

Insects as Raw Materials in Compound Feed for Aquaculture

[AU1](#) Erik-Jan Lock, Irene Biancarosa, and Laura Gasco

Abstract Already in the early phases of the development of an European insect industry, aquafeed was suggested as one of the first animal feeds where insect products could be implemented. Since then, substantial progress has been made by the research community and feed producers to test various types of insect species and insect products as part of a complete feed for aquaculture. These (mostly extruded) feeds are typically high in energy and protein content which demands specific characteristics of the raw materials. The role insects, high in protein and lipids, can play in these diets will be reviewed and discussed in this chapter. We will shortly touch on topics like the effect of insect feeding substrate, insect processing and chitin that all can have an effect on insect meal. Finally, feed safety considerations related to the use of insects in aquafeeds will be reviewed and discussed.

1 Introduction

Compound feed contains macro- and micronutrients in levels that fulfil the animal's requirements for healthy growth under intensive rearing conditions. Compound feed normally contains animal- and/or plant-based feed materials to which micronutrients (vitamins, minerals) are added. The most used feed ingredients are fishmeal, krill meal, soy protein concentrate, corn gluten meal, wheat gluten, fish oil and rapeseed oil amongst others. Animal by-products, like feather meal or blood meal are also used (not in Norway) and novel feed materials are investigated like, seaweed, microalgae, bacterial protein meal and insects. Diets for carnivorous fish like

E.-J. Lock (✉)

National Institute of Nutrition and Seafood Research (NIFES), Bergen, Norway

e-mail: elo@nifes.no

I. Biancarosa

National Institute of Nutrition and Seafood Research (NIFES)-University of Bergen, Bergen, Norway

e-mail: ibi@nifes.no

L. Gasco

Università degli studi di Torino, Torino, Italy

e-mail: laura.gasco@unito.it

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24 rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) are
25 high-energy diets, characterized by high contents of lipids and protein, and low
26 levels of carbohydrates. Animal-based feed ingredients, like insects, fit these con-
27 straints much better than vegetable-based feed ingredients. The nutrient content of
28 various insect species has been widely studied and is reviewed in several articles
29 (Rumpold and Schluter 2013; Barroso et al. 2014; Makkar et al. 2014; Sanchez-
30 Muros et al. 2014; Henry et al. 2015). Fish prey on insects in their natural environ-
31 ment and to include insects in a compound feed is self-evident from a natural
32 perspective. However, also from a nutritional perspective insects can be a valuable
33 feed ingredient and will be discussed in the following sections.

34 2 Inclusion of Insect Raw Materials in Compound Feed 35 for Fish

36 A large number of insect species can potentially be considered for their inclusion in
37 fish diets. However, the interest towards the use of insect ingredients in aquafeeds
38 focusses mainly around a few insect species that can be produced on a large scale.
39 The investigations conducted so far mainly concern the use of larvae meals obtained
40 from *Tenebrio molitor* (TM), *Hermetia illucens* (HI) and *Musca domestica* (MD).
41 While a relatively large number of research articles exists on insect meals in warm
42 water fish species (Henry et al. 2015), very few studies have investigated the effects
43 of insect meals (IM) in salmonids (Table 1) or marine species (Table 2).

44 The results of the existing studies differ, depending on fish species considered,
45 IM inclusion levels and types, and feed formulation. Including a new ingredient
46 means replacement of another ingredient in the diet. In most studies, fishmeal (FM)
47 is replaced; however other studies replaced plant-based ingredients, resulting in not
48 directly comparable results. Finally, a replacement of FM by IM is often expressed
49 as % replacement of FM. Since the amount of FM varies between studies, direct
50 comparisons on % replacement is not always possible.

51 2.1 Growth Performance and Feed Utilisation

52 The use of IM in salmonid diets was already investigated in the 1980s (Akiyama
53 et al. 1984) with the aim of stimulating feed ingestion or palatability. A part of the
54 FM was substituted by low levels (5%) of silkworm pupae or earthworm powder in
55 swim-up fry diets. The use of earthworm powder resulted in a weight gain (WG)
56 and feed efficiency improvement of 30% and 39% respectively. Silkworm meal
57 slightly improved feed efficiency while neither source increased the palatability of
58 the fish diet, measured as daily food consumption.

