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# UNIVERSITÀ DEGLI STUDI DI TORINO

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14 ***Review on the use of insects in the diet of farmed fish: Past and future***

15  
16 **Authors:** Henry M., Gasco, L., Piccolo, G. & Fountoulaki, E.

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Abbreviations: AA, amino acid; ADF, acid detergent fiber; BSF, black soldier fly; CP, crude protein; DM, dry matter; EAA, Essential amino acid; FA, fatty acid; FM, fishmeal; FO, fish oil; HUFA, Highly unsaturated fatty acid; Lys, lysine; Met, methionine; %FMr; Percentage of fishmeal replacement; PUFA, polyunsaturated fatty acid; SWP, silkworm pupae, Trp, tryptophan; WG, weight gain; Wf, fish final weight; Wi, fish initial weight

## **Review on the use of insects in the diet of farmed fish: past and future**

M. Henry<sup>\*a</sup>, L. Gasco<sup>b</sup>, G. Piccolo<sup>c</sup>, E. Fountoulaki<sup>a</sup>

<sup>a</sup>Laboratory of Fish Nutrition and Pathology, Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research, Aghios Kosmas, 16777 Elliniko, Greece.

<sup>b</sup> Department of Agricultural, Forest and Food Science, University of Turin, Grugliasco, 10095 Turin, Italy.

<sup>c</sup> Department of Veterinary Medicine and Animal Production University of Napoli Federico II, Via F. Delpino 1,  
80137 Naples, Italy.

\*corresponding author: [morgane@hcmr.gr](mailto:morgane@hcmr.gr); tel: +30 210 98 567 25; fax: +30 210 98 29 239

## ABSTRACT

The decrease in the availability and the increase in the prices of fishmeal and fish oil have prompted the search for sustainable alternatives for aquaculture feeds. Insects, which are part of the natural diet of fish, leave a small ecological footprint and have a limited need for arable land, may represent a good candidate. Over the last decade, studies on the replacement of fishmeal with insects in the diet of fish have emerged, and the promising results have encouraged further research. The present review presents these results in large tables and emphasizes the achievable dietary inclusion levels. It discusses the potential of locusts, grasshoppers, termites, yellow mealworms, Asiatic rhinoceros beetles, superworms, domesticated silkworms, common houseflies, common mosquitoes and black soldier flies for use as fishmeal and/or fish oil replacement in the fish diet. The review only succinctly compares the composition of the insects with the requirements of the fish (proteins and amino acids, lipids and fatty acids, vitamins and minerals). This review also discusses the potential hurdles of using insects in fish feeds (toxicity of insects through bioaccumulation, deficiencies in amino acids or fatty acids, chitin content, palatability, digestibility) and the available ways of avoiding these drawbacks (control of the dietary substrate of insects in mass rearing units, manipulation of the diet of insects, mixture of dietary proteins, use of aquatic insects, processing of insect meal). Finally, it suggests paths worthy of future research on these new fishmeal alternatives.

57 *Keywords:* Fishmeal alternative; Chitin; Orthoptera; Coleoptera; Lepidoptera; Diptera

59 1. Introduction

61 The steady decline in catches of wild fish (FAO, 2014), and the increased demands for livestock and  
62 aquaculture feeds have resulted in a rapid decrease in the availability of fishmeal (FM) and fish oil (FO) and in  
63 their concurrent price increase (FAO, 2014). The cost of aquaculture feeds represents 40 to 70% of the cost of the  
64 fish produced (Wilson, 2002; Rana et al., 2009), and is especially high in the aquaculture of carnivorous fish that  
65 require large amounts of FM (Manzano-Agugliaro et al., 2012). Soya and other terrestrial plants rich in proteins  
66 and lipids have been introduced into the diet of aquaculture fish to replace FM and FO (Hardy, 2002; Espe et al.,  
67 2006; Gatlin et al., 2007). However, the presence of anti-nutritional factors in plant meals (Tacon, 1993; Francis  
68 et al., 2001; Ogunji, 2004; Collins, 2014), the potential problems of the inflammation of the digestive tract  
69 (Merrifield et al., 2011) and the decreased palatability of the meal (Papatryphon and Soares, 2001) are of concern.  
70 Moreover, the rapid growth in the human population has put pressure on the use of arable land (Doos, 2002), and  
71 the ecological footprint of these protein-rich plants, related to the amount of energy and water necessary for their  
72 production, may alter the sustainability of such alternatives to FM and FO (Naylor et al., 2009).

73 Since insects are part of the natural diet of both freshwater and marine fish (Howe et al., 2014; Whitley and  
74 Bollens, 2014) (Table 1), and because they are rich in amino acids, lipids, vitamins and minerals (van Huis, 2013)  
75 and leave a small ecological footprint (no need for arable land, low need for energy and water) (Oonincx and de  
76 Boer, 2012), they have been considered as potential alternatives to FM and FO. Moreover, insect larvae can rapidly  
77 transform low quality organic waste into good quality fertilizer (van Huis et al., 2013), thus reducing the final mass  
78 of manure by 50%, of nitrogen waste by 30-50%, and of phosphorus waste by 61-70% (Newton et al., 2005; Diener  
79 et al., 2009; van Huis et al., 2013). They also reduce the load of pathogenic bacteria in the microflora of manure  
80 (Erickson et al., 2004; Liu et al., 2008). Furthermore, the final product of this very efficient bioconversion of  
81 manure is an abundant amount of insect prepupae rich in proteins (40%) and lipids (30%) (Sheppard et al., 1994;  
82 Newton et al., 2005), which is suitable for use in fish and animal feeds (Barroso et al., 2014). Many insects  
83 (Lepidoptera, Diptera, Hymenoptera, Coleoptera, Trichoptera, Hemiptera, Odonata) also show antifungal activity  
84 and/or antibacterial peptides (Ravi et al., 2011) that may increase the shelf-life of insect-containing feeds (Zhao et  
85 al., 2010). For all these reasons, nutritional studies on the use of insects in livestock and aquaculture feeds (mainly  
86 for freshwater fish) have been conducted, mainly in Asian, African and South American countries (Veldkamp et  
87 al., 2012). The present review article has the aim of describing the published results of experiments using insects  
88 (larvae, prepupae, pupae and adults) as FM and /or FO replacements in aquaculture feeds for freshwater and marine  
89 fish, after briefly presenting the general requirements of the fish and the composition of the insects used in these  
90 studies. The review also attempts to establish the best insect candidates and optimal incorporation rates in the fish

91 diets and discusses the potential hurdles and the different ways of improving the quality and acceptability of insect  
92 meal.

93 **Tables 1 and 2**

94

95 **2. Insect composition versus fish requirements**

96

97 The composition of insects has already been studied extensively and is available in various recent review  
98 articles (Rumpold and Schluter, 2013; Barroso et al., 2014; Makkar et al., 2014; Sanchez-Muros et al., 2014).  
99 Therefore, the present review will not focus on details and will summarize data available on the insect species  
100 studied in fish experiments (Table 2).

101 Before incorporating any insect species into the diet of a fish species, it is necessary to determine the exact  
102 composition of the insect, which varies according to its particular life stage, rearing conditions and diet, and to  
103 compare it with the requirements of the fish species of interest. These requirements are usually accurately reflected  
104 by the fish fillet composition (Oliva-Teles, 2000) and have been widely investigated for the main reared species  
105 (NRC, 2011). Table 1 describes the natural diets of the fish species studied for dietary insect inclusion.

106

107 *2.1. Proteins/Amino acids*

108

109 The protein requirements of different fish species range from 28 to 55 percent of dry diets. The protein  
110 requirement decreases as the fish grows (Lovell, 1989) and is highest in larvae and fry, especially in carnivorous  
111 fish (NRC, 2011). Marine species, which are usually carnivorous, require more dietary protein (40-55%) than most  
112 freshwater fish (25-40%) (Boonyaratpalin, 1997; Hasan, 2001; Sales and Janssens, 2003). According to literature,  
113 the average protein content of insects varies between 50 and 82% (dry matter, DM) (Rumpold and Schluter, 2013),  
114 depending on the insect species or on the method of processing the insect (Fasakin et al., 2003; Banjo et al., 2006).  
115 As an example, the protein content of a good quality FM can reach up to 73%, while soybean meal contains up to  
116 50% of proteins (Barroso et al., 2014).

