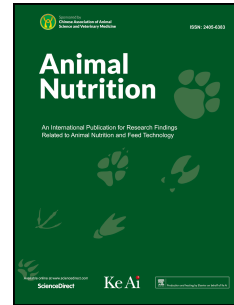


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Recent advances in the utilization of insects as an ingredient in aquafeeds: A review

Sahya Maulu, Sandra Langi, Oliver J. Hasimuna, Dagoudo Missinhoun, Brian P. Munganga, Buumba M. Hampuwo, Ndakalimwe Naftal Gabriel, Mabrouk Elsabagh, Hien Van Doan, Zulhisyam Abdul Kari, Mahmoud A.O. Dawood



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1 **Recent advances in the utilization of insects as an ingredient in aquafeeds: A review**

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39

#### 40 **Abstract**

41 The aquafeed industry continues to expand in response to the rapidly growing aquaculture sector.  
42 However, the identification of alternative protein sources in aquatic animal diets to replace  
43 conventional sources due to cost and sustainability issues remains a major challenge. Recently,  
44 insects have shown tremendous results as potential replacers of fishmeal in aquafeed. The  
45 present study aimed to review the utilization of insects in aquafeeds and their effects on aquatic  
46 animals' growth and feed utilization, immune response and disease resistance, and fish flesh  
47 quality and safety. While many insect species have been investigated in aquaculture, the black  
48 soldier fly (*Hermetia illucens*), and the mealworm (*Tenebrio molitor*) are the most studied and  
49 most promising insects to replace fishmeal in aquafeed. Generally, insect rearing conditions and  
50 biomass processing methods may affect the product's nutritional composition, digestibility, shelf  
51 life and required insect inclusion level by aquatic animals. Also, insect-recommended inclusion  
52 levels for aquatic animals vary depending on the insect species used, biomass processing  
53 method, and test organism. Overall, while an appropriate inclusion level of insects in aquafeed  
54 provides several nutritional and health benefits to aquatic animals, more studies are needed to  
55 establish optimum requirements levels for different aquaculture species at different stages of  
56 development and under different culture systems.

57

58 **Keywords:** Aquaculture; Aquafeed; Alternative protein source; Immune response; Insect;

59

#### 60 **1. Introduction**

61 The global population is expected to reach nearly 10 billion people by 2050 (United Nations,  
62 2019). Therefore, the food production sector, particularly animal protein production will play a  
63 critical role in food and nutrition security. In most global communities, fish is the cheapest and  
64 most readily available source of animal protein (Maulu et al., 2020). Aquaculture is not only the  
65 fastest-growing food production sector but also a major contributor to global food fish (Food and  
66 Agriculture Organization [FAO], 2020). Besides, aquaculture is the most sustainable and  
67 efficient way of producing aquatic products (FAO, 2020; Maulu et al., 2021a). However, the  
68 increasing costs of production in aquaculture due to the rising cost of feeding threaten the  
69 sustainability of the sector (Dawood, 2021). This is primarily caused by the overdependence of  
70 intensive aquaculture production on fishmeal and fish oils as major feed ingredients whose prices  
71 continue to rise due to declining production (Dawood, 2022; Hazreen-Nita et al., 2022). In  
72 addition, the food-feed-fuel competition for the limited resources under the current changing  
73 climatic conditions has drastically affected the availability of conventional feedstuffs such as  
74 fishmeal, soybean and cereals, leading to a decline in availability and high volatility in feed  
75 ingredient prices (Mugwanya et al., 2022). Besides, plant-based ingredients have been reported  
76 to cause negative side effects in the gut of carnivorous fish due to the presence of anti-nutritional  
77 factors (Zhou et al., 2018) and complex indigestible carbohydrates (Gaudioso et al., 2021),  
78 consequently impacting growth and welfare of the fish. Therefore, recent studies have focused  
79 on evaluating potentially sustainable alternatives including insects (Li et al., 2019; Alves et al.,  
80 2020; Terova et al., 2021), bacteria (Maulu et al., 2021b; Li et al., 2021) and organic by-products  
81 (Mo et al., 2014; Cheng et al., 2017). Among these, insects have attracted the most attention due  
82 to their wide application in aquaculture and ease of production.

83 Insects are reported to contain high crude protein content of 34% to 74% dry matter, DM  
84 (Freccia et al., 2020; Gasco et al., 2020). However, most whole-insects contain 42% to 63.3%  
85 crude protein on a dry matter basis (Alfiko et al., 2022) with up to 74% reported when insect  
86 meal is defatted (Alfiko et al., 2022). Additional nutritional value includes a well-balanced  
87 essential amino acid (EAA) profile resembling that of fishmeal, high lipid (10% to 30%), DM  
88 level (albeit high variability in fatty acid profiles), a good source of vitamins like vitamin B<sub>12</sub>,  
89 and some bio-available minerals like iron and zinc (Alegbeleye et al., 2012; Gasco et al., 2020).  
90 Furthermore, insect meal contains bioactive compounds (e.g., chitin, fatty acids and  
91 antimicrobial peptides) with prebiotic, antioxidant and antimicrobial properties that promote

92 animal health and counteract antimicrobial resistance (Gasco et al., 2018; Borrelli et al., 2021;  
93 Veldkamp et al., 2021). However, the nutritional composition varies with insect species  
94 (DeFoliart, 1995; Barroso et al., 2014), the rearing process (Zarantoniello et al., 2020) and the  
95 production process of the protein (Ramos-Elorduy et al., 2002), suggesting that the proximate  
96 composition could be modified to suit specific requirements. For instance, defatting increases the  
97 protein content in insect meal, (Alfiko et al., 2022) while rearing the insects on substrate rich in  
98 n-3 polyunsaturated fatty acids (PUFAs) could increase the PUFA profile content in insects  
99 (Zarantoniello et al., 2020). Unlike fishmeal and plant-based protein, insects can be produced  
100 intensively within a short time with little need for arable land, reduced water  
101 consumption/utilization, lower greenhouse gas (GHG) emissions, and bio-waste conversion  
102 (Gasco et al., 2020; Pulido-Rodriguez et al., 2021). Thus, insect farming is considered  
103 sustainable due to its low ecological footprint. Also, when used in diets with multiple ingredients  
104 including plant-based proteins, insects have shown potential to counteract the negative effects on  
105 growth and gut health in carnivorous species, which are usually common when fishmeal is  
106 replaced with plant protein (Randazzo et al., 2021; Pulido-Rodriguez et al., 2021; Gaudioso et  
107 al., 2021).

108 The unique properties of insects and their suitability for application in aquafeeds as fishmeal and  
109 fish oil replacement have become a hotspot for research in aquaculture. Numerous authors have  
110 reviewed the existing literature to identify findings that provide a map for future development.  
111 Most of these studies have focused on the nutritional composition of different insects used in  
112 aquafeeds, their production technology, and prospects (Henry et al., 2015; Nogales-Merida et al.,  
113 2018; Ameixa et al., 2020; Gasco et al., 2020; Oonincx and Finke, 2021; Alfiko et al., 2022).  
114 Others have further highlighted the effect of insects in aquafeed on aquatic animals (Wang and  
115 Shelomi, 2017; Freccia et al., 2020; Hawkey et al., 2021). English et al. (2021) reviewed the  
116 advancements in the production methods for BSF and their application in salmonids, while  
117 Priyadarshana et al. (2021) reviewed the application of BSF meal focusing on growth  
118 performance and body composition in finfish. Mousavi et al. (2020) reviewed the functional  
119 properties of insects focusing on their immunomodulatory and physiological effects on aquatic  
120 animals. Other reviews, such as those of Zarantoniello et al. (2020) and Shafique et al. (2021)  
121 only focused on a single insect species and its effect on fish. Hodar et al. (2020) broadly looked  
122 at a range of alternative protein alternatives (including insects) as potential fishmeal and fish oil

123 replacement in aquafeeds while Liland et al. (2021) performed a meta-analysis on the nutritional  
124 value of insects in aquafeeds. More recently, Alfiko et al (2022) reviewed the status and trends in  
125 the application of insects in fish feeds. In this study, we provide a more comprehensive overview  
126 of the most recent advances in the utilization of insects as a promising aquafeed ingredient. This  
127 paper attempts to bridge the gap in the existing literature by presenting information on the  
128 response of different aquaculture species to insect-based feeds and opportunities for further  
129 improvement.