Table 1 Growth performances of salmonids fed insect meals compared to FM (or other protein sources) control diet

Specie	Insect meal	IBW (g)	Rearing substrate	Insect processing	FM in diet (%)	Other protein source (%)	% CP in diet (%DM)	FM (or other protein source) substitution (%)	% Insect meal dietary inclusion	FBW	WG3%	WG	FC	FR	FE	PER	FCR	SGR	Reference
t1.1	Control	0.2	-	-	69.2	-	45.63	-	-	-	-	b	a	-	c	-	-	-	Akiyama et al. (1984)
t1.2	Control	0.2	-	-	69.2	-	45.63	-	-	-	-	b	a	-	c	-	-	-	Akiyama et al. (1984)
t1.3	SW	-	-	Commercial products	64.8	-	49.53	6.36	5	-	-	b	b	-	b	-	-	-	-
t1.4	EW	-	-	Commercial products	64.4	-	46.44	6.94	5	-	-	-	c	-	a	-	-	-	-
t1.5	Control	22.5	-	-	36.0	CGM	39.1	-	a	a	-	a	nd	-	-	-	a	-	St-Hilaire et al. (2007)
t1.6	HI	-	Pig manure	-	27.0	SBM (8)	37.4	25	ab	ab	-	ab	nd	-	-	-	a	-	St-Hilaire et al. (2007)
t1.7	HI	-	Pig manure	-	18.0	SBM (16)	37.5	50	b	b	-	b	nd	-	-	-	b	-	St-Hilaire et al. (2007)
t1.8	MD	-	Cow manure	-	27.0	-	41.0	25	b	b	-	-	nd	-	-	-	a	-	-
t1.9	Control	146	-	-	29.1	CGM (7)	46.00	0	-	-	a	-	nd	-	-	-	nd	-	Sealey et al. (2011)
t1.10	NHI	-	Cow manure	Freeze grinded + dried full fat prepupae	21.8	SBM (16)	48.50	25	16.4	-	b	-	nd	-	-	-	nd	-	Sealey et al. (2011)
t1.11	NHI	-	Cow manure	Freeze grinded + dried full fat prepupae	14.5	SBM (16)	50.40	50	32.80	-	b	-	nd	-	-	-	nd	-	Sealey et al. (2011)
t1.12	EHI	-	Cow manure + fish offal	Freeze grinded + dried full fat prepupae	21.8	WGM (7.8)	51.20	25	18.12	-	ab	-	nd	-	-	-	nd	-	Sealey et al. (2011)
t1.13	EHI	-	Cow manure + fish offal	Freeze grinded + dried full fat prepupae	14.5	WGM (7.8)	52.50	50	36.24	-	ab	-	nd	-	-	-	nd	-	Sealey et al. (2011)
t1.14	Control	115.6	-	-	75	-	45.2	0	0	nd	nd	nd	-	a	-	b	a	b	Belforti et al. (2015)
t1.15	TM	-	Wheat bran	Oven dried (full fat)	49	-	44.6	34.66	25	nd	nd	nd	-	ab	-	a	b	a	Belforti et al. (2015)
t1.16	Control	115.6	-	-	25	CGM (5)	44.8	66.67	50	nd	nd	nd	-	b	-	a	b	a	Belforti et al. (2015)
t1.17	TM	-	Wheat bran	Oven dried (full fat)	25	CGM (5)	44.8	66.67	50	nd	nd	nd	-	b	-	a	b	a	Belforti et al. (2015)

(continued)

Specie	Insect meal	IBW (g)	Rearing substrate	Insect processing	FM in diet (%)	Other protein source (%)	% CP in diet (%DM)	FM (or other protein source) substitution (%)	% Insect meal dietary inclusion	FBW	WG%	WG	FC	FR	FE	PER	FCR	SGR	Reference
Rainbow trout (<i>Oncorhynchus mykiss</i>)	Control	179	Plant substrate	Dried partially defatted larvae meal	60	WM (4)	45.20	0	0	nd	-	nd	-	nd	-	nd	nd	nd	Rema et al. (2017)
	HI				45		44.86	25	20	nd	-	nd	-	nd	-	nd	nd	nd	
					30		45.00	50	40	nd	-	nd	-	nd	-	nd	nd	nd	