117 To date, all studies on finfish have shown that they require the usual essential amino acids (EAA) (NRC, 2011;  
118 Oliva-Teles, 2012; Takeuchi, 2014). The development of commercial aquatic feeds has traditionally been based  
119 on FM as the primary protein source, due to its high protein content and well balanced EAA profile (Nguyen et  
120 al., 2009; NRC, 2011; Oliva-Teles, 2012) with high levels of digestible EAA, such as lysine (Lys), methionine

121 (Met) and leucine (Hall, 1992). Plant feedstuffs usually have lower protein contents and EAA imbalances, and  
122 they are often deficient in Lys and Met. Such deficiencies can primarily lead to an increase in feed intake, because  
123 the fish will eat more to cover its basic needs, as exemplified with/as has been seen for Lys (Dabrowski et al.,  
124 2007). Subsequently, fish growth and the feed conversion rate may be affected (Ostaszewska et al., 2011) and  
125 deficiency symptoms may appear, possibly followed by an increased sensitivity to diseases (Cowey, 1994; Helland  
126 and Grisdale-Helland, 2006; Kiron, 2012).

127 In general, the AA patterns of insects are taxon-dependent, with the profiles of Diptera considered close to FM  
128 profiles and the profiles of Coleoptera and Orthoptera close to those of soybean, with potential deficiencies in Lys  
129 or Met (Barroso et al., 2014). However, the AA profiles of most insects studied for fish diets, which are presented  
130 in Figure 1, show a good correlation with fish requirement values (Hasan, 2001; NRC, 2011; Alegbeleye et al.,  
131 2012), and, in some cases, even exceed these requirements (domesticated silkworms, *Bombyx mori* and yellow  
132 mealworm, *Tenebrio molitor*) (Hossain et al., 1997; Barker et al., 1998; Finke, 2002; 2007; Longvah et al., 2011;  
133 Rumpold and Schluter, 2013; Yi et al., 2013; Barroso et al., 2014). Other species (1 orthopterous species, the  
134 cricket, *Acheta domesticus* and 2 hymenopterous species, ants and bees) have also been included in Figure  
135 1although they have not been studied in fish diets before, because they also show valuable EAA profiles (Barker  
136 et al., 1998; Finke, 2002; 2007; Rumpold and Schluter, 2013; Barroso et al., 2014) and may be worthy of further  
137 investigation. Lepidoptera (silkworms) and Hymenoptera are particularly interesting, due to their richness in Met  
138 (Hossain et al., 1997; Finke, 2007; Longvah et al., 2011; Rumpold and Schluter, 2013), whereas the termites,  
139 *Macrotermes*, and the variegated grasshopper, *Zonocerus variegatus*, have shown several AA deficiencies (Fig.1).

#### 140 **Figure 1**

141 Plant feedstuffs lack certain FM components, such as taurine and hydroxyproline, which are beneficial for fish  
142 growth and fish health (Aksnes et al., 2008; Kousoulaki et al., 2009; Pinto et al., 2013). Many insects, such as  
143 locusts, houseflies, mealworms and mosquitoes, but also the honeybee (*Apis mellifera*), the Eri silkworm (*Attacus*  
144 *ricini*), the common fruit fly (*Drosophila melanogaster*) and the discoid cockroach (*Blaberus discoidalis*), instead  
145 contain valuable levels of taurine (up to 26µmol/g) (Clark and Ball, 1952; Whitton et al., 1987; Bicker, 1992;  
146 Whitton et al., 1995) and hydroxyproline (Briggs, 1962; Pant and Agrawal, 1964; Sowa and Keeley, 1996).

147

#### 148 **2.2. Lipids/ Fatty acids**

149

150           The energy requirements in fish are lower than those of mammals (Finke, 2002). Cold-water  
151          carnivorous fish, such as salmonids, can adapt to dietary lipid levels as high as 35%, but high dietary lipids can  
152          lead to a reduction in fish growth or fat deposition (New and Wijkstroem, 2002), and the dietary protein/lipid  
153          ratio (DP/DL) can be as low as 16 g CP/MJ (Bowyer et al., 2013). Warm-water fish require a higher DP/DL  
154          level (25-26 g CP/MJ) (Bowyer et al., 2013), and the maximum dietary lipid levels for herbivorous and  
155          omnivorous warm-water fish appear to be lower: in general, 10-20 percent leads to optimal growth rates without  
156          producing an excessively fatty carcass (Cowey and Sargent, 1979; Boonyaratpalin, 1997; Hasan, 2001; Sales and  
157          Janssens, 2003). High dietary lipids (20%) can impair the immune status of some marine omnivorous species  
158          (Henry and Fountoulaki, 2014). The lipid level in FM (8.2%) and soya meal (3.0%) is lower than that of insects,  
159          in which it usually ranges from between 10 and 30%, even though it is extremely variable (DeFoliart, 1991). It is  
160          the diet of insects that is mainly responsible for the variations in the lipids and fatty acids (FAs) composition  
161          (Barroso et al., 2014).

162          The requirements of essential fatty acids in fish depend on the bioconversion and elongation capacity of the  
163          FAs of a given fish species (Sargent et al., 1999). Freshwater fish usually require the dietary inclusion of  
164          polyunsaturated FAs (PUFA) (Tocher et al., 2008; Tocher, 2010), whereas marine fish generally require the dietary  
165          inclusion of highly unsaturated FAs (HUFA) (Rainuzzo et al., 1997; Hasan, 2001; Sargent et al., 2002; Tocher et  
166          al., 2008; Tocher, 2010). Aquatic insects that feed on aquatic algae are generally richer in HUFA (Bell et al., 1994;  
167          Yang et al., 2006) than terrestrial insects (Rumpold and Schluter, 2013; Sanchez-Muros et al., 2014) and are  
168          considered to be a better source of FA for marine fish (Sushchik et al., 2003). HUFA-deficiencies in terrestrial  
169          insects could impair both fish growth and health, due to the involvement of the HUFAs in multiple functions such  
170          as membrane structure or eicosanoid production (Tocher, 2003). However, a HUFA and/or PUFA deficiency or  
171          sub-deficiency in the diet of marine fish may stimulate an adaptation mechanism by enabling a level of  
172          bioconversion of C18 to C20 or even to C22 FA (Seiliez et al., 2003; Robin and Skalli, 2007). Thus, the differences  
173          in FA composition between diets containing black soldier fly (BSF) and fillets of rainbow trout, *Oncorhynchus*  
174          *mykiss*, fed these diets with different levels of n-3 FA could reflect an adaptive mechanism of the fish to equilibrate  
175          their FA profile (Sealey et al., 2011).

176

177          2.3. *Minerals and vitamins*

178

179 Minerals (potassium, calcium, iron, magnesium, zinc and selenium) are found in insects (DeFoliart, 1992;  
180 Finke, 2002; Banjo et al., 2006; Schabel, 2010; Rumpold and Schluter, 2013). However, the Ca and P levels are  
181 usually lower than that of FM, except for Ca in BSF and P in housefly (Makkar et al., 2014). The vitamin  
182 requirements of fish can be found in Lall (1991). Insects provide several vitamins, as can be seen in a recent review  
183 (Schabel, 2010). However, as far as the FA composition is concerned, the vitamin and mineral profiles of insects  
184 depend to a great extent on the composition of the insect diet. For example, feeding yellow mealworm larvae with  
185 different diets containing organic wastes has resulted in different vitamin and mineral compositions of/in the larvae  
186 (Ramos-Elorduy et al., 2002).