130

## 131 **2. Insect species utilized in aquafeed**

132 Nowadays, insects are viewed as the most promising and sustainable source of animal protein  
133 mainly because of their nutritional value, amino acid composition and ease of propagation  
134 (Iaconisi et al., 2019; Gasco et al., 2016; Biancarosa et al., 2019; Gasco et al., 2020; Tilami et al.,  
135 2020; Were et al., 2021). Many of these insects have shown beneficial conversion factors and  
136 productivity, fast life cycles and the ability to grow on a variety of available substrates, yielding  
137 high quality and readily assimilated proteins and highly unsaturated fatty acids (HUFA), as well  
138 as vitamins and functional compounds (Tacon and Metian, 2008; Gasco et al., 2016; Turek et al.,  
139 2020; Shafique et al., 2021). Consequently, some have been incorporated into aquafeed  
140 formulations for different aquatic species, yielding interesting results. The most promising insect  
141 species whose meal has been used to replace fish meal and/or fish oils include the black soldier  
142 fly (BSF: *Hermetia illucens*), the yellow mealworm (TM: *Tenebrio molitor*) and the common  
143 housefly (MD: *Musca domestica*) (Belforti et al., 2015; Gasco et al., 2020; Iaconisi et al., 2019;  
144 Sogari et al., 2019). So far, BSF, TM and MD have well-documented production processes.  
145 Although some of the insects like the house fly are known to be parasitic and disease vectors,  
146 other species like the BSF are considered symbiotic (Menino and Murta, 2021) as they can be  
147 propagated without causing any known harm to humans. Unlike animals, the feed conversion  
148 rate and GHG emissions of insects are much lower in a certain temperature range since insects  
149 do not use energy to maintain their body temperature in a strict range (Belforti et al. (2015).  
150 Irrespective of the different methods of propagation and production of different species, insects  
151 have shown promising results for potential use as a protein and oil source in aquafeed.  
152 Many studies have revealed that insect meals and oil can partially or completely replace the fish  
153 and soybean meals and oils that are commonly used in aquaculture production (Henry et al.,

154 2015; Nogales-Merida et al., 2019; Fawole et al., 2020; Tilami et al., 2020; Xu et al., 2020;  
155 Hender et al., 2021). Insects such as the BSF have been extensively studied, not only in fish  
156 culture but also in poultry and swine (Sogari et al., 2019). In aquaculture, many studies have  
157 revealed positive results when BSF meal was used as a substitute for fish meal for many species  
158 such as whiteleg shrimp (*Litopenaeus vannamei*) (Richardson et al., 2021), barramundi (*Lates  
159 calcarifer*) (Hender et al., 2021), climbing perch (*Anabas testudineus*) (Mapanao et al., 2021),  
160 Nile tilapia (*Oreochromis niloticus*) (Were et al., 2021), African catfish (*Clarias gariepinus*),  
161 (Fawole et al., 2020), Japanese sea bass (*Lateolabrax japonicus*) (Wang et al., 2019), Atlantic  
162 salmon (*Salmo salar*) (Lock et al., 2016; Stenberg et al., 2019), Siberian sturgeon (*Acipenser  
163 baerii*) (Zarantoniello et al., 2021), gilthead sea bream (*Sparus aurata*) (Randazzo et al., 2021),  
164 clownfish (*Amphiprion ocellaris*) (Vargas-Abúndez et al., 2019) and in rainbow trout  
165 (*Oncorhynchus mykiss*) (Cardinaletti et al., 2019; Bruni et al., 2020) to produce food fish. In the  
166 aforementioned species, BSF improved various growth parameters as well as the immune  
167 response to some diseases affecting aquatic species. Also, TM has shown positive results when  
168 utilized in the diets of many aquatic species, such as yellow catfish (Su et al., 2017; 2019),  
169 gilthead seabream (Fabrikov et al., 2021), largemouth bass (*Micropterus salmoides*) (Su et al.,  
170 2022), seabass (*Dicentrarchus labrax*) (Reyes et al., 2020), narrow-clawed crayfish  
171 (*Pontastacus leptodactylus*) (Mazlum et al., 2021), olive flounder (*Paralichthys olivaceus*)  
172 (Jeong et al., 2021), black porgy (*Acanthopagrus schlegelii*) (Jeong et al., 2022) and rainbow  
173 trout (Su et al., 2017; Melenchón et al., 2021). Furthermore, TM has a relatively high nutritional  
174 value as well as being a rich source of essential amino acids (methionine), lipids and fatty acids,  
175 that vary based on the developmental stage of the larvae (Shafique et al., 2021).

176 Other species of insects that have yielded promising results in aquatic animals include the  
177 superworm (*Zophobas morio*) in Nile tilapia (Alves et al., 2020) and the silkworm (*Bombyx  
178 mori*) in Pacific white shrimp (*L. vannamei*) (Rahimnejad et al., 2019) as well as its application  
179 in many other cultured fish and shrimp species (Sankian et al., 2018; Feng et al., 2019; Ido et al.,  
180 2019; Su et al., 2019). Despite limited information existing, the nutritional properties of insects  
181 for use in aquafeed are likely to vary across and within aquaculture species depending on  
182 developmental stage, culture media and rearing conditions (Liu et al., 2017; Yu et al., 2021).  
183 Overall, the proximate composition of most insects decreases with advancement in the  
184 developmental stage.

185

### 186 **3. Insect biomass production and processing for aquafeed**

187 The conversion of insects into aquafeed ingredients is an important step that determines their  
188 required level and effectiveness in aquatic animals. With the discovery of insects and their  
189 potential for replacing fishmeal in animal feeds, there is a danger that natural harvests could have  
190 serious biodiversity conservation-related issues. Hence, the mass production of insects for  
191 commercial-scale industry from agricultural organic residues and biowaste for feed purposes or  
192 food is a promising and sustainable approach (Varelas et al., 2019). However, due to variations  
193 that occur during culturing under controlled environments for insect mass production, the  
194 nutritional value is also expected to vary (Varelas et al., 2019). For example, Cortes Ortiz et al.  
195 (2016) noted that the artificial diets required by insects differ not only in presentation, from  
196 liquid to solid, but also in nutritive value, the feeding adaptation of the insect, insect species and  
197 the pre-manufacture method. Additionally, insects have been reported to have variable fatty acid  
198 profiles, particularly having a low level of PUFAs. PUFAs have important health benefits in  
199 humans and are required for optimal growth and development in children (Maulu et al., 2021c).  
200 Therefore, it is important to incorporate PUFA enrichment methods such as rearing insects in n-3  
201 PUFA-rich substrates as demonstrated by Zarantoniello et al. (2020). Erbland et al. (2019)  
202 reported that insects can accumulate eicosapentaenoic acid (EPA) and docosahexaenoic acid  
203 (DHA) by modifying their rearing substrates. The authors supplemented a chicken feed diet with  
204 increasing concentrations of salmon oil (0% to 42%) to the substrate over an increasing number  
205 of days (0 to 8 d) to improve the concentration of omega-3 fatty acids in BSF larvae. The  
206 accumulation of EPA and DHA was achieved in BSF and TM larvae by feeding the insects with  
207 round sardinella (*Sardinella aurita* Valenciennes, 1847) and blackspot seabream (*Pagellus*  
208 *bogaraveo*, Brünnich, 1768) discards. Similar findings were reported by Romero-Lorente et al.  
209 (2022) in TM larvae, however, the authors suggested that longer pre-treatment, for 5 days, would  
210 be required. Tirtawijaya and Choi (2021) fortified BSF larvae substrate with squid liver at  
211 different concentrations of 2.5% to 20% and a concentration of 5% was reportedly required to  
212 achieve a better accumulation of EPA and DHA in the insect. Very long-chain polyunsaturated  
213 fatty acids (VLCPUFAs) could be altered in BSF larvae by modifying the diet of the insect  
214 (Barroso et al., 2017). Overall, these studies indicated the possibility of improving the nutritional  
215 value of insects for use in aquafeed by modifying their rearing conditions. However, the fatty



216 acid composition of the substrate and the weight of the insect larvae are determinants of the fatty  
217 acid profile (Ewald et al., 2020).

218 In a review by Varelas et al., (2019), the different methods of insect biomass production from  
219 food waste are presented in more detail. For insects to remain a sustainable protein source in  
220 aquafeed, low-cost production technology for commercial industries is very crucial. Thus,  
221 various biomass processing methods have recently been reviewed. The most recent one is that of  
222 Parniakov et al. (2021) where a comprehensive overview of insect biomass processing methods  
223 is discussed.