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t1.62 *FM* Fishmeal, *SW* Silkworm, *EW* Earthworm, *HI* *Hermetia illucens*, *MD* *Musca domestica*, *NHI* *Hermetia illucens* prepupae reared in normal cow manure, *EHI* *Hermetia illucens* prepupae reared in cow manure enriched with fish offal, *HIA* *Hermetia illucens* larvae meal containing 25.5 lipid, *HIB* *Hermetia illucens* larvae meal containing 17% lipid, *TM* *Tenebrio molitor*, *SBM* soybean meal, *CGM* Corn gluten meal, *WGM* Wheat gluten meal, *SPC* Soy protein concentrate, *CP* crude protein, *IBW* Initial body weight (g), *FBW* Final body weight (g), *WG* Weight gain (g), *WG (%)* = $\frac{FBW - IBW}{IBW} * 100$; *FC* Feed consumption = grams feed consumed per 100 g body weight per day, *FR* feeding rate (%/day) = $\frac{[total\ feed\ supplied\ (g\ DM) * 100\% / number\ of\ feeding\ days]}{e^{(ln IBW + ln FBW) * 0.5}}$, *FE* Feed efficiency = $\frac{WG}{dry\ food\ intake}$, *PER* Protein efficiency ratio = $\frac{wet\ weight\ gain\ (g)}{total\ protein\ intake\ (g)}$, *FCR* Feed conversion ratio = $\frac{Ingested\ feed\ (g)/wet\ weight\ gain\ (g)}{SGR}$ Specific growth rate (%/day) = $\frac{[ln\ final\ fish\ weight \pm ln\ initial\ fish\ weight]/days}{* 100}$. Columns with different superscripts (a, b) are significantly different at $P < 0.05$; * S; significantly different from control diet ($P < 0.05$) - no information; *nd* no differences

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Table 2 Growth performances of marine species fed insect meals compared to FM (or other protein sources) control diet

Specie	Insect meal	IBW (g)	Rearing substrate	Insect processing	FM in diet (%)	Other protein source (%)	% CP in diet (%DM)	FM (or other protein source substitution) (%)	% Insect meal dietary inclusion	FBW	WG%	WG	FIFC	FR	PER	FCR	SGR	Reference
Turbot (<i>Psetta maxima</i>)	Control	54.9	-	-	68.70	WP (2)	54.80	0	0	nd	-	-	a	-	-	a	a	Kroeckel et al. (2012)
	HI		Green house waste	Frozen, partially defatted, oven dried prepupae	55.00	BM (5)	54.90	20	16.5	nd	-	-	a	-	-	a	b	
					42.20		53.70	40	33.2	nd	-	-	a	-	-	ab	b	
					30.50		53.90	55	48.6	nd	-	-	b	-	-	b	c	
					18.00		53.3	74	64.0	nd	-	-	b	-	-	c	d	
European sea bass (<i>Dicentrarchus labrax</i>)	Control	5.22	-	-	8.00	WGM (5)	52.70	88	75.6	nd	-	-	c	-	-	d	e	Gasco et al. (2016)
	TM		Wheat bran	Oven dried (full fat)	45	WGM (7.5) CGM (2.8)	59.04	35.71	25	ab	-	ab	-	ab	nd	nd	ab	
					20	WGM (15)	59.54	71.42	50	b	-	b	-	b	nd	nd	b	
Gilthead sea bream (<i>Sparus aurata</i>)	Control	105	-	-	50	15 (CGM)	46.05	0	0	b	b	-	-	-	b	a	b	Piccolo et al. (2017)
	TM		Wheat bran	Oven dried (full fat)	33.3	12.5 (CGM)	45.69	33.4	25	a	a	-	-	-	a	b	a	
					13	13 (CGM)	45.16	74	50	b	b	-	-	-	b	a	b	

FM Fishmeal, HI *Hermetia illucens*, TM *Tenebrio molitor*; WP Wheat protein, BM Blood meal, CGM Corn gluten meal, WGM Wheat gluten meal, CP crude protein, IBW initial body weight (g), FBW Final body weight (g), WG Weight gain (g); FBW – IBW; WG (%) = FBW – IBW/ IBW * 100; FC Feed consumption = grams feed consumed per 100 g body weight per day, FR feeding rate, (%/day) = [total feed supplied (g DM) * 100%/(number of feeding days)]/(e(lnFBW + lnFBW)*0.5); PER Protein efficiency ratio = wet weight gain (g)/total protein intake (g), FCR Feed conversion ratio = Ingested feed (g)/wet weight gain (g), SGR Specific growth rate (%/day) = {(ln final fish weight ± ln initial fish weight)/days} * 100. Columns with different superscripts (a, b, c) are significantly different at P < 0.05. - no information; nd no differences