187

#### 188 2.4. *Nitrogen Free Extract - Chitin*

189

190 Nitrogen-free extracts include carbohydrates, sugars, starches, fibre and chitin. Insects are usually poor in  
191 carbohydrates, but they contain chitin, a primary component of the exoskeleton of arthropods (crustacean shells,  
192 insect exoskeletons), which is composed of an unbranched polymer of N-acetylglucosamine (Lindsay et al., 1984;  
193 Ng et al., 2001). Chitin is discussed in more detail in section 4.5.

194

### 195 3. Incorporation of insects in fish feeds

196

197 The first studies on the incorporation of insects in fish feeds mainly investigated herbivorous/omnivorous  
198 catfish, tilapia and carp. Over the last decade, interest has increased, and many feeding experiments have looked  
199 at different fish, including carnivorous species (see Table 1). The review by Makkar et al., (2014) discusses the  
200 use of insects in livestock, fish and crustaceans feeds. Another recent brief and informative review has looked at  
201 the incorporation of insects in the diet of juvenile fish and crustacean (Riddick, 2014). The present review is  
202 extended to some insect species that were overlooked in the previous reviews and it refines the analysis of all the  
203 fish studies through the use of large tables in which the optimal and detrimental dietary inclusion levels of  
204 Orthoptera, Isoptera and Coleoptera (Table 3), of Lepidoptera (Table 4) and of Diptera (Table 5) are given. The  
205 first studies were mostly conducted in Asia and Africa, where the locally available FM is often of poor quality,  
206 with a lower protein content than that of the tested insects (Heuzé and Tran, 2013). These studies investigated the  
207 supplementation of these cheap reference diets with insect larvae used whole, cut into pieces or transformed as a  
208 meal (Jeyachandran and Raj, 1976; Boscolo et al., 2001; Achionye-Nzeh and Ngwudo, 2003; Idowu et al., 2003;

209 Rangacharyulu et al., 2003; Ossey et al., 2012). Later studies looked at isonitrogenous and isoenergetic  
210 experimental diets, with well-balanced reference diets adapted to the needs of the tested fish. The protein  
211 requirements of each studied fish species, at the stage of development of the tested fish, are included in Tables 3-  
212 5, in order to assess the quality of the control diet used in the feeding experiments described hereafter.

213

214 3.1. *Use of whole or cut, live or frozen insects in freshwater fish diets*

215

216 Common house mosquitoes, black soldier flies, common houseflies and yellow mealworms have been tested  
217 whole or chopped, live or frozen, on mudfish, *Clarias anguillaris*, African catfish, *Clarias gariepinus*, Channel  
218 catfish, *Ictalurus punctatus*, blue tilapia, *Oreochromis aureus*, Nile tilapia, *Oreochromis niloticus* and rainbow  
219 trout. The growth of rainbow trout fed exclusively on frozen common house mosquito, *Culex pipiens*, has been  
220 shown to be affected, probably because the diet was unbalanced and/or the particulate size of the diets was different  
221 (Ostaszewska et al., 2011) (Table 5). Feeding 120g channel catfish or 32g blue tilapia in monoculture settings with  
222 100% of BSF larvae (whole or chopped) was also shown to strongly suppress fish growth, compared to a  
223 commercial diet (Bondari and Sheppard, 1987) (Table 5, trials 2 and 3). Partial substitution of FM could be more  
224 successful. This was verified in a polyculture settings, where 64g channel catfish and 30g blue tilapia were fed  
225 successfully on chopped BSF larvae (50 or 75% larvae with 50 or 25% commercial diet) combined with high-  
226 protein (45% crude protein, CP) or low-protein (30% CP) commercial diets (Bondari and Sheppard, 1981) (Table  
227 5). No differences were found in the body weight of these two species between the experimental diets, thus  
228 suggesting that BSF is suitable for use in the diets of both fish species (Bondari and Sheppard, 1981) (Table 5).  
229 All the other studies using whole or cut insect larvae (maggots or yellow mealworms) have been very successful,  
230 often giving better growth results than the control fish: Nile tilapia fed a mixture of wheat bran and 20% of live  
231 maggots showed a better growth performance, specific growth rate, feed conversion ratio and survival than fish  
232 fed wheat bran alone (Ebenso and Udo, 2003) (Table 5), probably because the diets were more balanced and better  
233 adapted to the needs of the fish. Feeding mudfish with whole frozen maggots has led to better growth than a  
234 soybean diet or a commercial diet, but the composition of these two diets was not stated and it is not known whether  
235 they actually provided all the necessary nutrients for optimal fish growth (Achionye-Nzeh and Ngwudo, 2003)  
236 (Table 5). Similarly, African catfish fed 50% live maggots in conjunction with 50% of an artificial diet poor in  
237 FM (3.5%) grew better than fish fed an artificial diet alone (Oyelese, 2007) (Table 5). This was also true when  
238 this diet was compared to a diet rich in FM (40% crude protein, CP) (Madu and Ufodike, 2003). Interestingly, an

239 alternation of feeding catfish pellets and whole yellow mealworms cut into small pieces, which is very palatable  
 240 to African catfish, enhanced both feed efficiency and protein utilization, compared to a control diet containing  
 241 34% CP (Ng et al., 2001) (Table 3). However, all the fish fed live yellow mealworms (50 or 100%) had a higher  
 242 body lipid content than that of the control fish, due to the high lipid content of mealworms (Ng et al., 2001). Fish  
 243 fed only yellow mealworms demonstrated a reduced FI and weight gain (WG), thus suggesting the advisability of  
 244 using an alternation of pellets and whole insects (Ng et al., 2001). Feeding fish with maggots or yellow mealworms  
 245 live or chopped is therefore a good option, especially in countries where the usual feeds used are of poor quality.

246

247       3.2. *Use of insect meal as an FM replacement in fish diets*

248

249       In order to assess the use of insects in fish diets, it is scientifically more convincing to compare a control diet  
 250 that covers all the fish requirements with an isonitrogenous and isoenergetic diet containing insect meal. The  
 251 results of such a feeding experiment, pertaining to Orthoptera, Isoptera and Coleoptera, are summarized in Table  
 252 3.

253 **Table 3**

254       3.2.1. *Use of Orthoptera as an FM replacement in fish diets*

255       Orthoptera, including many species of locusts, grasshoppers and crickets, are major crop pests. The adult  
 256 insects are highly nutritious and have already been described accurately in a review paper (Makkar et al., 2014).  
 257 Briefly, a dietary inclusion of 13-25% of adult Orthoptera did not reduce the digestibility or growth of catfish or  
 258 tilapia (Alegbeleye et al., 2012; Emehinaiye, 2012) (Table 3). Catfish growth was actually improved by the 13%  
 259 inclusion of variegated grasshopper meal, a diet that showed an EAA profile very close to the requirements of  
 260 African catfish and high in PUFA (Alegbeleye et al., 2012). On the contrary, the dietary inclusion of dried  
 261 grasshopper significantly reduced the specific growth rate of walking catfish, *Clarias batrachus*, after 60 days of  
 262 feeding (Johri et al., 2011). Growth reduction was only found for higher inclusion levels than 34% of grasshoppers,  
 263 and this result may be associated with reduced protein and lipid digestibility and/or deficiencies in arginine (Arg)  
 264 and Lys (Alegbeleye et al., 2012).