224 So far, several parts of insects, including meal (Ido et al., 2019), the pulp (Xu et al., 2020), paste  
225 (Weththasinghe et al., 2021), frass (Yildirim-Aksoy et al., 2020) and oil (Kumar et al., 2020)  
226 have been used as ingredients in aquafeed. However, the meal, either full-fat or defatted is the  
227 most commonly used form of insects in aquafeed. For example, the BSF is processed mainly  
228 into a dry meal using partially defatted or full-fat larvae (English et al., 2021). In yellowtail  
229 (*Seriola quinqueradiata*), feeding the fish with completely defatted BSF larvae meal enhanced  
230 the growth of the fish compared with using partially defatted larvae (Ido et al., 2021). Therefore,  
231 defatting insects before milling for inclusion in aquafeed could yield better results in many fish  
232 species. For commercial use, BSF is processed using technologies like drying ovens (Dortmans  
233 et al., 2017) while defatting is achieved using an oil press or centrifuge (English et al., 2021).  
234 Drying at the right temperature is important particularly for storage purposes as it prevents  
235 microbial activity from affecting the nutritional value of the product (Dortmans et al., 2017).  
236 Defatting is recommended because it renders a product with high protein content and low  
237 moisture which is ideal for feeding and storage (Dortmans et al., 2017). As BSF larvae are  
238 usually dried at high temperatures (>90 °C), there are concerns that there will be a decrease in  
239 the nutrient (protein) digestibility coefficient which could negatively affect fish growth  
240 (Weththasinghe et al., 2021; Xu et al., 2020). However, few studies have comprehensively  
241 investigated the effect of processing such as drying temperature and pressure of partially  
242 defatted, fully defatted, or full-fat BSF larvae meals in diets for fish (English et al., 2021).  
243 Therefore, further research is needed in that regard. Most studies simply investigate the effect of  
244 one processing method e.g., partial defatting in comparison to the fishmeal control diet, although  
245 they try to regulate temperature below 90 °C when drying the larvae. The TM larvae can be fed  
246 fresh (Henry et al., 2015) or prepared as a meal by oven drying, sun drying or freeze-drying the

247 larvae before grinding. As with BSF, processing of TM by defatting or utilizing it with full fat  
248 could affect acceptability and consequently maximum inclusion levels in diets (Shafique et al.,  
249 2021). However, defatted TM has been reported to provide the benefit of increased protein  
250 content and a more stable pelleting process of the feed (Shafique et al., 2021). Other insects such  
251 as the MD and earthworm (*Perionyx excavates*) could be processed by boiling in hot water  
252 followed by drying in an oven before being milled (Gbai et al., 2018). Interestingly, the mopane  
253 worm (*Imbrasia belina*) has been processed by first gutting it before boiling in brine and later  
254 sun-drying in preparation for grinding into a meal (Rapatsa and Moyo, 2017). Although  
255 degutting is mainly used for preservation purposes, the authors observed that the plant matter in  
256 the gut of the mopane worm could contain amylase activity. Further studies are necessary to  
257 investigate the effect of degutting the mopane worm before use in aquafeed. Different processing  
258 methods and recommended levels of different insect meals are summarized in Table 1.

259

#### 260 **4. Utilization in aquafeeds**

##### 261 **4.1 Recommended levels in diets for aquatic animals**

262 The incorporation of insects in aquafeed has been investigated and is considered a breakthrough  
263 in the efforts to replace fishmeal in many aquaculture species. Currently, a very limited number  
264 of studies have determined the optimal requirement levels of insect meals in aquafeed (Katya et  
265 al., 2017; Shekarabi et al., 2020; Tippayadara et al., 2021). What is available are mostly  
266 recommended levels based on the results yielded from insect meal inclusion in the diets mainly  
267 as replacements for fishmeal. The results reported so far regarding insect incorporation levels in  
268 aquafeeds have shown conflicting results depending on factors such as fish species, growth  
269 stage, feed formulation, insect biomass processing method and dietary administration period. A  
270 recent review of the meta-analysis studies on the nutritional value of insects in aquafeed  
271 indicates a high degree of variation regarding the maximum inclusion levels of insects in  
272 aquafeed based on these factors (Liland et al., 2021). Hence, the authors observed that 20% to  
273 30% could be the maximum range for insect meal inclusion levels without adverse effects. Also,  
274 whether the diet is plant-based or animal-based appears to influence the insect requirement level  
275 of different species. Earlier reviews predominantly focused on inclusion levels of insect meals in  
276 freshwater species (Henry et al., 2015) but recent reviews are broadening the scope to include  
277 marine species (English et al., 2021; Priyadarshana et al., 2021). This is because of the growing

278 evidence that insect meal requirement levels between freshwater and marine aquaculture species  
279 could vary. However, there are no studies that have critically compared this in aquatic animals  
280 even though it is obvious that the nutritional requirements between the two are different. In some  
281 marine fish species, such as the European seabass (*D. labrax*), optimal growth had previously not  
282 been achieved by replacing fishmeal with full-fat insect meal at levels higher than 50% (Basto et  
283 al., 2021). This was attributed to n-3 long-chain polyunsaturated fatty acids (LC-PUFA)  
284 deficiencies (<0.7 % DM) at higher fishmeal replacement levels (Skalli and Robin, 2004). A  
285 recent study by Basto et al. (2021), however, showed that up to 80% (360 g/kg) of fishmeal  
286 could be replaced by TM in the diet of *D. labrax* fingerlings without detrimental effects on  
287 growth and nutrient digestibility. As aquaculture is a diverse industry in terms of cultured species  
288 and their developmental stages, production systems used and culture conditions, more studies are  
289 required to investigate insect meal requirement levels in aquatic animals.

290 Most of the progress made in the utilization of insects in aquafeed has focused on replacing  
291 fishmeal due to rising costs and sustainability issues. As such, most of the existing studies have  
292 investigated the effect of replacing fishmeal at different levels in the diets of aquatic animals  
293 with a view to partially or fully replace fishmeal. This has been done either by combining some  
294 insect species (Hoffmann et al., 2021) or singly, with amino acids supplemented to meet the  
295 EAA requirements of fish (Chemello et al., 2020). However, when used in combination,  
296 Hoffmann et al. (2021) reported that the type of insect meal had a crucial impact on fish growth  
297 and feed utilization parameters. In their study, the authors noted that combinations of full-fat  
298 larval stage TM and BSF meal performed better than combinations of imago stage tropical house  
299 cricket (*Grylloides sigillatus*) and Turkestan cockroach (*Blatta lateralis*) in diets of sea trout  
300 (*Salmo trutta*) larvae. In Eurasian perch (*Perca fluviatilis*), fingerlings fed an experimental diet  
301 containing a combination of 50 g/kg house cricket and 50 g/kg of superworm (25% fishmeal  
302 replacement) had significantly lower growth compared to the control (Tilami et al., 2020). This  
303 was attributed to several factors including reduced feed intake (palatability), presence of chitin  
304 and oxidized fat. Insects have also been used singly or in combination with other ingredients to  
305 replace plant-based proteins in animal diets. For instance, BSF inclusion at 324 g/kg (47%  
306 replacement of vegetable mix) and BSF and protein by-product meal (PBM) inclusion levels at  
307 81 and 206 g/kg, respectively (49% replacement of vegetable mix), led to faster growth of  
308 gilthead seabream in comparison to the vegetable mix and fishmeal only controls (Randazzo et

309 al., 2021). This study is of interest because while it is important to look at studies in which insect  
310 meals are used to replace fishmeal in aquafeeds; it is also beneficial to compare the effects with  
311 insect replacement in commonly utilized plant-based diets.

312 The presence of chitin in insect meals could have beneficial effects on fish by shaping the gut  
313 microbial community and boosting the innate immune response when incorporated at moderate  
314 quantities ranging from 25 to 50 mg/kg (Esteban et al., 2001; Bruni et al., 2018). On the other  
315 hand, the effect of higher inclusion levels of insect meal has been reported to yield negative  
316 results in most species, and this has been associated with the increased level of chitin at higher  
317 levels (Kroeckel et al., 2012; Renna et al., 2017). For instance, BSF larvae meal incorporated at  
318 400 g/kg (corresponding to a chitin level of 2 g/100 g DM) was reported to reduce dry matter and  
319 crude protein digestibility but did not affect growth in rainbow trout (*O. mykiss*) (Renna et al.,  
320 2017). BSF pre-pupae meal incorporated in diets of juvenile turbot (*Psetta maxima*) at levels  
321 higher than 332 g/kg (chitin level ranging from 47 to 73 g/kg DM) led to reduced feed intake and  
322 feed conversion and subsequently reduced growth (Kroeckel et al., 2017). According to  
323 Soetemans et al. (2020), the crystalline nature of chitin present in some insects is what limits its  
324 utilization in aquafeed. Wang et al. (2020) found that this crystalline nature increases with the  
325 advance in developmental state of insects, particularly BSF from larvae to adults. For example,  
326 in Siberian sturgeon juveniles, the inclusion of highly defatted BSF meal from 185 to 375 g/kg  
327 (25% to 50% fishmeal replacement; 0.72 to 1.92 g/100 g chitin in feed) reduced the feed intake  
328 and apparent digestibility coefficient (ADC) of protein, while inclusion at 750 g/kg (100%  
329 fishmeal replacement; 3.75 g/100 g chitin in feed) led to complete rejection of the feed (Caimi et  
330 al., 2020). Feeding sea trout (*S. trutta*) fingerlings with hydrolyzed TM at an inclusion level of at  
331 least 100 g/kg (9.3 g/kg chitin in feed; 42% fishmeal replacement) resulted in a significantly  
332 reduced protein efficiency ratio (Mikołajczak et al., 2020). However, whether insect biomass  
333 processing methods affect the chitin content in the meal is not yet clear and as such, further  
334 studies are required. Although Gasco et al. (2018) reported that the content level of chitin can be  
335 reduced through the extraction process or dietary enzyme inclusion to improve its digestibility;  
336 appropriate technologies have not yet been fully applied. Jayanegara et al. (2017) were able to  
337 completely remove chitin from cricket (*Gryllus assimilis*) by chemical digestion while reducing  
338 chitin levels from 7.7% dry matter to 3.5% by exoskeleton removal.