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St-Hilaire et al. (2007) investigated the use of a full fat pre-pupae HI meal used in partial substitution of FM and fish oil (FO) in the diet of rainbow trout. HI meal was included at two levels (15 and 30%) leading to a FM substitution of 25% and 50% and to a FO substitution of 36 and 72% respectively. No significant differences on growth performances were reported at the lowest level of inclusion allowing a valuable FO saving. St-Hilaire et al. (2007) suggested that above this level, the chitin contained in the pre pupae may have decreased the digestibility, thus the availability of nutrients, resulting in lower fish performances. On the other hand, the dietary inclusion of HI meal lead to a modification of the dietary fatty acid profile (increase and decrease of saturated and polyunsaturated fatty acids respectively) that could have influenced lipid digestibility. In the same trial, authors studied the effects of a whole MD larvae meal included at 9.2% in the fish's diet (25% of FM substitution). The inclusion resulted in a decrease of production parameters (St-Hilaire et al. 2007). Renna et al. (2017) showed that a partially defatted HI larvae meal can be used as feed ingredient in rainbow trout diets up to 40% of inclusion level (50% of FM substitution) without impacting growth performance. Sealey et al. (2011) highlighted the possible influence of larva rearing substrate on the quality of the insect meal in a trial with rainbow trout. IM produced from HI larvae fed a diet enriched with fish offal performed better than IM produced from HI larvae fed a diet without the fish offal enrichment. Rainbow trout fed a diet with the enriched HI meal (at 50% FM replacement) performed equally well as the control FM based diet, whilst the non-enriched HI meal performed less at already 25% FM replacement.

A full fat TM larvae meal was tested as a FM substitution (up to 50%) in rainbow trout diets by Belforti et al. (2015). No significant changes in fish performance parameters were found up to 50% FM replacement. A reduced voluntary feed intake was reported with the increase of TM meal. The effects of dietary FM replacement (0, 25%, 50% and 100%) by super worm (*Zophobas morio*) meal on rainbow trout fingerlings growth performance was investigated by Doğankaya (2017). Fish fed diets containing up to 25% of FM substitution performed better than the fish fed the control diet, while no differences were observed between 0% and 50% of FM substitution. Highest IM level induced a dramatic worsening in performance parameters.

Concerning marine species, Kroeckel et al. (2012) tested partially defatted HI prepupae meal in diets of juvenile turbot (*Psetta maxima*), and found a general worsening of performances at the inclusion levels higher than 33%. Moreover, authors found a decrease of feed intake with increasing HI meal incorporation, due to low palatability. Authors suggested that the presence of chitin might have influenced the feed intake, availability, and digestibility of the nutrients and therefore growth performance. Nevertheless, as HI was produced on local greenhouse waste streams, the authors concluded that it could be a sustainable alternative protein source in partial substitution of FM (Kroeckel et al. 2012). Karapanagiotidis et al. (2014) evaluated a pre-pupae full fat HI meal (crude protein, CP: 31.6%; ether extract, EE: 27.2) in gilthead seabream (*Sparus aurata*) diets. Four diets were formulated substituting FM (0, 9%, 17% and 25%) with HI meal at 0%, 9.5%, 19.4%

104 and 27.6% of HI inclusion. Fish fed diets containing HI meal recorded a significant
 105 decrease in final fish weight and WG due to a significant decrease of total feed con-
 106 sumption. On the other hand, feed conversion rate (FCR), protein efficiency ratio
 107 (PER) and protein retention as well as specific growth rate (SGR) parameters did
 108 not show differences among treatments.

109 Gasco et al. (2016) evaluated the effects of dietary inclusion of a full-fat TM
 110 larvae meal on European sea bass (*Dicentrarchus labrax*) juveniles. Dietary TM
 111 meal inclusion level of 50% led to a worsening of final body weight, WG, SGR and
 112 feeding rate (FR). Using the same substitution protocol and the same full-fat TM
 113 larvae meal, Piccolo et al. (2017) found improved final weight, SGR, PER and FCR
 114 in fish fed 25% of TM meal dietary inclusion.

115 The importance of insect processing becomes evident in a study by Lock et al.
 116 (2016). Two different HI meals (IMA and IMB), obtained through different nutrient
 117 isolation and processing techniques, were evaluated in diets for Atlantic salmon.
 118 IMA substituted 25, 50 and 100% of FM in the control diet while IMB was used at
 119 25 and 100% FM replacement rate. Diets containing IMA performed equally well
 120 as the control group at all inclusion levels, however diets produced with IMB
 121 reduced fish performance parameters already at 25% FM replacement.