265

266       3.2.2. *Use of Isoptera as an FM replacement in fish diets*

267       Isoptera are termites, *Macrotermes*, and are rich in proteins (37-49% CP) and lipids (30%), but poor in minerals  
 268 (Sogbesan and Ugwumba, 2008). A study on the use of termites in the diet of fish showed that they were very

269 palatable to vundu catfish, *Heterobranchus longifilis*: a 15% dietary inclusion produced the best growth  
270 performance, which, however, was not significantly different from the growth of fish fed FM or a 7.5% termite  
271 meal. Fish growth was significantly reduced for higher inclusion levels (22.5 and 30%) (Sogbesan and Ugwumba,  
272 2008). Solomon et al (2007) fed fingerlings of African catfish, *Heterobranchus bidorsalis*, with FM based diets  
273 supplemented with termites and soybean meal (SBM) at different ratios. They found increased growth performance  
274 and better nutrient utilization when termites were included in the blend. However, the results were not very reliable,  
275 given/considering the errors in the calculations of the percentages of termite inclusion and the variation in SBM  
276 and FM between the experimental diets, which made it difficult to establish whether the improved fish growth was  
277 due to the dietary termites or to the increased dietary FM (Solomon et al., 2007). Despite the good results of the  
278 partial replacement of FM, even compared with a very high quality FM (71% CP), termites (46% CP), being high  
279 producers of methane (Martius et al., 1993), being seasonal (Jamali et al., 2008) and being very difficult to rear,  
280 may not be a very good choice for large-scale use in aquafeeds.

281

### 282 3.2.3. Use of Coleoptera as an FM replacement in fish diets

283 3.2.3.1. *Mealworms*. Mealworms, such as the easily bred yellow mealworm, *Tenebrio molitor*, are beetles  
284 (Coleoptera), and are grain and flour pests (Ramos-Elorduy et al., 2002). Although the adults may not be used in  
285 feeds as they contain quinones, their larvae, mealworms, are a high quality feed that is rich in protein and lipids  
286 and poor in ash; their composition can be found in a previous/another review (Makkar et al., 2014). Mealworm  
287 larvae are usually fed live (see section 3.1), but can also be used as meal (Aguilar-Miranda et al., 2002; Veldkamp  
288 et al., 2012). They are currently being produced at a large industrial scale in China (Veldkamp et al., 2012). When  
289 the highly palatable yellow mealworm larvae was sun-dried or oven-dried, low inclusion levels of 9% of mealworm  
290 meal (20% FM replacement) led to the optimal growth of African catfish, and showed a significantly improvement  
291 compared to fish fed the FM based diet. Mealworm larvae could even replace up to 60% of dietary FM (26%  
292 dietary inclusion), without significantly affecting the growth or feed utilization of African catfish, but higher  
293 inclusion rates (35-43% corresponding to 80-100% FM replacement) have been shown to decrease fish growth  
294 performance, as well as feed and protein efficiency (Ng et al., 2001). In a pre-fattening trial performed on common  
295 catfish, *Ameiurus melas*, the total replacement of FM with dried mealworm larva meal significantly reduced fish  
296 growth performances, compared to control fish fed 50% FM, but growth was still considered satisfactory for this  
297 species (Roncarati et al., 2014). Yellow mealworm larvae have also been successfully used in rainbow trout, where  
298 they could be included at dietary levels of up to 50% (Gasco et al., 2014a) and in marine carnivorous fish, gilthead

299 seabream, *Sparus aurata*, and European sea bass, *Dicentrarchus labrax*, where 25% FM replacement did not affect  
300 fish growth significantly (Gasco et al., 2014b; Piccolo et al., 2014). The growth of both these marine fish was  
301 significantly affected at a 50% FM replacement level (Gasco et al., 2014b; Piccolo et al., 2014), and European sea  
302 bass showed a reduced n-3 HUFA concentration in the fillets (Gasco et al., 2014b).

303

304 *Asiatic rhinoceros beetles (Oryctes rhinoceros) or palm weevil (Rhynchophorus spp.)*. The former, which is  
305 another member of the Coleoptera order, and is known to be a beetle pest of palm trees, was overlooked in  
306 previous review papers on the subject ( see Makkar et al., 2014; Riddick, 2014). It was described in two  
307 publications as “palm grub”, but was associated with/erroneously given the Latin name of the Asian rhinoceros  
308 beetle (*O. rhinoceros*). It could also be confused with the palm weevil (*Rhynchophorus spp.*). The larvae of *O.*  
309 *rhinoceros* have very successfully been used to replace large amounts of FM in the diet of catfish (Table 3). A  
310 dietary inclusion of 14% (16% FM replacement) led to the optimal growth of both African and Vundu catfish  
311 (Fakayode and Ugwumba, 2013), but higher dietary inclusions of 33, 57, 80 or even 100% palm grub meal did  
312 not affect fish growth significantly (Fakayode and Ugwumba, 2013). However, the results are not very reliable:  
313 the FM replacement levels were overestimated (erroneous calculations) in this publication. *O. rhinoceros*  
314 processed into a meal and fed to Nile tilapia reduced both the WG and the survival of the fish (Omoyinmi and  
315 Olaoye, 2012), but both parameters were also very low in the control fish (2g WG in 10 weeks), perhaps because  
316 of the low quality of the used FM (35% CP) or the stressful conditions / disease of the fish.

317

318 3.2.3.2. *Superworm (Zophobas morio)*. The superworm is very rich in protein and contains adequate quantities of  
319 the EAA required for optimum fish growth, except for Met, which seems to be low (Yi et al., 2013; Barroso et al.,  
320 2014), but it is poor in minerals such as calcium and phosphorus (Ghaly and Alkoak, 2009). The dietary inclusion  
321 of 7.5 and 15% (25 and 50% FM replacement) of superworm meal (47% CP) has led to the optimal growth of Nile  
322 tilapia. Even a 22.5% inclusion (75% FM replacement) was tolerated well, in terms of growth, feed efficiency and  
323 protein digestibility, and showed no significant difference from FM-fed fish. Only a total FM replacement (30%  
324 dietary inclusion) reduced fish growth significantly, compared to FM-fed fish (Jabir et al., 2012). Although the  
325 diets decreased in proteins and increased in lipids with the increasing inclusion of superworms, the fillet  
326 composition was enriched in proteins and poorer in lipids at all the dietary levels tested, compared to the fish fed  
327 good quality FM (57% CP) (Jabir et al., 2012) , a result that was surprising.

328 Overall, Coleoptera have been used successfully as a partial replacement of FM in fish diets, although the  
 329 reliability of the studies on palm grub is questionable. Meals made from superworm led to the optimal growth of  
 330 Nile tilapia, while mealworm larvae showed positive results in the diets of both freshwater and marine fish.

331

332 *3.2.4. Use of Lepidoptera as an FM and FO replacement in fish diets*

333 **Table 4**

334 The domesticated silkworm, *Bombyx mori*, is mainly produced in India (Heuzé et al., 2014). Dried silkworm  
 335 pupa (SWP) meal is a valuable source of protein (50-71% dry matter, DM) and lipids (30%) (Wei and Liu, 2001;  
 336 Rumpold and Schluter, 2013). SWP meal has been used in many fish nutrition studies, which are summarized in  
 337 Table 4. . Silkworm meal, unlike other insect meals, seems to lead to good results, whether defatted or not, and  
 338 the fat of SWP could even be considered an asset. SWP oil was reported to attract and stimulate the appetite of  
 339 common carp, *Cyprinus carpio* (Begin et al., 1994). A study showed comparable growth and organoleptic  
 340 parameters when sardine oil was replaced with SWP oil in the diet of common carp, suggesting that SWP lipids  
 341 are satisfactory for use in cyprinids and that SWP could cheaply replace both FM and FO (Nandeesh et al., 1999).  
 342 Increasing dietary non-defatted SWP meal or SWP oil led to an increase in lipid digestibility, without significantly  
 343 increasing fat deposition (Nandeesh et al., 1990; Nandeesh et al., 1999). Moreover, the dietary addition of  
 344 defatted or non-defatted SWP meal led to very good digestibility values, not only in cyprinids (Jayaram and Shetty,  
 345 1980; Begin et al., 1994), but also in tilapia (Hossain et al., 1992; Boscolo et al., 2001) and in catfish (Hossain et  
 346 al., 1991; Borthakur and Sarma, 1998), even in the case of the total replacement of FM (Habib et al., 1994). In  
 347 cyprinids, the digestibility of both defatted and non-defatted SWP was even better than that of FM (Hossain et al.,  
 348 1997), although the defatted SWP was shown to be less digestible than FM in another study on common carp  
 349 (Kim, 1974). Numerous studies have shown the potential of the dietary inclusion of SWP in the diets of fish larvae  
 350 or juveniles, mainly in cyprinids, but also in many other fish species (Table 4). The growth, the feed performances  
 351 and the organoleptic quality of the fish were not affected by the dietary inclusion of SWP at levels below 50%,  
 352 even when not defatted (Begin et al., 1994; Rahman et al., 1996; Hossain et al., 1997; Nandeesh et al., 2000; Ji  
 353 et al., 2013). Low inclusion levels (5-12%) were successfully achieved for the carnivorous chum salmon,  
 354 *Oncorhynchus keta* (Akiyama et al., 1984) and for the olive flounder, *Paralichthys olivaceus*, when supplementary  
 355 AAs Lys and Met were added to the SWP-containing diet (Lee et al., 2012). The omnivorous snakeskin gourami,  
 356 *Trichopodus pectoralis*, was fed a diet containing 15% of SWP, without it affecting fish growth, but higher levels  
 357 than 22% decreased both protein digestibility and fish growth (Jintasatapom et al., 2011). In several studies on