339 Besides the presence of chitin, negative effects observed in aquatic animals when insect meals  
340 are incorporated in aquafeeds can be attributed to lower levels of fatty acids in the diets in  
341 comparison to the fishmeal control diet (Zarantoniello et al., 2021). Insects have been reported to  
342 have lower levels of n-3 PUFA (Zarantoniello et al., 2020; Bruni et al., 2020) and therefore  
343 without sufficient enrichment processes in the insect rearing process, this might translate to  
344 lower n-3 PUFA levels in the aquafeeds. For instance, in a study by Zarantoniello et al. (2021),  
345 diets in which 50% fishmeal was replaced by BSF had significantly lower n-3 fatty acids.  
346 Consequently, Siberian sturgeon fed these diets had significantly lower growth and specific  
347 growth rate than those fed the control diet. According to the authors, the fish spent energy  
348 converting linoleic acid and  $\alpha$ -linolenic acid to EPA and DHA instead of utilizing the energy all  
349 for growth. Additionally, the authors reported lower diet acceptance in the fish-fed diets  
350 containing 50% insect meal thus, requirement levels might be affected by the palatability of the  
351 diets (Zarantoniello et al., 2021). However, several studies have shown that the absence of n-3  
352 long-chain polyunsaturated fatty acids (LCPUFA) in terrestrial insects can be alleviated by  
353 feeding insects with diets rich in n-3 LCPUFA (Barroso et al., 2017; Fabrikov et al., 2020; 2022;  
354 Tirtawijaya et al., 2021).

355 In other studies, the negative effects when aquatic animals are fed with higher dietary levels of  
356 insect meal were attributed to the presence of non-protein nitrogen in some insects, which could  
357 lead to the overestimation of protein (Janssen et al., 2017). Nevertheless, the recommended  
358 levels of different insect meals under different processing methods for different fish species are  
359 presented in Table 1 below.

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## 361 4.2 Effects of insect meal on aquatic animals

### 362 4.2.1 Growth and feed utilization

363 The growth performance and feed utilization effects of several insects have been studied in  
364 aquaculture. These include BSF (Fawole et al., 2020; Peng et al., 2021b), yellow mealworm (*T.*  
365 *molitor*) (Sankian et al., 2018), housefly (*M. domestica*) (Hashizume et al., 2019), mopane worm  
366 (*I. belina*) (Rapatsa and Moyo 2017), chironomid (Roncarati et al., 2019) and cricket (*G.*  
367 *bismasculatus*) (Taufek et al., 2016), with BSF being the most studied insect in aquaculture.  
368 Insects can be utilized either as dry meals (Jeong et al., 2021; Kamarudin et al. 2021), pulps  
369 (Peng et al., 2021b; Peng et al., 2021a), or oils (Belghit et al., 2018; Xu et al., 2020; Abu Bakar  
370 et al., 2021). For example, Fawole et al. (2020) carried out a 60-day experiment to examine the  
371 effect of fish meal substitution with BSF larvae meal at 25%, 50% and 75% on the growth  
372 performance, nutrient utilization and health parameters of African catfish (*C. gariepinus*). This  
373 study discovered that black soldier fly larvae meal at 50% presented the highest final body  
374 weight, weight gain and specific growth rate compared to other groups. Feed conversion ratio,  
375 protein efficiency ratio and protein productive value were better in fish fed 50% BSF larvae meal  
376 (Fawole et al., 2020). According to Kamarudin et al. (2021), a black soldier pre-pupae meal  
377 inclusion level of 75% was needed to increase the growth performance of lemon fin barb hybrid  
378 fingerlings. A study by Belghit et al. (2019) indicated that a total replacement of fish meal with  
379 BSF meal was possible in Atlantic salmon (*S. salar*) without compromising their growth and  
380 nutrient digestibility. Furthermore, the dietary inclusion of black soldier fly pulp reportedly  
381 improved the growth performance of largemouth bass (*M. salmoides*) (Peng et al., 2021b; Peng  
382 et al., 2021a). Xue et al. (2020) compared the dietary effect of BSF, TM and Silkworm oils on  
383 the growth and other metabolic parameters of the juvenile mirror carp (*Cyprinus carpio*). The  
384 results showed that BSF oil alone or in combination with two of the other insect oils in fish diets  
385 significantly enhanced the growth and feed utilization of the fish.

386 TM is the second most widely studied insect in aquaculture after BSF, with the potential to be  
387 utilized as an optional protein ingredient in aquafeed. A study by Rema et al. (2019) reported that  
388 graded inclusion of defatted TM increased the growth and feed utilization of rainbow trout (*O.*  
389 *mykiss*) and showed the potential to completely replace fish meal. Improved growth and feed  
390 utilization parameters were also reported in freshwater prawns, (*Macrobrachium rosenbergii*)  
391 (Feng et al., 2019) and mandarin (*Siniperca scherzeri*) (Sankian et al., 2018) fed TM diets. On

392 the contrary, no significant effect on the growth and feed utilization parameters was observed  
393 when mealworm was used to partially substitute fish meal at 25% and 50% for 131 days in  
394 blackspot seabream (*P. bogaraveo*) (Iaconisi et al., 2017). The same was reported in *O. mykiss*  
395 (Iaconisi et al., 2018) and yellow catfish (*Pelteobagrus fulvidraco*) (Su et al., 2017). However,  
396 negative effects on growth performance and feed utilization of TM were reported in some fish  
397 species (Coutinho et al., 2021; Jeong et al., 2021). These findings may call for better processing  
398 of the ingredient and the need for further studies to optimize this ingredient in aquaculture.

399 Furthermore, the housefly (*M. domestica*) (Hashizume et al. 2019), mopane worm (*I. belina*)  
400 (Rapatsa and Moyo, 2017), chironomid (Roncarati et al. 2019) and cricket (*G. bismasculatus*)  
401 (Taufek et al. 2016) are some of the insects that showed potential to be used as protein  
402 ingredients to improve fish growth, however, more research is deemed important.

#### 403 **4.2.2. Antioxidant capacity**

404 The effect of insect utilization in aquafeed on the antioxidant capacity of fish has been reported  
405 in numerous studies with promising results. A summary of the results reported by different  
406 studies is presented in Table 2. However, the results vary depending on the insect species and  
407 parts used in aquafeed. For example, dietary insect (BSF) meal as a replacement for fishmeal  
408 showed deleterious effects on the transcription of antioxidant enzymes and stress-related genes in  
409 the leukocytes of the head kidney (Stenberg et al., 2019). In the African catfish, substituting  
410 fishmeal with BSF at 75% did not impair the antioxidant status of the fish (Fawole et al., 2020).  
411 In rainbow trout, Elia et al. (2018), reported that dietary inclusion of at least 20% BSF could  
412 adversely affect the fish's oxidative homeostasis, particularly in the liver and kidney by lowering  
413 the glutathione peroxidase (GPx) activity while enhancing the activities of ethoxyresorufin O-  
414 deethylase (EROD), glutathione S-transferase (GST) and total glutathione (GSH). Therefore, the  
415 authors suggested adding levels of BSF that are lower than 20% in the fish's diets. In Atlantic  
416 salmon, increasing the levels of BSF paste from 6.25% to 25% in fishmeal and plant-based diets  
417 improved the antioxidant capacity in the blood of the fish (Weththasinghe et al., 2021). In Pacific  
418 white shrimp, dietary replacement of fishmeal with defatted silkworm (SW) (*B. mori L.*) pupae  
419 meal enhanced the serum antioxidant capacity of the shrimp (Rahimnejad et al., 2019). Recently,  
420 Xu et al. (2020) reported the effect of insect oils on the antioxidant status of juvenile mirror carp  
421 (*C. carpio var. specularis*). In this study, the combined inclusion of BSF oil, silkworm pupae oil  
422 and TM oil at the same level improved the antioxidant capacity in the liver of the fish. When



423 individual insect oils were compared, the authors observed that BSF oil could provide better  
424 results compared to the other two oils. Furthermore, Xu et al. (2020) reported significantly  
425 improved serum antioxidant capacity in mirror carp fed dietary BSF pulp at low levels. Other  
426 insect meal proteins that have shown similar results include cricket (*G. bimaculatus*) meal in the  
427 diet of African catfish (Taufek et al., 2016) and maggot meal in the diet of common carp (Ogunji  
428 et al., 2011). Dietary inclusion of TM in the diet of rainbow trout improved the intestinal  
429 antioxidant enzyme activity and a led to a decline in lipid peroxidation (Henry et al., 2018a). The  
430 antioxidant capacity of the hybrid tilapia was not affected when the fish was fed a diet containing  
431 maggot meal as a full replacement for fishmeal (Qiao et al., 2019).

