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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

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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 frequirements 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.

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

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.   Icorporation 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 Alkoaik, 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* (Begun 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 (Nandeesha 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 (Nandeesha et al., 1990; Nandeesha 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; Begun 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 (Begun et al., 1994; Rahman et al., 1996; Hossain et al., 1997; Nandeesha 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). Hoever, 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 (Nsofor 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   (Begun 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 ot 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 that 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^{-1}, 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 hydrolysation or ensilage of insect meal*

605   Although hydrolysation 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.