358 rohu, *Labeo rohita*, common carp or rainbow trout, positive results have even been obtained for inclusion levels  
 359 of 30-50% (up to 100% FM replacement) of defatted or not-defatted SWP (Jayaram and Shetty, 1980; Rahman et  
 360 al., 1996; Hossain et al., 1997; Nandeesha et al., 2000; Dheke and Gubhaju, 2013). Successful total FM  
 361 replacement has also been achieved in common carp or in Japanese sea bass, *Lateolabrax japonicus*, but the control  
 362 diets were deficient in protein (11% FM, 21% CP) and the diets were not isonitrogenous (Jeyachandran and Raj,  
 363 1976), or no details on the reference diet were available (Ji et al., 2010). SWP has not been so successful in tilapia,  
 364 where a low inclusion level (5%) significantly reduced fish growth, compared to FM-fed fish (Boscolo et al.,  
 365 2001). Similarly, a study on Jian carp (*Cyprinus carpio* var Jian) has shown significantly reduced fish growth,  
 366 accompanied by decreased superoxide dismutase, intestinal protease activities and increased heat shock protein,  
 367 even for very low inclusion levels (6-9%) of SWP (Ji et al., 2013). The authors suggested that low levels of SWP  
 368 caused oxidative stress in Jian carp (Ji et al., 2013). However, walking catfish, fed 58% SWP, showed the same  
 369 level of protease activity as fish fed FM (Venkatesh et al., 1986). Overall, SWP seems to be a very good potential  
 370 protein source for use in fish diets, except, according to a few studies, for Nile tilapia, Jian carp and gourami.  
 371

## 372 **Table 5**

### 373 3.2.5. Use of Diptera as an FM replacement in fish diets

374 Protein replacement in fish diets has also been investigated using meals obtained from the larvae of two species  
 375 of Diptera: the housefly and BSF (Table 5). Both flies have been extensively described (distribution, rearing,  
 376 environmental impact, nutritional attributes) in a published review (Makkar et al., 2014).  
 377

#### 378 3.2.5.1. Common house fly larva (maggot) meal (magmeal)

379 Most studies on fish fed common house fly larva (maggot) meal, have not shown any improved fish growth,  
 380 except when maggots were fed live to fish (see section 3.1). However, three studies showed improved fish growth  
 381 when maggot meal was included in the fish diet: 50% FM replacement improved the growth of Nile tilapia, but  
 382 the inclusion levels were not mentioned in the publication (Ajani et al., 2004). The supplementation of the basal  
 383 diet with 2.5% of maggot meal improved fish growth and the fish immune status and resistance to diseases in the  
 384 mostly carnivorous black carp, *Mylopharyngodon piceus*, but the composition of the basal diet was not given and  
 385 may have been low in FM (Ming et al., 2013). Hybrid catfish fed 7.5% maggot meal also showed improved growth  
 386 compared to a control diet containing 30% of FM (isonitrogenous diets containing 40% CP) (Sogbesan et al.,  
 387 2006). In other studies, African catfish fed 12.5 or 25% of maggot meal (corresponding to 50 or 100% of FM

388 replacement, respectively) for 10 weeks grew well and showed good protein efficiency values (Nsor et al., 2008).  
389 Many other studies have shown the potential of maggot meal for use as an FM replacement in African and vundu  
390 catfish (7.5 to 32% inclusion) (Fasakin et al., 2003; Idowu et al., 2003; Aniebo et al., 2009; Adewolu et al., 2010),  
391 in common and Gibel carp (30-39% inclusion) (Ogunji et al., 2009; Dong et al., 2013) and in Nile tilapia (15-68%  
392 dietary inclusion) (Ogunji et al., 2007; Ogunji et al., 2008a; Ogunji et al., 2008b; Omoyinmi and Olaoye, 2012),  
393 without affecting fish growth (Table 5). A study using a low-protein maggot meal (29% CP) showed a reduction  
394 in growth of Nile tilapia for a 15-30% dietary inclusion (Ogunji et al., 2008c), but the different Protein/Energy  
395 ratios in the diets may have biased the results. Higher inclusion levels or similar levels of non-defatted maggot  
396 meal have been shown to affect fish growth in African catfish (Fasakin et al., 2003; Idowu et al., 2003; Oyelese,  
397 2007). When the maggot meal diet was supplemented with AAs, an inclusion level of 81% compared favourably  
398 with a soybean meal diet, but no FM control diet was included in this study (Ossey et al., 2012). Moreover, an  
399 increased cannibalism of the fish larvae was observed in fish fed the maggot meal diet (Ossey et al., 2012), which  
400 could have significantly improved the balance of the diet. Maggot meal has also been tested in rainbow trout, but  
401 was not very successful, as a 9.2% dietary inclusion significantly affected fish growth, and the fish fillets were  
402 poorer in n-3 FA than the control fish fed 36% of FM (St-Hilaire et al., 2007b). Although studied under the same  
403 conditions, dietary maggot meal was shown not to affect the growth of Gibel carp, *Carassius gibelio*, but to  
404 significantly reduce the growth, FCR and PER of darkbarbel catfish, *Pelteobagrus vachelli*; this reduction was  
405 linked to a decreased antioxidant activity of the latter fish (Dong et al., 2013). It is therefore not possible to  
406 generalise the positive results to other fish species, as fish requirements may vary from one fish species to another,  
407 and this may explain the high interspecies variability of the results.

408

#### 409 3.2.5.2. BSF larvae meal

410 Another species of Diptera that has been widely studied in fish is the BSF larva. The fish nutrition experiments  
411 were not as successful as expected (Table 5). They have already been described extensively (Makkar et al., 2014),  
412 and are therefore only described briefly hereafter: no inclusion level of BSF has led to a better fish performance  
413 than fish fed FM-rich control diets. However, some inclusion levels of BSF prepupae have shown a similar WG  
414 to that of fish fed FM: 6% dietary inclusion in channel catfish (Newton et al., 2005), 15% (St-Hilaire et al., 2007b)  
415 or 18-36% inclusion in rainbow trout (Sealey et al., 2011), with improved n-3 HUFA and PUFA contents in the  
416 fillets of fish fed BSF (Sealey et al., 2011), 5-25% inclusion in Atlantic salmon, *Salmo salar*, where the BSF diets  
417 were supplemented with AAs (Lys and Met) (Lock et al., 2014). Higher inclusion levels (12-30%) reduced fish

418 growth significantly in channel catfish, in rainbow trout, and in turbot, *Psetta maxima*, (Newton et al., 2005; St-  
419 Hilaire et al., 2007b; Kroeckel et al., 2012). Juvenile turbot accepted diets containing up to 33% defatted BSF  
420 prepupa meal, without any significant effects on FI or feed conversion. However, the specific growth rate was  
421 significantly lower for all the inclusion levels tested. At inclusion levels higher than 33%, the palatability of the  
422 diet and protein digestibility decreased, and resulted in a reduced FI and lower growth performance (Kroeckel et  
423 al., 2012). The protein and lipid digestibility results have been shown to be very good in Atlantic salmon (Lock et  
424 al., 2014).