#### 432 **4.2.3. Immune response and disease resistance**

433 The response of immune function in aquatic animals to dietary supplementation has become an  
434 important criterion for evaluating the suitability of feed ingredients in aquaculture. Insect  
435 utilization in aquafeed has been evaluated on several immune-related parameters including blood  
436 biochemical composition, histopathology of related organs, gut health, related gene expression  
437 and disease resistance in numerous aquaculture species. The results of these parameters are  
438 included in Table 2. In Atlantic salmon diets, full fishmeal replacement with BSF meal could be  
439 achieved without negative effects on liver histology and the transcription of pro-inflammatory  
440 genes in the fish's head kidney (Belghit et al., 2019; Stenberg et al., 2019). In juvenile Japanese  
441 seabass, dietary BSF meal supplementation did not alter the intestinal histomorphology of the  
442 fish (Wang et al., 2019). The substitution of fish meal with BSF meal in the diets of the African  
443 catfish did not alter the fish's blood biochemical parameters and differential leucocyte counts  
444 (Fawole et al., 2020). Also, no significant effect was observed on the gut histology, stress levels,  
445 and immune response in zebrafish when fishmeal was replaced by BSF at 25% and 50%  
446 (Zarantoniello et al., 2019). In juvenile Japanese seabass (*L. japonicus*), partial replacement of  
447 fishmeal up to 64% with defatted BSF larvae meal did not affect the histomorphology of the  
448 intestine and liver, or intestinal antioxidant status and immune response of the fish (Wang et al.,  
449 2019). However, replacing fishmeal with TM in the diets of juvenile Pacific white shrimp  
450 improved the survival rates of the shrimp after being challenged with pathogenic bacteria (*Vibrio*  
451 *parahaemolyticus*) (Motte et al., 2019). In yellow catfish, dietary TM at 18% could improve the  
452 immune response and disease resistance of the fish against a bacterial (*Edwardsiella ictalurid*)  
453 challenge (Su et al., 2017). In juvenile mandarin fish, the inclusion of TM in the diets could

454 enhance the immune system of the fish (Sankian et al., 2018). In Siberian sturgeon, BSF meal  
455 enhanced the morphology of the intestine, although higher inclusion levels of more than 18.5%  
456 were likely to negatively impact the health status (Józefiak et al., 2019; Caimi et al., 2020).  
457 Interestingly, the resistance of Pacific white shrimp against *V. parahaemolyticus* and stress was  
458 not affected after feeding the shrimp with BSF meal (Richardson et al., 2021). In zebrafish  
459 grown from larvae to adult, fishmeal could be replaced by 25% and 50% BSF meal without  
460 adversely affecting the immune response and stress resistance of the fish (Zarantoniello et al.,  
461 2019). The health status of the African catfish was not negatively affected when fishmeal was  
462 replaced by 75% (Fawole et al., 2020). In giant freshwater prawn (*M. rosenbergii*), replacing  
463 fishmeal with TM at 12% improved immune response and the resistance of the prawn against  
464 *Lactococcus garvieae* and *Aeromonas hydrophila* (Feng et al., 2019). Existing studies show  
465 slight variations with regard to the insect species and meal status used in aquafeed. For example,  
466 replacing fishmeal with a 50% partially-defatted BSF meal did not yield a significant effect on  
467 the histology of the spleen, liver and gut of rainbow trout (Elia et al., 2018). While 28% to 67%  
468 full-fat TM meal as a replacement for fishmeal could improve the immune response in the fish  
469 (Henry et al., 2018a; Jeong et al., 2020). In Nile tilapia, a total replacement of fishmeal was  
470 achieved using BSF meal with observed improvement in the haematology and immunity of the  
471 skin mucus (Tippayadara et al., 2021), while only 15% could be replaced with superworm (SW)  
472 larvae (*Z. morio*) for enhanced innate immunity of the fish (Alves et al., 2020). Few studies have  
473 also reported the combined effect of multiple species of insect meals in aquaculture (Józefiak et  
474 al., 2019), but further investigations are required.

475 In Pacific white shrimp, dietary replacement of fishmeal with defatted silkworm pupae meal at  
476 higher (over 75%) levels could have adverse effects on the integrity of the hepatopancreas in the  
477 shrimp (Rahimnejad et al., 2019). In the findings of Motte et al. (2019), replacing fishmeal with  
478 50% defatted TM improved the disease resistance of the pacific white shrimp against EMS (*V.*  
479 *parahaemolyticus*). In rainbow trout, Bruni et al. (2018) reported the effect of replacing fishmeal  
480 with partially defatted BSF meal on the intestinal microbial community of the fish. In this study,  
481 the authors concluded that 50% of BSF meal in the diets could improve biodiversity and modify  
482 the microbial community structure in the intestine of rainbow trout. In red seabream (*Pargus*  
483 *major*), feeding the fish with diets containing TM after challenge with a bacterial pathogen  
484 (*Edwardsiella tarda*) improved the fish's survival (Ido et al., 2019).

485 The utilization of insect meals in aquaculture could promote the use of plant-based protein,  
486 particularly soybean meal whose application in the culture of high-value species has declined  
487 because it causes intestinal enteritis. In rainbow trout, the inclusion of BSF meal in soybean  
488 meal-based diets successfully prevented soybean meal-induced intestinal enteritis (Kumar et al.,  
489 2020). This was accompanied by down-regulated prostaglandin and interferon regulatory factor 1  
490 (IRF-1) in the fish's intestine. However, the mechanism through which insect meal prevents  
491 soybean meal-induced enteritis in fish is not clearly understood. According to Xiang et al (2020),  
492 insect meal contains bioactive peptides that could be attributed to the prevention of this disease.  
493 Therefore, insect meal presents the potential prevention of intestinal inflammation in  
494 aquaculture. However, as observed by Kumar et al. (2020), this requires further investigation to  
495 characterize the bioactive peptides present in insect meals.

496 In some aquaculture species, the component of the insect used in the diet could yield different  
497 results. Furthermore, different organs of aquatic animals could respond differently to varying  
498 levels of insect meal included in the diets. For example, in the diets of Atlantic salmon, the  
499 inclusion of 6.25% and 12.5% of BSF meal in fishmeal and plant-based diets could reduce the  
500 enterocyte steatosis in the pyloric caeca of the fish (Weththasinghe et al., 2021). On the contrary,  
501 3.7% and 6.7% of BSF paste would be required to yield similar results (Weththasinghe et al.,  
502 2021). The authors further observed that increasing the inclusion levels to 25% (BSF meal) and  
503 6.7% (BSF paste) improved the histology of the distal intestine. A minor effect of BSF meal and  
504 BSF paste on the skin mucus proteome and immune response in Atlantic salmon has also been  
505 reported (Weththasinghe et al., 2021). In the hybrid tilapia (Nile x Mozambique, *O. niloticus* x  
506 *O. mossambicus*), Yildirim-Aksoy et al. (2020) used frass, a by-product of BSF fly larvae, as a  
507 protein source to replace plant-based protein in the diet. The authors reported improved innate  
508 immune response and disease resistance against bacterial (*Flavobacterium columnare* and  
509 *Streptococcus iniae*) infection. Some studies have also reported the effect of BSF oil in aquatic  
510 animals and studies in this area are likely to increase as aquaculture moves towards  
511 sustainability. In rainbow trout, BSF oil showed beneficial effects on the immunity of the fish in  
512 the liver, intestine and kidney (Kumar et al., 2020). In the findings of Dumas et al. (2018), the  
513 inclusion of BSF oil in the diet of rainbow trout did not affect the histology of the posterior  
514 intestine, although the villus height in the anterior intestine was reduced. In juvenile mirror carp,

515 Xu et al. (2020) reported that dietary inclusion of BSF pulp at low levels (less than 131 g/kg) did  
516 not significantly affect the health status of the intestine.