122 2.2 Whole Body and Fillet Composition

123 The influence of the use of IM on whole body composition (WBC) and fillet com-
 124 position is not univocally. While an effect on the protein content has been shown
 125 (Belforti et al. 2015), the majority of the existing studies report a decrease in lipid
 126 and moisture increase in either WBC or fillet of fish when fed diets containing IM
 127 (St-Hilaire et al. 2007; Sealey et al. 2011; Kroeckel et al. 2012; Belforti et al. 2015).
 128 A reduced fat and energy digestibility of some IM could be the reason for the
 129 observed decreasing carcass fat content. Conversely, Akiyama et al. (1984) reported
 130 an increase in body energy reserves using earthworm. This effect was considered as
 131 very valuable as that could increase the fingerlings survival once released. Renna
 132 et al. (2017) found an increase of fat content in rainbow trout fillets using a partially
 133 defatted HI meal, but only at the highest level of inclusion. Similar results have been
 134 found in feeding Atlantic salmon diets containing high levels of defatted HI meal
 135 (Lock et al. unpublished results). High HI meal inclusion results in a higher satu-
 136 rated lipid content of the whole fish and fillet.

137 It has been ascertained that the dietary fatty acid (FA) profile dramatically influ-
 138 ences the fish FA composition. IM are rich in saturated and monounsaturated FA,
 139 and do not contain the marine omega-3 long chain polyunsaturated FA (PUFA) such
 140 as eicosapentaenoic acid (EPA, C20:5 n3) or docosahexaenoic acid (DHA, C22:6
 141 n3), which are well known for their beneficial effects on human health. St-Hilaire
 142 et al. (2007) reported a deterioration in fish nutritional quality using both MD and
 143 HI meals in diets for rainbow trout. The inclusion of IM led to a significant decrease
 144 of n3 FA such as EPA and DHA, which is confirmed in other studies (Belforti et al.

2015; Gasco et al. 2016; Lock et al. 2016; Renna et al. 2017). Sealey et al. (2011) and Liland et al. (under revision) were able to modify the HI meal FA profile by enriching the larvae rearing substrate with fish offal and seaweed, respectively. Sealey et al. (2011) performed a trial with trout using the enriched HI meal and reported increased EPA (significant) and DHA (not significant) content in the fish. Up to 20% inclusion of a de-fatted HI meal while maintaining FO in the diet does not change the FA profile of trout compared to fish fed a control diet based on FM and FO (Renna et al. 2017).

2.3 Sensory Analyses

As the change of body composition and fatty acid profile can influence fish flavour, aroma and consumer acceptance (Turchini et al. 2011), some studies investigated the effect of diets containing IM on the sensory aspects of the fish fillet.

In a triangle difference test, untrained panellists did not highlight different sensory perception in samples of fillets of trout fed diets containing HI meal (enriched or not using fish offal in larva rearing substrate) compared to a FM based diet with no inclusion of HI pre-pupae meal (Sealey et al. 2011). Lock et al. (2016) investigated the sensory attributes of fillets of fish from diets containing FM (control) or 25% of inclusion of HI meal (100% of FM substitution) after 105 days of feeding. Trained panellists were asked to score attributes such as odour, taste and flavour, and texture scoring them in a scale from 1 to 9. The analysis did not highlight any significant differences in odour, flavour/taste or texture between groups.

Borgogno et al. (2017) utilised descriptive analysis (DA) and Temporal Dominance of Sensations (TDS) to investigate the effects of replacing 25 and 50% of FM with HI meal on sensory properties of rainbow trout. Results indicated that diets significantly affected fillets sensory profile. In fact, significant changes in perceived intensity of aroma, flavour and texture descriptors as a function of diet composition was indicated by DA. Concerning TDS, the first sensations perceived as dominant were related to texture attributes, followed by flavours. Dominance of fibrousness (or toughness) decreased with the increasing of HI meal in diet. Boiled fish, algae flavours and umami taste clearly dominated the fish fed control diet dynamic profile. The onset of metallic flavour dominance characterized fish fed diets where FM was substituted by 25 and 50% of HI meal. No differences in physical parameters were detected. Principal component analysis highlighted the relationship between sensory attributes and physico-chemical parameters.