425

### 426 *3.3. Quality of the fish fed insects*

427

428 Since marine fish richness in n-3 HUFA is associated with a health benefit for human consumers, modifying  
429 their FA profile could affect the perception of consumers and subsequently the market value of fish cultured for  
430 human consumption (Amberg and Hall, 2008). Moreover, the modification of fillet lipids and FA composition  
431 directly affects the total volatile compounds, and thus affects the aroma and flavor of the fish (Turchini et al.,  
432 2003). The replacement of FM with insect meal can increase the amount of fat or change the nature of lipids in  
433 fish, and could therefore change the taste of the fish fillets. Thus, less preference has been shown for catfish and  
434 tilapia fed solely with whole BSF larvae, due to the different aroma and texture from that of fish fed a commercial  
435 diet or fed partly with BSF larvae (25 or 50%) (Bondari and Sheppard, 1981). On the other hand, despite the  
436 differences in the FA profiles, no significant difference was obtained in a blind comparison of rainbow trout fed  
437 the FM-containing control diet with fish fed normal BSF or fish offal-enriched BSF pre-pupa diets (Sealey et al.,  
438 2011). Similarly, no difference in organoleptic properties was found in African catfish fed maggot meal (Aniebo  
439 et al., 2011), in Atlantic salmon fed defatted BSF (Lock et al., 2014), in cyprinids fed SWP oil (Jayaram et al.,  
440 1980; Nandeesha et al., 1999; Nandeesha et al., 2000) or non-defatted SWP meal (Nandeesha et al., 2000)  
441 compared to control fish. These results suggest that the partial inclusion of insect meal (10-50%) in the diet of fish  
442 does not affect the FA profiles, aroma or flavor enough to be detected by consumers.

443

## 444 **4. Potential hurdles and solutions**

445 The many studies using insects in the diet of fish have underlined potential problems and suggested some solutions,  
446 which are discussed hereafter.

447

448    4.1. *Mass rearing to avoid toxicity of insects through bioaccumulation*

449

450    Potential hurdles, such as the bioaccumulation of insecticides, heavy metals and natural toxins in insects  
451 harvested from the wild can be monitored more easily in mass rearing setups through controls of their rearing  
452 substrates (van der Spiegel et al., 2013), especially when organic by-products are proposed as diets for the insects.  
453 A study using high dietary levels of maggot meal (>35%), obtained from larvae fed on poultry droppings, has  
454 shown increased levels of liver Glutathione S-transferase (GST) in Nile tilapia, suggesting the presence of  
455 pesticides, medical drugs or toxic residues in the hen manure (Ogunji et al., 2007). As large quantities of insect  
456 meal should be available before aquafeed companies envisage their inclusion in the feed of fish (I. Karakostas,  
457 Biomar, Greece, Personal Communication), the supply chain would need to rely on the mass rearing of the insects  
458 on quality-controlled substrates in order to avoid any potential toxicity problem. The mass rearing of insects has  
459 spread worldwide, especially for the production of silk, fishing bait and pet food (for fish, birds and reptiles) and  
460 for the biological control of pest species (Schabel, 2010; FAO, 2013). Small production units of BSF, mealworms,  
461 locusts, house crickets and grasshoppers are currently being set up in Europe and in the rest of the world (Ynsect,  
462 France; Protix, Netherlands; Hermetia, Germany; Insagri, Spain; Enviroflight, USA; Next Millenium farm, USA;  
463 Enterra Feed, Canada; Entologics, Brazil) (Kroeckel et al., 2012; Rumpold and Schluter, 2013), and a very large  
464 production unit of maggot meal has already been set up in South Africa (AgriProtein; 7t maggot meal/day, 3t  
465 magoil/day). Moreover, the production of mealworms is already very important in China (HaoCheng: 50t  
466 mealworms and superworms/month) and in India (Veldkamp et al., 2012; Ji et al., 2013; van Huis et al., 2013).  
467 The production of silkworms in China accounts for approximately 80% of the world's production, with the annual  
468 capacity for dry pupa production being approximately 200 000 mt (Dong and Wu, 2010). Producers throughout  
469 the world are becoming organized (VENIK, IPIFF) in order to raise interest in the use of insects as feeds or food.  
470 As FM prices regularly increase (FAO, 2013), the price of insect meal obtained from large production units could  
471 very rapidly become competitive (Drew et al., 2014).

472

473    4.2. *Mixture of dietary proteins or dietary supplementation in order to better satisfy fish requirements*

474

475    On the basis of studies on FM replacement with plant proteins, it appears that the use of a mixture of dietary  
476 proteins may help to prevent the deficiencies caused by the use of a single ingredient (Hansen et al., 2007;  
477 Torstensen et al., 2008; Hansen et al., 2011; Zhang et al., 2012a; Zhang et al., 2012b; Hu et al., 2013). Some

478 insects, such as *Z. variegatus* and *M. bellicosus*, show deficiencies in AAs (Met, Lys, threonine or tryptophan,  
479 Trp). The use of a mixture of insect meals may equilibrate the AA profiles of the diet. A species slightly deficient  
480 in Trp, such as BSF, could be mixed with the housefly or with SWP, both of which are richer in Trp. Insect meals  
481 may also be mixed with other protein sources. Nutritional studies have shown that combining superworm meal  
482 with 10% of a prebiotic mushroom in a fish diet further improves the performances of tiapia (better Specific  
483 Growth Rate, Feed Conversion Ratio, Protein Efficiency Ratio and fish survival) (Mohd Din et al., 2012), possibly  
484 due to a balancing of the AA composition of the diet (Kim et al., 2009) or to the prebiotic properties of the  
485 mushroom. The total replacement of dietary FM protein with a mixture of SWP (43% inclusion) and clam meat  
486 (31% inclusion) protein showed improved feed utilization in rohu fingerlings, compared to fish fed 54% of FM  
487 (Begin et al., 1994). Mineral dietary supplementation may also be necessary to cover the basic needs of the fish,  
488 particularly for freshwater species that require more minerals than seawater species (Hasan, 2001). Salt and mineral  
489 supplementation made it possible to overcome the growth depression of broilers, caused by 50% FM dietary  
490 replacement (Reddy et al., 1991), but, to the best of the authors's knowledge, no trial has been performed on  
491 freshwater fish.

492

#### 493 *4.3. Improving insect lipids to better satisfy fish requirements*

494

##### 495 *4.3.1. Manipulation of lipids in the insect diet to improve the FA profile for use in aquafeeds*

496 The quality of the FA profile of terrestrial insects is usually thought to be poor in HUFA for use in the feeds  
497 of marine fish, but it may be enhanced by manipulating the diet of the insects in order to increase the n-3 and n-6  
498 FAs (Ogunji et al., 2008b). For example, the lipids of BSF larvae fed on cow manure consisted mainly of saturated  
499 or monounsaturated FAs and low levels of omega-3 FA (0.2%), whereas larvae fed cow manure and 22% fish  
500 offal for only 24h contained 4% n-3 FA (St-Hilaire et al., 2007a). Feeding BSF prepupae partly (25 or 50%) with  
501 fish offal for a month led to a 25 to 50% increase in FM replacement in the diet of rainbow trout, without any  
502 growth reduction (Sealey et al., 2011). However, it seems more economically viable to use n-3 HUFA rich  
503 components (by-products of the fish filleting industry, microalgae, phytoplankton...) to feed the fish directly rather  
504 than enriching insects in order to feed them to the fish.