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#### 518 4.2.4. Effect of insect meal on fish flesh quality and safety

519 Fish flesh quality and safety are of primary importance for consumers and thus those parameters  
520 should be evaluated in fish fed insect-derived products. The fatty acid profile of fish flesh is of  
521 utmost importance for human health, particularly the n-3 PUFA; EPA and DHA. The fatty acid  
522 profile of insect-derived products varies greatly with insect species, production system and  
523 product processing method (Gasco et al., 2018, 2019; Oonincx and Finke, 2020). Generally,  
524 insect meals are deficient in EPA and DHA and rich in saturated fatty acids (SFA); a limitation  
525 that compromises the n-3/n-6 ratio when insect products are included at increasing levels in fish  
526 diets (Belforti et al., 2015; Iaconisi et al., 2017; Iaconisi et al., 2018; Gasco et al., 2019). Fatty  
527 acid profiles in fish-fed insect-derived products are not quite consistent and the general trend  
528 regarding EPA and DHA (and other fatty acid missing in insects) in trials with insect-based diets  
529 show a decrease of these fatty acids when they are not supplemented otherwise. Feeding high  
530 levels of BSF larvae meal to rainbow trout (40% inclusion level; Renna et al., 2017; Mancini et  
531 al., 2018; Secci et al., 2019) or Jian carp (14% inclusion level; Zhou et al., 2018) has been shown  
532 to decrease both n-3 and n-6 PUFA but increase the SFA content. Similar findings were observed  
533 in rainbow trout (*O. mykiss*) fed live adult house cricket *Acheta domestica* or live superworm *Z.*  
534 *morio* larva (at 25% and 100% of gross energy, single or in combination); EPA and DHA  
535 content in muscle of fish fed insects was 45% and 63% of the control fish, respectively (Turek et  
536 al., 2020). Similarly, there was a reduction in the n-3/n-6 ratio and the relative content of EPA  
537 and DHA (% total fatty acids) in the muscle of European seabass fed defatted TM larvae meal  
538 that replaced fish meal at increasing levels (0%, 50%, and 100%), however, the absolute value of  
539 EPA + DHA in a fillet portion of 100 g for human consumption remained above the  
540 recommended level for human consumption (>0.25g / 100 g of wet weight) in all fish and did not  
541 vary significantly among treatments (Sousa, 2020). In contrast, the n-3/n-6 ratio and the EPA and  
542 DHA content were increased in sea-water Atlantic salmon fed diets in which BSF larvae meal  
543 completely substituted fish meal (Belghit et al., 2019). Overall, 10% of HM, corresponding to  
544 17% of FM replacement, might be included in meagre diets without major adverse effects on  
545 growth, feed utilization, whole-body composition and fatty acid profile. Furthermore, despite  
546 high dietary inclusion of BSF larvae (*H. illucens*) meal (0%, 9.2%, 18.4% and 27.6%,  
547 corresponding to 0%, 25%, 50% and 75% of fishmeal substitution) reduced the n-3 PUFA in  
548 gilthead sea bream fillets, it did not reduce the overall n-3 PUFA positioned in the *sn-2* of fillet

549 triglycerides, nor EPA percentage (Pulido et al., 2022). Replacement of 25% fishmeal by a  
550 mixture of house cricket (*Acheta domesticus*) and superworm (*Z. morio*) in the diet of perch  
551 (*Perca fluviatilis*) increased the linoleic fatty acid and the total content of n-6 fatty acids in fish  
552 fillets but did not affect the nutritional value of the fish with the insect-based diet for human  
553 consumption, despite a decrease in growth performance and an increase in feed intake (Tilami et  
554 al., 2020). The effects of insect-derived product feeding on the content of heavy metals and  
555 mycotoxins in fish flesh are rarely investigated and warrant further work.

556  
557 Regarding fish texture properties, fishmeal replacement using insect meals might have an impact.  
558 Texture parameters are technologically important (Wang et al., 2017) therefore, need not be  
559 overlooked. Incorporation of maggot meal in diets of Nile tilapia (*O. niloticus*) at levels ranging  
560 from 110 g/kg to 430 g/kg (25% to 100% fishmeal replacement) significantly increased hardness  
561 and reduced thaw loss in comparison to the control (Wang et al., 2017). Incorporation of TM in  
562 diets of yellow croaker (*Larimichthys crocea*) led to increased muscle hardness and significantly  
563 lower shear force in fillets in which fishmeal was replaced at 426.2 to 568.3 g/kg (75% to 100%  
564 fishmeal replacement) (Yuan et al., 2022). Fillet composition was not affected by the inclusion  
565 of BSF (*H. illucens*) pre-pupae larvae meal at 65 to 195 g/kg (15% to 45% fishmeal replacement)  
566 in diets for European seabass (*D. labrax*) (Moutinho et al., 2021). There were no significant  
567 differences in texture properties of fillets of barramundi (*L. calcarifer*) fed diets supplemented  
568 with tuna hydrolysate and BSF (*H. illucens*) larvae meal (50 to 100 g/kg insect meal inclusion  
569 levels) (Chaklader et al., 2021).

#### 570 **4.2.5. Consumer opinion on the consumption of aquaculture products fed with insect meal**

571 The use of insects as feed ingredients in aquaculture is a relatively new but highly promising  
572 technology for mitigating the rising cost of aquafeed due to sustainability issues of fishmeal  
573 (Baldi et al., 2019; Hasimuna et al., 2019; Kord et al., 2021). However, the wider adoption of  
574 insect utilization in aquafeed will likely depend, to a larger extent, on aquaculture producers and  
575 consumer acceptance. Despite few existing studies investigating people's perception concerning  
576 the use of insects as feed ingredients, the majority of the aquatic animal product consumers have  
577 shown favourable responses for various reasons, including risk-free (Popoff et al., 2017; Szendrő  
578 et al., 2020), sustainability considerations (Verbeke et al., 2015; Rumbos et al., 2021), as well as  
579 availability and access to information about the products (Baldi et al., 2021; Rumbos et al.,

2021). Product awareness and information availability are considered the most important factors that could accelerate the acceptance and positive perception of aquatic products produced on insects-based feeds (Baldi et al., 2021; Rumbos et al., 2021). According to Baldi et al. (2021) reducing information asymmetry could promote wider consumer acceptance. Interestingly, a study conducted in Italy revealed that men and young consumers are more likely to accept aquatic products given insect-based feeds (Baldi et al., 2021), suggesting that gender and age could play a role. Further, the authors observed that well-informed respondents had a higher acceptance rate compared with those that had little to no information. Sogari et al. (2019) also noted that, in Australia, males were more likely to accept insect products as food compared with their female counterparts. However, in Belgium, age and gender did not appear to significantly affect the perception of the aquatic products. As observed by Verbeke (2015), consumer perception regarding insect use in aquafeed is likely to evolve with time and vary based on culture, familiarity and past experiences, meaning that acceptance of aquatic products fed insect-based diets will improve with accumulation of information about the product. Additionally, different cultures and beliefs are likely to affect perception although further studies are required to confirm it. Currently, the majority of existing studies were conducted in developed countries, particularly the European Union (EU), with no current information for developing countries. This lack of information makes it difficult to predict perception in developing countries and among different social groups. Therefore, more studies are required in different countries, at least the major producers, and among consumers from different cultures to ascertain the future of insect utilization in aquafeed.

## 5. Conclusion

Insects have emerged as a potentially sustainable alternative protein source to the conventional fishmeal whose production continues to be unsustainable, resulting in rising costs. Significant progress has so far been made in the efforts to unlock the potential of insects for use in aquafeed. Our review of existing studies in this area has shown promising results, particularly with regards to enhanced growth performance, nutrient utilization, antioxidant capacity, immune response as well as disease resistance in many aquaculture species. Mechanisms have also been identified to enrich the nutritional value of insects, making them more effective as an aquafeed ingredient. Besides, the low carbon footprint associated with their production makes them an even more



610 interesting protein source candidate in aquafeed. However, there are still many areas that require  
611 further investigation to fully understand the utilization and benefits of insects in aquafeed.

