg^{-1})					
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^{-1})					
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[1]	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[2]	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[3]	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[4]	4.6	5.1	3.5	2.7	2.5
Total Polyunsaturated[5]	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 [1]Includes 15:0; 22:0 and 24:0 ; [2]Includes 16:1n-9; 20:1n-11; 20:1n-7; 22:1n-9 and 24:1n-9 ; [3]Includes 18:3n-6; 20:3n-6 and 22:4n-6
486 ; [4]Includes 20:3n-3; 20:4n-3 and 22:5n-3 ; [5]Includes 16:2 and 16:3
487

488 **Table 2** Growth performance and feed utilisation indices determined for nursing tilapia

489 fingerlings fed control and experimental diets for 32 days

	Dietary treatments			
	PG40	**MM30**	**MM50**	**MM80**
Initial live weight (g $fish^{-1}$)	5.5±0.2	5.1±0.2	6.1±0.7	6.1±0.3
Final live weight (g $fish^{-1}$)	16.0±0.8	16.9±1.8	17.0±1.1	16.5±0.9
Live weight gain (g $fish^{-1}$)	10.4±0.9	11.8±1.9	10.9±1.5	10.4±0.6
SGR (% day^{-1})	3.3±0.2	3.7±0.4	3.2±0.5	3.1±0.1
Total feed distributed (kg $cage^{-1}$)	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^{-1})	4.4±0.1	4.3±0.4	4.0±0.1	4.1±0.1
Survival rate (%)	$86.1±0.3^{b}$	$81.7±1.9^{c}$	$89.5±2.2^{ab}$	$90.1±0.5^{a}$

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^{-1} of fish, wet weight basis) and fatty acid
493 composition (g kg^{-1} 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**
Proximate composition (g kg^{-1})					
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
Fatty acid composition (g kg^{-1} fish)					
14:0	0.50±0.03	1.57±0.09^{c}	1.70±0.13^{c}	2.32±0.21^{b}	2.91±0.25^{a}
16:0	4.99±0.20	15.92±0.93^{ab}	14.57±0.57^{b}	16.65±1.99^{ab}	18.01±1.03^{a}
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
***Total saturated*[1]**	7.56±0.30	22.68±1.35^{ab}	21.34±0.84^{b}	24.35±2.78^{ab}	26.57±1.64^{a}
16:1n-7	0.93±0.05	2.71±0.16^{b}	2.58±0.12^{b}	2.94±0.37^{ab}	3.32±0.12^{a}
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^{ab}	1.96±0.06^{b}	2.28±0.32^{ab}	2.52±0.20^{a}
22:1n-11	0.08±0.00	0.36±0.02^{a}	0.18±0.02^{b}	0.20±0.02^{b}	0.18±0.01^{b}
***Total monounsat.*[2]**	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^{b}	0.60±0.04^{b}	0.66±0.08^{ab}	0.76±0.05^{a}

Total n-6[3]	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[a]	0.07±0.00[b]	0.07±0.01[b]	0.07±0.00[b]
20:5n-3 (EPA)	0.07±0.00	0.13±0.02[a]	0.08±0.01[b]	0.08±0.01[b]	0.09±0.01[b]
22:5n-3	0.16±0.01	0.47±0.04[a]	0.32±0.03[b]	0.29±0.04[b]	0.32±0.02[b]
22:6n-3 (DHA)	0.89±0.03	1.85±0.19[a]	1.38±0.12[b]	1.16±0.17[b]	1.24±0.05[b]
Total n-3[4]	1.43±0.06	3.55±0.32[a]	2.66±0.18[b]	2.38±0.35[b]	2.56±0.12[b]
Total polyunsat.[5]	5.30±0.34	14.77±1.09	13.19±0.53	13.79±1.82	15.21±0.82
Total fatty acids	22.58±1.08	70.42±4.71	63.46±2.17	70.68±8.42	76.04±4.22

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

[1]Includes 15:0; 22:0 and 24:0

[2]Includes 16:1n-9; 17:1; 20:1n-11; 20:1n-9; 20:1n-7; 22:1n-9 and 24:1n-9

[3]Includes 18:3n-6; 20:3n-6; 22:4n-6 and 22:5n-6

[4]Includes 18:4n-3and 20:3n-3

[5]Includes 16:2; 16:3 and 16:4