505

##### 506 *4.3.2. Use of aquatic insects to supply marine fish with adequate amounts of n-3 FA*

507 Since aquatic insects are more predatory than the usually herbivores or omnivores terrestrial insects (Yen,  
 508 2014), and because they eat aquatic organisms richer in n-3 FA, aquatic insects are usually richer in n-3 HUFA  
 509 (mainly EPA) (Bell et al., 1994; Yang et al., 2006; Fontaneto et al., 2011). This makes them better candidates to  
 510 feed marine fish, which are more dependent on HUFA than freshwater fish (see section 2.2). There are numerous  
 511 aquatic insects (McCafferty, 1981). They were already semi-cultivated in Mexico in the pre-Hispanic era (Van  
 512 Itterbeeck and Van Huis, 2012), by means of environmental manipulation, through the arrangement of bundles of  
 513 aquatic weeds to host the eggs of *Neopomorpha* Hemiptera aquatic tree bugs (Parsons, 2010). Lion water bugs  
 514 and aquatic bees are used for medicinal purposes in Zaire (Tango, 1994; Srivastava et al., 2009; Yen, 2014). The  
 515 intensive harvesting of insect delicacies, such as giant water bugs in Thailand (Clutterbuck, 1993), lake fly,  
 516 *Chaoborus*, adults in Uganda, the larvae of dragonflies in Papua New Guinea (Srivastava et al., 2009) or diving,  
 517 backswimmer and water scavenger beetles in China and Laos, can occasionally lead to a decline in wild insect  
 518 populations (Hanboonsong and Durst, 2014; Yen, 2014). To the authors' knowledge, mass rearing of aquatic  
 519 insects is restricted to the larvae of mosquitoes, which are mainly used for biological controls, and only one  
 520 bibliographical reference is available about their use in fish nutrition (Ostaszewska et al., 2011) (Table 5). It would  
 521 be interesting to study the use of aquatic insects in marine fish diets.

522

#### 523 4.4. Chitin

524

##### 525 4.4.1. Process to improve chitin digestibility

526 It is commonly assumed that monogastric animals, including fish, cannot digest chitin (Rust, 2002; Sanchez-  
 527 Muros et al., 2013). In order to enable the inclusion of chitin in animal feeds, it could be removed from insect meal  
 528 via alkaline extraction (DeFoliart, 1992; Belluco et al., 2013; Sanchez-Muros et al., 2014), or chitinase and  
 529 chitinolytic bacteria could be included simultaneously to improve the digestibility of chitin-protein complexes  
 530 (Kroeckel et al., 2012), as has been shown for tilapia fed diets containing crustacean shell (Zhang et al., 2014).  
 531 Alternatively, chitin could be degraded by chemical or enzymatic methods before being added to fish diets as  
 532 chito-oligosaccharides (COS), acetylglucosamine (GlcNAc) or chitosan ((Shiau and Yu, 1999; Se-Kwon and  
 533 Niranjan, 2005; Lin et al., 2012a; Lin et al., 2012b). Like chitin, low levels of these metabolites have been shown  
 534 to immunostimulate fish (Hoffman et al., 1997; Lin et al., 2012a). However, these processes could substantially  
 535 increase the costs of insect meal.

536

## 537 4.4.2. Is chitin really a problem?

538 Growth experiments on the inclusion of insects in fish diets that lead to a reduction in fish growth and protein  
539 and lipid digestibility have very often blamed chitin, without any real evidence (Lindsay et al., 1984; Köprücü and  
540 Özdemir, 2005; Balogun, 2011; Longvah et al., 2011; Alegbeleye et al., 2012). The digestion of chitin requires the  
541 action of 3 enzymes, chitinase, chitobiase and lysozyme, all of which are present in both carnivorous and  
542 omnivorous fish (Lindsay et al., 1984; Fines and Holt, 2010). Chitinase, which has been found in the fish stomach,  
543 disrupts the chitinous exoskeletons, whereas chitobiase, which is present in the fish intestine, plays a nutritive role  
544 (absorption of nutrients) (Jeuniaux, 1993). The presence of these enzymes has been found in both freshwater  
545 (Lindsay et al., 1984; Jeuniaux, 1993) and marine fish (Fänge et al., 1979; Danulat and Kausch, 1984; Kono et al.,  
546 1987; Clark et al., 1988; Kurokawa et al., 2004; Fines and Holt, 2010). However, a study on turbot fed BSF larvae  
547 failed to detect any chitinase activity, but the study looked at the enzymatic levels in the mid-gut rather than in the  
548 stomach of the fish (Kroeckel et al., 2012). Despite the presence of these enzymes in fish, it is commonly assumed  
549 that fish cannot digest chitin ? fish?Do you need the word “fish” here? (Rust, 2002; Sanchez-Muros et al., 2013).  
550 The chitin found in crustaceans is encompassed in a matrix of proteins and minerals (mostly calcium) (Johnson  
551 and Peniston, 1982; No et al., 1989), whereas the cuticle of insects is composed of chitin in a matrix of proteins,  
552 lipids and other compounds (Kramer et al., 1995). It has been hypothesized that these matrix forms of chitin may  
553 reduce the access of chitinases or proteinases to their substrates and may prevent the absorption of proteins and  
554 lipids by the intestine (Tanaka et al., 1997), thus reducing not only chitin digestibility but also lipid and protein  
555 digestibility, with a subsequent reduction in nutrient utilization and fish growth performance. Dietary crustacean  
556 chitin was shown to reduce digestibility of the diet and the growth of rainbow trout fed 25% chitin (Lindsay et al.,  
557 1984) and of tilapia hybrids, *O. niloticus*×*O. aureus* fed 2, 5 and 10% chitin (Shiau and Yu, 1999). However, in  
558 contrast to these two studies, many more studies on fish naturally feeding on crustaceans, insects or benthic  
559 invertebrates have shown a high digestibility of shrimp and crab meals. In these studies, fish performances were  
560 either improved or were not affected by dietary chitin: an increased protein digestibility was obtained in cobia fed  
561 crab meal containing 3% chitin or shrimp meal containing 10% chitin (Fines and Holt, 2010);, 1% of dietary chitin  
562 did not affect the growth of common carp (Gopalakannan and Arul, 2006). Ten percent of dietary chitin stimulated  
563 the growth of both Japanese eel (*Anguilla japonica*) and yellowtail kingfish (*Seriola quinqueradiata*) (Kono et al.,  
564 1987); 5 and 10% of dietary chitin stimulated the growth of red seabream (*Pagrus major*), while 20% reduced it  
565 (Kono et al., 1987). In addition, low levels of dietary chitin have been reported to increase the activity of the  
566 innate immune system in seabream (<0.01%) (Esteban et al., 2001) and carp (1%) (Gopalakannan and Arul, 2006)

567 and to increase the resistance of common carp to bacterial disease (1% chitin) (Gopalakannan and Arul, 2006).  
568 Chitin may therefore not be the problem it was first suggested to be.