612

## 613 **6. Prospects**

614 Despite the promising results reported from the inclusion of insects as ingredients in aquafeed,  
615 important gaps still exist concerning their full utilization in aquaculture. For example, the  
616 majority of effects of insect utilization in aquafeed reported so far have important biases towards  
617 adult species. A large gap still exists with regards to the effects in the initial ontogenetic stages of  
618 fish such as embryos, fingerling and larvae. Additionally, the insect requirement levels in  
619 aquafeed for different aquatic animal species and stages of development under different culture  
620 systems are unclear. This knowledge is very important for commercializing the utilization of  
621 insects in aquafeed. Also, given the numerous insect species currently reported as ingredients in  
622 aquafeed, there is a need to explore value addition methods during biomass production to  
623 improve the nutritional value. This will ensure the diets are easily utilized by the aquatic animals  
624 while reducing waste in culture facilities. Emerging studies show that different parts of insects  
625 such as meal, oil, pulp and paste can be used in aquafeed. However, the majority of studies in the  
626 literature have focused on insect meals to a larger extent and oils to a lesser extent, while very  
627 little is known regarding the utilization of pulp and paste. Furthermore, important bioactive  
628 compounds such as chitin, fatty acids and antimicrobial peptides have been reported in insects,  
629 however, their role in aquatic animal growth and physiology is not very clear. Besides, chitin has  
630 shown detrimental effects at higher insect inclusion levels in the diets of aquatic animals. Future  
631 studies are required to explore how different parts and compounds of insects could be utilized in  
632 aquafeed. Finally, studies evaluating the effect of insects on flesh safety and quality of fish and  
633 other aquatic food for human consumption are necessary. Addressing these gaps is relevant for  
634 the commercialization of insect utilization in aquafeed.

## 635 **Author contributions**

636 **Sahya Maulu** conceptualized the study, developed the objectives and coordinated the  
637 manuscript writing, **Sandra Langi, Dagoudo Missinhoun, Oliver Jolezya Hasimuna, Buumba**  
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639 **Hien Van Doan, Zulhisyam Abdul Kari, and Mahmoud A.O. Dawood** wrote the draft

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642

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644

645

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651

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**Table 1. Maximum recommended levels of insect meal for inclusion in aquafeed based on different insect biomass processing methods**

Fish species	Initial weight, g	Insect meal	Processing method	Recommended (Fish meal replacement)	Administration period	References
Red seabream ( <i>Pargus major</i> )	24.9 ± 0.71	Yellow mealworm larvae ( <i>Tenebrio molitor</i> , TM)	Ground and defatted	650 g/kg (100%)	4 wk	Ido et al. (2019)
Seabass ( <i>Dicentrarchus labrax</i> )	10.7	Black soldier fly (BSF, <i>Hermetia illucens</i> ) larvae	Larvae fed on a broiler diet	109 g/kg (30%)	49 d	Reyes et al. (2020)
	24.2 ± 7.59	Defatted BSF	Defatted using mechanical method	185 g/kg (25%)	118 d	Caimi et al. (2020)
	53.39 ± 3.74	Full-fat TM larvae	Air-dried and ground	200 g/kg (40%)	71 d	Józefiak et al. (2019)
	201.8 ± 13.9	Full-fat BSF	Larvae dried and ground	300 g/kg (30%)	5 wk.	Huyben et al. (2019)
	137 ± 10.5	Full-fat BSF prepupae meal	Frozen and ground	210 g/kg (50%)	98 d	Cardinaletti et al. (2019)
Rockfish ( <i>Sebastes schlegeli</i> )	3.11 ± 0.01	TM	Dried and ground	160 g/kg (19%)	8 wk.	Khosravi et al. (2018)
Pacific white shrimp ( <i>Litopenaeus vannamei</i> )	1.55 ± 0.5	Defatted TM larvae	Freeze dried, ground, and dried	205 g/kg (100%)	8 wk	Motte et al. (2019)
White shrimp ( <i>L. vannamei</i> )	2.39 ± 0.49	Partially defatted or full defatted TM larvae	Oven dried and milled	100 g/kg (50%)	8 wk	Choi et al. (2018)
Sea trout ( <i>Salmo trutta</i> )	5.08 ± 0.9	TM larvae	Hydrolyzed	100 g/kg (42%)	8 wk	Mikołajczak et al. (2020)

Nile tilapia ( <i>Oreochromis niloticus</i> )	14.77 ± 2.09	BSF larvae	Dried in hot air and ground	100 g/kg (100%)	12 wk	Tippayadara et al. (2021)
	0.66	Maggot meal ( <i>Musca domestica</i> , MD)	Killed in hot water, oven-dried, and ground	200 g/kg (100%)	90 d	Gbai et al. (2018)
	68.85 ± 0.24	MD larvae meal	Oven dried and milled	330 g/kg (75%)	10 wk	Wang et al. (2017)
	8.65	MD larvae meal	Killed in hot water, oven-dried, and ground	250 g/kg (66.7%)	12 wk	Alofa and Abou (2020)
Mandarin fish ( <i>Siniperca scherzeri</i> ) juveniles	20.8 ± 0.05	TM larvae	Freeze-dried and ground	300 g/kg (30%)	12 wk	Sankian et al. (2018)
Butter catfish ( <i>Ompok pabda</i> )	0.6	Earthworm meal ( <i>Perionyx excavates</i> )	Boiled, oven-dried, and ground	260.4 g/kg (75%)	8 wk	Chakraborty et al. (2021)
Asian sea bass ( <i>Lates calcarifer</i> )	12.52 ± 0.52	Defatted superworm larvae ( <i>Zophobas morio</i> )	Ground, defatted, oven-dried, and reground	120 g/kg (44%)	12 wk	Prachom et al. (2021)
Mozambique tilapia ( <i>Oreochromis mossambicus</i> )	40 ± 2.5	Mopane worm ( <i>Imbrasia belina</i> )	Gutted, cooked in brine & sundried	24 g/kg (60%)	51 d	Rapatsa and Moyo (2017)
African catfish ( <i>Clarias gariepinus</i> )	4.00 ± 0.8	Field cricket ( <i>Gryllus bimaculatus</i> ) meal	Oven-dried and ground	300 g/kg (100%)	56 d	Taufek et al. (2018)
Red seabream ( <i>P. major</i> )	10.4	Defatted MD larvae meal	Boiled, air-dried and milled	700 g/kg (100%)	4 wk	Hashizume et al. (2019)
	12.8	Defatted MD larvae meal	Boiled, air-dried and milled	400 g/kg (100%)	4 wk	Hashizume et al. (2019)

**Table 2. A summary of some studies that have shown effects when insects were used as a protein source in aquafeed**

Insect species	Used part	Aquaculture species	Fish weight, g	Period	Inclusion level, %	Effect	References
Black soldier fly ( <i>Hermetia illucens</i> )	Frass	Hybrid tilapia, Nile × Mozambique ( <i>Oreochromis niloticus</i> × <i>O. mozambique</i> )	2.6 ± 0.04	12 wk	5 to 30	Improved protein efficiency, serum complement activity and resistance against <i>Flavobacterium columnare</i> and <i>Streptococcus iniae</i>	Yildirim-Aksoy et al. (2020)
	Meal	Rice field eel ( <i>Monopterus albus</i> )	24.0 ± 0.02	10 wk	15.78	Improved growth performance and gut microbiota balance	Hu et al. (2020)
	Meal	Atlantic salmon ( <i>Salmo salar</i> )	17.5 ± 7.5	8 wk	66 to 100	Down-regulation of stress and antioxidant-related gene expression in the leucocytes.	Stenberg et al. (2019)
	Defatted meal	Japanese seabass ( <i>Lateolabrax japonicus</i> )	14.1 ± 0.17	8 wk	64	Enhanced feed intake but lowered serum properties, blood lipid and inhibited lipid deposition	Wang et al. (2019)
	Meal	African catfish ( <i>Clarias gariepinus</i> )	4.0 ± 0.01	60 d	50	Improved growth performance and feed utilization and antioxidant enzymes.	Fawole et al. (2020)
	Meal	European sea bass ( <i>Dicentrarchus labrax</i> )	50.0 ± 0.50	62 d	22.5	Reduced lipid oxidation in the fillet	Mountinho et al. (2020)
	Meal	Rainbow trout ( <i>Oncorhynchus mykiss</i> )	32.0	10 wk	8 to 16	Successful prevention of soybean meal (SBM)-induced enteritis in the intestine and enhanced immune response	Kumar et al. (2020)
	Oil	Rainbow trout	32.0	10 wk	16	Improved serum-peroxidase activity and upregulation of kidney interleukin-8 (IL-8), tumor necrosis factor (TNF), and interferon regulatory factor 1 (IRF-1)	Kumar et al. (2020)