179 2.4 Chitin

180 It is commonly assumed that, due to its complex matrix, insect chitin is poorly
181 digestible by fish, albeit the chitinase activity has been observed in some fish spe-
182 cies (Henry et al. 2015). It has been hypothesized that these matrix forms of chitin
183 may reduce the access of chitinases or proteinases to their substrates and thus pre-
184 vent the absorption of proteins and lipids by the intestine. As such, reducing lipid
185 and protein digestibility resulting in a subsequent reduction in nutrient utilization
186 and fish growth performance (Belforti et al. 2015; Henry et al. 2015; Gasco et al.
187 2016). Some studies investigated the nutrient apparent digestibility coefficients
188 (ADC) of diets containing IM. In general a lower crude protein ADC is found com-
189 pared to FM based diets (Kroeckel et al. 2012; Belforti et al. 2015). Nevertheless,
190 not all studies find a decrease in ADC (Lock et al. 2016; Renna et al. 2017), high-
191 lighting once again the high variability in type and quality of insect meal available
192 on the market.

193 Chitin as a stimulant of intestinal function, much like a fibre, has also been sug-
194 gested. The use of alternative protein sources has often showed to induce histologi-
195 cal changes of the fish gastrointestinal tract (Merrifield et al. 2009; Gai et al. 2012;
196 Oliva-Teles et al. 2015). Very few studies have investigated this aspect using insect
197 meals and results obtained so far are promising as no negative effects are reported
198 (Lock et al. 2016; Doğankaya 2017; Renna et al. 2017).

199 3 Feed Safety

200 Feed safety regulations are in place to secure that feed and feed materials do not
201 pose any danger to human health, animal health or the environment, aiming to pro-
202 vide healthy and safe food products to the public. To achieve this, the European
203 Union has set maximum allowed levels for undesirable substances in animal feed
204 and feeding stuffs (EC Directive 2002/32 and amendments) (EU 2002). This covers
205 a wide range of toxic compounds such as heavy metals, arsenic, polychlorinated
206 biphenyl (PCBs), pesticides, plant and fungal toxins, amongst others. Safety consid-
207 erations need to be taken into account when insects are destined to animal feed.

208 The uptake of contaminants by insects in the wild is well known, therefore they
209 have been successfully used as bioindicators for environmental pollution (Azam
210 et al. 2015). The chemical safety of farmed insects for feed and food purposes has
211 been reviewed (Belluco et al. 2013; Charlton et al. 2015; van der Spiegel et al.
212 2013). Although little data is available, major potential chemical hazards associated
213 with farmed insects are heavy metals, and in particular cadmium. Accumulation of
214 metals in insects is dependent on species, life stage, and metal considered. Larval
215 stages of insects have been shown to contain higher concentrations of metals than
216 adults (Lindqvist 1992; Diener et al. 2015).

Studies on the feed safety of farmed insects are very limited. Charlton et al. (2015) investigated a variety of insect species cultivated in several geographical locations, using different rearing substrates and conditions. The heavy metals cadmium, lead, mercury and the metalloid arsenic were found in larvae of MD, Blue bottle (*Calliphora vomitoria*), Blow fly (*Chrysomya spp.*) and HI. The EU maximum allowed levels for cadmium, lead, mercury and arsenic in complete fish feed and feed materials are set at 0.5, 5, 0.1, 2 and 2, 10, 0.1, 2 mg/kg (88% dry matter), respectively (EU 2002). The concentrations of these undesirables in the fly larvae analysed by Charlton et al. (2015) were all below the maximum limits.

During rearing, insects could accumulate contaminants present in their feeding media. However, only few studies have investigated the influence of different feeding substrates on metal accumulation in insect larvae (Biancarosa et al. under revision; Diener et al. 2015; Vijver et al. 2003). HI larvae accumulate heavy metals when these are present in the diet, and a direct correlation exists between dietary and larval metal concentrations. This was shown using either feeding substrates spiked with heavy metals (cadmium, lead or zinc) (Diener et al. 2015) or media naturally containing these undesirable elements such as seaweeds (Biancarosa et al. under revision). Rearing insect larvae on substrates containing marine materials (seaweeds, tunicates, FM) resulted in the uptake of cadmium, lead, mercury and arsenic also in TM and super worms (Biancarosa et al. unpublished results). Vijver et al. (2003) previously documented accumulation of cadmium and lead in mealworms, when fed on soils contaminated with these contaminants.