569

570 *4.4.3. Quantification of chitin in insects*

571 Until recently, the amount of chitin in insects has been considered to correspond to acid detergent fiber (ADF),  
572 but a study has shown that the ADF fraction actually contains high amounts of AAs (6.7 to 32.7% of the ADF),  
573 which suggests that previous studies overestimated the chitin content of insects (Finke, 2007). Using ADF,  
574 adjusted for its amino acid content, the estimated chitin content of insects ranged from 11.6 to 137.2 mg/kg (DM)  
575 (Finke, 2007). Thus, a diet containing insect meal will only transfer a very small amount of chitin to the fish.  
576 Moreover, the first suggestions that soft-bodied insects contained less chitin and were more digestible than hard-  
577 bodied insects (Frye and Calvert, 1989) were biased, as more recent studies have shown that the chitin content of  
578 SWP larvae is similar to that of adult crickets (Finke, 2007). In fact, the hardness of the cuticle could primarily be  
579 a function of the degree of sclerotization, a process that links protein to fibers, and could thus be proportional to  
580 the AA content of the cuticles rather than to its chitin content (Nation, 2002). Subsequently, the digestibility of the  
581 insects may decrease when the proportion of AAs increases in their ADF fraction (Finke, 2007). Thus, the  
582 reduction in fish growth is more likely to be related to the proportion of AAs in the cuticle or to a potential AA  
583 imbalance, FA deficiency or other inadequacies of the diets than to the chitin content. With a caloric content of  
584 17.1 kJ g<sup>-1</sup>, chitin could constitute a substantial percentage of the total energy intake of marine fish feeding on  
585 crustaceans (Gutowska et al., 2004), and it represents an under-utilized biomass resource, despite the potential  
586 commercial benefits of using it as a prebiotic or as a carbohydrate source for aquafeeds (Tharanathan and Kittur,  
587 2003). This ? Laura – what does “This” refer to? may also be applicable to insect chitin, and further studies are  
588 needed to determine this potential.

589

590 *4.5. Processing of insect meal to improve palatability and digestibility*

591

592 The low palatability of insect meal may be caused by different factors: a potential chemical or microbiological  
593 contamination, the presence of anti-nutrient factors, flavonoids and terpenoids in the feedstuff of insects (Finke,  
594 2002) and/or the richness in monounsaturated FAs of terrestrial insects (Barroso et al., 2014), which makes them  
595 susceptible to oxidation, with resulting rancidity problems (Finke, 2002). The palatability, nutrient availability,

596 digestibility and composition of insect meal may be more suitable for fish nutrition after some processing of the  
597 meal, such as drying, hydrolyzing, ensiling or defattening (Newton et al., 2005).

598

599 *4.5.1. Drying of insect meal*

600 The sun- or oven-drying of mealworms, directly or after boiling in water, has improved the palatability of  
601 mealworms (Ng et al., 2001). Oven-drying is usually recommended over sun-drying, as the latter usually yields a  
602 fattier maggot meal (with an increased risk of lipid oxidation) that is poorer in proteins (Aniebo and Owen, 2010).

603

604 *4.5.2. Acid hydrolysis or ensilage of insect meal*

605 Although hydrolysis using acids has been shown to successfully increase the protein and decrease the lipid  
606 contents of insect (silkworm and maggots) meal (Anon, 1999; Fasakin et al., 2003), it may also partially or totally  
607 destroy some EAAs such as cystine, Met and Trp (Castell, 1986). Ensiling with molasses, acids and antioxidants  
608 (Rangacharyulu et al., 2003) seems more promising. During fermentation, the bacteria predigest the components  
609 of the feed, which may be difficult to digest by the fish. Fermentation converts sugars into acids, thus lowering the  
610 pH, and competes with bacteria that might cause spoilage (Manikandavelu et al., 1992). Optimised ensilage can  
611 improve palatability, as shown in mammals, but the conditions must be closely monitored (Buchanan-Smith,  
612 1990). Fermented SWP silage has been shown to be nutritionally superior (better nutrient utilization and fish  
613 survival, leading to a higher production) than untreated SWP or even FM (Rangacharyulu et al., 2003).

614

615 *4.5.3. Defattening*

616 Given the presumed good quality of insect EAA profiles and the low quality of insect FA profiles for marine  
617 fish, it could be a good idea to defatten insect meal and use the proteins for fish feeds and the lipids for other  
618 processes that do not require richness in n-3 HUFA. For example, the lipids extracted from BSF or mealworms  
619 have been suggested to be good candidates for use in biofuel (Belforti et al., 2014; Schneider and Llecha, 2014).  
620 Defattening insects by cutting the frozen BSF larvae and applying them to a press to enable the leakage of  
621 intracellular fat (Kroeckel et al., 2012) or using petroleum ether extraction of the insect meal (Fasakin et al., 2003)  
622 has increased the crude protein content of the insect meal (Hossain et al., 1997; Fasakin et al., 2003). Defattening  
623 maggot meal has led to an increase in the inclusion level of maggot meal in the diet of African catfish, without  
624 affecting fish growth (Fasakin et al., 2003). However, a recent study on Atlantic salmon has shown that highly  
625 defatted BSF, dried at a conventional temperature, surprisingly reduced fish growth compared to fish fed lightly

626 defatted BSF dried at a low temperature (Lock et al., 2014). The problem with lipids may be related more to their  
627 oxidation (at a high temperature) than to the actual high dietary lipid content.

628

629 *4.5.4. Addition of antioxidants*

630 Rancidity has been proposed as a limiting factor to the use of fatty insect meal, but some studies using SWP  
631 have shown better results with non-defatted than with defatted insect meal (Hossain et al., 1997). It was suggested  
632 that lipophilic growth stimulants may be lost when insect meal is defattened (Tsushima and Ina, 1978; Nandeesha  
633 et al., 1999). Adding antioxidants to insect meal could therefore increase palatability and reduce the negative  
634 effects of the insect lipid content on fish growth.

635 The advantages of processing meal (digestibility/ palatability) must be weighed against the cost of the processing,  
636 as an increase in the price of the final insect meal should not surpass the gains provided by the increase in weight  
637 of the commercialized fish.

638

639 **5. Conclusion**

640 This review has highlighted the good potential of using insects as a replacement of FM in finfish diets, thanks  
641 to their high protein content and adequate EAA profiles, which are only deficient in a few AAs. The use of a  
642 mixture of different protein sources (different insects or insects with prebiotics, with plants or with animal  
643 proteins) or dietary AA supplementation could reduce the potential nutrient deficiencies and better balance the  
644 AA profiles. Lipid oxidation or an inadequate FA composition of some insect meals can limit their use in  
645 aquafeeds as replacements of FO, and defatted insect meal could be more useful, although this topic needs  
646 further research. A total FM replacement with insect meal has usually not been successful, probably because of a  
647 dietary unbalance or deficiencies. However, when insects (mealworm, maggots, BSF) were fed whole to fish,  
648 they usually compared positively with control fish fed low quality commercial pellets. The partial replacement  
649 with insect meal seems possible, mainly for herbivorous / omnivorous species, but also for some carnivorous fish  
650 (black carp, rainbow trout, Japanese sea bass, chum salmon, gilthead seabream and European sea bass).

651 Mealworms, SWP and maggots have been shown to be the most successful insects in fish feeding experiments so  
652 far, with good digestibility results and fish growth performances that match and even exceed FM in some cases,  
653 even for high dietary inclusion levels or for whole fat insect meal. In order to achieve a positive and significant  
654 ecological and economic impact, further studies need to be conducted, preferentially focusing on the use of these  
655 insects in the diet of fish species with high protein requirements. Digestibility issues also require further studies,

656 as the chitin content may not be the only factor responsible for these problems, and may even not be involved at  
657 all. In order to enhance the achievable percentage of FM substitution, without compromising fish growth or fish  
658 health, different strategies (drying, defattening, ensilage, manipulation of the insect diet and rearing conditions,  
659 the addition of antioxidants) could be adopted to enhance the nutritive value, the palatability and/or digestibility  
660 of the insect meal, and to reduce the potential toxicity or contamination level of the meal. Defattening insect  
661 meal may also be a means of separating the highly proteic insect meal for use in fish feeds, and insect lipids for  
662 use, for example, as biofuel. A finishing diet rich in FM and FO could be used in marine fish fed insects, before  
663 the commercialization of the fish, in order to restore the richness in n-3 FA of the fillets. Alternatively, aquatic  
664 insects could be used in the diet of marine fish, but their mass rearing needs to be mastered first. The sheer  
665 multitude of availablr insect species, with different habitats, developmental stages, and feeding habits as well as  
666 the optimal rearing conditions that can affect their composition, portend a great deal of potential future research  
667 on this exciting subject in which entomologists and fish nutritionists will have to join forces.