Meal	Pre-smolt salmon	Atlantic	$49.0 \pm 1.50$	8 wk	85	Reduced the deposition of excess lipids in the pyloric caeca and stimulated xenobiotic metabolism.	Li et al. (2019)
Meal	Rainbow trout		$137.0 \pm 10.50$	98 d	50	Activation of immune related genes such as interleukin 10 (IL-10), TNF- $\alpha$ and toll-like receptor 5 (TLR-5)	Cardinaletti et al. (2019)
Meal	Pacific white shrimp ( <i>Litopenaeus vannamei</i> )		$0.67 \pm 0.15$	4 wk	7.5	Improved weight gain, feed conversion ratio (FCR) and specific growth rate (SGR)	Richardson et al. (2021)
Meal	Barramundi ( <i>Lates calcarifer</i> )		$1.74 \pm 0.15$	42 d	30	Improved growth and feed utilization, bactericidal activity and upregulation of immune-related genes such as interleukin 1 (IL-1) and IL-10	Hender et al. (2021)
Oil	Barramundi		$1.74 \pm 0.15$	42 d	30	Enhanced growth performance and upregulation of immune-related genes (IL-1 and IL-10)	Hender et al. (2021)
Partially defatted meal	Rainbow trout		$178.9 \pm 9.81$	78 d	50	Sensitivity and modulation of intestinal bacterial community and structure.	Bruni et al. (2018)
Meal	Atlantic salmon		$49.0 \pm 1.5$	8 wk	60	Modulation of intestinal microbiota, enrichment of beneficial bacteria	Li et al. (2021a)
Meal	Atlantic salmon		$1400 \pm 43$	16 wk	15	Improved microbial richness and diversity related to immune responses and barrier function in the distal intestine	Li et al. (2021b)
Oil	Mirror carp ( <i>Cyprinus carpio var. Specularis</i> )		2.74	8 wk	50 to 100	Enhanced growth and feed utilization and health parameters	Xu et al. (2020)

Meal	Atlantic Salmon	34	7 wk	12.5	Reduction in enterocyte steatosis in pyloric caeca improved distal intestine histology and enhanced plasma lysozyme content	Weththasinghe et al. (2021)
Meal	Rainbow trout	201.8 ± 13.9	5 wk	30	Increased diversity and modulation of gut bacteria composition	Huyben et al. (2019)
Pulp	Mirror carp	13.68 ± 0.02	8 wk	50	Decreased whole-body lipid content and increased antioxidant enzyme activity	Xu et al. (2019)
Meal	Rainbow trout	100	131 d	50	Modulation of the gut microbial community by enhancing the abundance of bacteria taxa related to fish health	Rimoldi et al. (2021)
Meal	Baltic prawn ( <i>Palaemon adspersu</i> )	0.49 ± 0.1	60 d	18	Improved growth performance and survival	Mastoraki et al. (2020)
Meal	Siberian sturgeon ( <i>Acipenser baerii</i> )	640 ± 3.9	60 d	15	Improved gut microbiota composition and intestinal morphology but reduced mucosa thickness in the gastrointestinal tract.	Józefiak et al. (2019)
Meal	Siberian sturgeon		60 d	50	Lowered diet acceptance results in lowered growth and survival, decreased hepatic lipids and glycogen content, adverse effects on gut histology, but with a higher hepatic heat shock protein 70.1 (hsp70.1) gene expression	Zarantoniello et al. (2021)
Meal	Rainbow trout	53.4 ± 3.74	71 d	20	Improved growth performance and an increased count of beneficial bacteria in the intestine	Józefiak et al. (2019)



Silkworm ( <i>Bombyx mori</i> L.) pupae	Defatted meal	Pacific white shrimp ( <i>L. vannamei</i> )	0.2 ± 0.02	8 wk	75 to 100	Improved digestibility, antioxidant capacity and reduced molting time.	Rahimnejad et al. (2019)
Mealworm beetle, (TM)	Meal	Giant river prawn ( <i>Macrobrachium rosenbergii</i> )	3.26 ± 0.13	10 wk	12	Improved growth performance, immune response, disease resistance against <i>Lactococcus garvieae</i> , and <i>Aeromonas hydrophila</i> .	Feng et al. (2019)
	Defatted meal	Pacific white shrimp	1.5–1.6	8 wk	50	Improved growth and feed conversion ratio, enhanced resistance against early mortality syndrome ( <i>Vibrio parahaemolyticus</i> )	Motte et al. (2019)
	Partially defatted meal	Rainbow trout	78.3 ± 6.24	154 d	50 to 100	Reduced apparent digestibility of crude protein	Chemello et al. (2020)
	Meal	Gilthead seabream ( <i>Sparus aurata</i> )	105.2 ± 0.17	163 d	50	Establishment of novel nutritional niches in the gut	Antonopoulou et al. (2018)
	Meal	Gilthead seabream	86.97 ± 2.3	163 d	25	Best final weight, specific growth rate, weight gain, protein efficiency ratio and a lower feed conversion ratio	Piccolo et al. (2017)
	Meal	European sea bass	5.2 ± 0.82	70 d	50	Establishment of novel nutritional niches in the gut	Antonopoulou et al. (2018)
	Meal	Rainbow trout	115.2 ± 14.21	90 d	60	Improved specialized gut bacterial community	Antonopoulou et al. (2018)
	Defatted meal	Red seabream ( <i>Pargus major</i> )	30.4	8 wk	10	Increased resistance against pathogenic <i>Edwardsiella tarda</i> bacteria	Ido et al. (2019)
Meal	Rainbow trout	115.6 ± 14	90 d	50	Increased activity of the antioxidant enzymes in the intestine and reduction of lipid peroxidation.	Henry et al. (2018a)	

					Also increased antibacterial activity of the serum	
Meal	European sea bass	65.3 ± 5.7	6 wk	25	Enhanced lysozyme antibacterial activity and serum trypsin inhibition linked to the anti-parasite activity of the fish.	Henry et al. (2018b)
Meal	Rainbow trout	105.2 ± 0.17	163 d	50	Reduction in some essential amino acids (Ala, Ile, Leu, and Lys).	Iaconisi et al. (2019)
Meal	Rainbow trout	1.11 ± 0.01	8 wk	14	Improved growth performance and lysozyme activities	Jeong et al. (2020)
Meal	Baltic prawn ( <i>P. adspersu</i> )	0.49 ± 0.1	60 d	18	Higher protein and energy contents in the muscles	Mastoraki et al. (2020)
Meal	Siberian sturgeon	640 ± 3.9	60 d	15	Increased thickness of the muscular layer in the gastrointestinal tract and decreased the total number of bacteria	Józefiak et al. (2019)
Partially defatted	Rainbow trout	78.3 ± 6.24	22 wk	100	Slight modulation observed in the gut and skin microbiota by reducing pathogenic bacteria count	Terova et al. (2021)
Meal	Mandarin fish ( <i>Siniperca scherzeri</i> )	20.8 ± 0.05	8 wk	30	Improved growth and feed efficiency and enhanced serum lysozyme and glutathione peroxidase (GPx) activities.	Sankian et al. (2018)
Meal	Yellow catfish ( <i>Pelteobagrus fulvidraco</i> )	10.0 ± 0.03	5 wk	18	Enhanced immune response and disease resistance against <i>Edwardsiella ictaluri</i>	Su et al. (2019)
Meal	Rainbow trout	53.4 ± 3.74	71 d	20	Improved growth performance, reduced villus height and increased count of beneficial bacteria in the intestine	Józefiak et al. (2019)

	Meal	White shrimp	2.39 ± 0.49	8 wk	100	Enhanced the weight gain, specific growth rate and feed conversion rate	Choi et al. (2018)
	Meal	Narrow-clawed crayfish ( <i>Pontastacus leptodactylus</i> )	0.011 ± 0.002	80 d	50	Improved weight gain, specific growth rate, protein efficiency ratio, apparent net protein utilization, molting frequency, and feed conservation ratio, but lower survival rate as compared to the other diet groups. Also improved protein and lipid content of the whole body	Mazlum et al. (2021)
	Meal	Black porgy, ( <i>Acanthopagrus schlegelii</i> )	6.43 ± 0.00	12 wk	60	Improved serum lysozyme activity and upregulation of antioxidant enzyme-related genes but with declined fillet lipid content	Jeong et al. (2022)
Superworm larvae ( <i>Zophobas morio</i> )	Meal	Nile tilapia	3.00 ± 0.2	12 wk	15	Enhanced innate immune parameters (thrombocytes and neutrophils, liver and serum lysozyme activity, and complement system activity)	Alves et al. (2020)
Housefly ( <i>Musca domestica</i> )	Meal	Baltic prawn ( <i>P. adspersu</i> )	0.49 ± 0.1	60 d	18	Enhanced growth performance but lowered survival	Mastoraki et al. (2020)
Cricket ( <i>Gryllus bimaculatus</i> )	Meal	African catfish	13.2 ± 0.3	7 wk	100	Improved growth performance, haemoglobin, haematocrit and catalase activity	Taufek et al. (2016)
Tropical house cricket ( <i>Gryllodes sigillatus</i> )	Meal	Rainbow trout	53.4 ± 3.74	71 d	20	Lowered growth performance, reduced villus height and increased count of beneficial bacteria in the intestine	Józefiak et al. (2019)
Turkestan cockroach ( <i>Blatta lateralis</i> )	Meal	Rainbow trout	53.4 ± 3.74	71 d	20	Improved growth performance, increased villus height and count of beneficial bacteria in the intestine	Józefiak et al. (2019)

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