The transfer of heavy metals and arsenic from feeding substrates to insect larvae highlights the need to carefully choose the material that is used to rear the larvae. However, there are currently big knowledge gaps related to the influence of different substrates on the metal content of farmed insects, thus further studies are required. Moreover, besides exploring the metal content of non-processed insects (e.g. whole larvae), documentation of the occurrence of these undesirable elements in processed larvae products (e.g. IM and insect lipid (IL)). Processing of the insect raw materials could potentially reduce metal contaminations prior to feeding. Further research is also needed to investigate whether heavy metals (or other potential risks) present in insects used for feed, are transferred to farmed fish.

Other chemical hazards may be present in rearing substrates for insects, thus may end up in insects and products thereof. In respect of the EU feed legislation (EC Directive 2002/32 and amendments) (EU 2002), Charlton et al. (2015) investigated the presence of dioxins, PCBs, polyaromatic hydrocarbons (PAHs), pesticide residues, veterinary drugs and mycotoxins in farmed insects destined to animal feed (house fly, blue bottle, blow fly and black soldier fly). These contaminants were found in the insect species tested, although in concentrations generally below current regulatory limits for these compounds in animal feed. Only the veterinary medicine nicarbazin (4,4'-dinitrocarbanilide) was detected at concentrations above the maximum allowed in animal feed (500 µg/kg) in one sample of MD due to the use of contaminated animal manure as growth medium for the larvae. Risks of this kind are minor in the EU, where feeding manure to farmed insects is currently prohibited. However, outside the EU other regulations apply. Insect meals produced

262 outside the EU can be imported however they have to fulfil the same requirements
263 set by the abovementioned EC Directive when used in feeds. For some of the com-
264 pounds detected by Charlton et al. (2015) (e.g. PAHs and the pesticide residue
265 chlorpyrifos), no maximum limits are currently established for animal feed.

266 Microbiological hazards related to the use of insects for feed purposes have been
267 taken into account in the first “Risk profile related to the production and consump-
268 tion of insects as food and feed” by EFSA (2009). Like other famed animals, micro-
269 organisms can be naturally associated with insects (e.g. the microbiota in the guts or
270 on the surface), or can be introduced during rearing processes. However, very little
271 studies on the microbiological safety of insects for food and feed are currently avail-
272 able (Klunder et al. 2012) to support such risk analysis.

273 4 Conclusion

274 Studies on IM inclusion in aquafeeds so far have focussed on FM replacement and
275 growth performance, which is a logical first step for any new feed ingredient. Other
276 aspects (both positive and negative) of IM on fish health are expected to be addressed
277 over time, e.g. intestinal health, changes in microbiota, immunology, etc. There is
278 also clearly an important role for insect processing (de-fattening, protein isolation,
279 hydrolysis, etc), which can affect the properties of a meal into a great extent. The
280 effect of chitin is still under investigation, and no conclusive evidence exists of chi-
281 tin functioning as an anti-nutrient, immunostimulant, or any other function that has
282 been proposed. Moreover, the role of the substrate on the quality of the meal is of a
283 major importance as both the nutrient composition and content of undesirables are
284 (partly) dictated by the composition of the insect feeding substrate. The approval of
285 the EU Commission of the use of insect PAP in aquafeeds on the 13th December
286 2016 most likely triggers a surge in both demand and supply of IM and exiting
287 developments in this field of research are expected. Signals from feed producers
288 indicate a strong interest in using this resource if volumes are reaching 40.000 MT
289 or more and the price is competitive. The increase in IM demand will inevitably lead
290 to a decrease in IM selling price that is until now, still not competitive if compared
291 with other protein sources commonly used in aquaculture feeds. Finally, initial stud-
292 ies on consumer acceptance of insect-fed fish showed a positive consumer attitude
293 (Verbeke et al. 2015; Mancuso et al. 2016), but additional studies will be needed
294 when insect products will reach the market.

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Author Queries

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Queries	Details Required	Author's Response
AU1	Please confirm corresponding author.	
AU2	Please provide the term for "FBW-IBW" definition.	
AU3	Reference Lock et al. (unpublished results), Biancarosa et al. (unpublished results) have been cited in text but not provided in reference list. Please check.	
AU4	Please provide volume number for reference EFSA (2009).	
AU5	Please provide volume and page range for reference Renna et al. (2017).	

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