

Chapter 1

Fishmeal Alternative Protein Sources for Aquaculture Feeds



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Abstract Aquaculture currently accounts for approximately 50% of fish consumed by humans. The future development of aquaculture will be greatly constrained by the increasing costs of fishmeal and fish oil. To remedy this situation, scientific research and feed manufacturers have made a significant progress by looking for alternative protein sources for use in fish diets in order to develop feeds that provide adequate nutrition for animals' growth, while reducing to minimum the use of traditional sources of protein. This chapter aims at critically reviewing recent studies, carried out worldwide, about the effects of the inclusion of new protein sources as insect, poultry by-products, meat and bone meals and other protein sources alternative to fishmeal in aquafeeds. In particular, the impacts of these protein sources in terms of growth, nutrient digestibility, fillet quality traits and sensorial perception in the most important farmed marine and freshwater fish species are evaluated.

Keywords Alternative proteins • Aquaculture • Fishmeal • Insects
Poultry By-Products • Processed animal proteins

1.1 Introduction

The global demand for fish products is expected to increase significantly in the next 35 years due to the increase in world population that, according to the last Food and Agriculture Organization of the United Nations (FAO) evaluations, will reach 9.5 billion people in 2050 (FAO 2016). Although there is a slight improvement in the state of certain fish stocks due to improved fisheries management, the expected increase will be possible only through aquaculture production that already provides half of all seafood for human consumption (FAO 2016). Aquaculture is claimed to be the fastest growing food production sector in the world. Fish from fisheries and

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aquaculture provide important nutrients (energy, protein, vitamins and minerals) and fish supply reached a new record of about 20 kg in 2014 accounting for about 17% of the global population's intake of animal protein and 6.7% of all consumed proteins (FAO 2014).

While in the past, aquaculture feeds largely used fishmeal (FM) and fish oil (FO), these ingredients are no longer sustainable (Hardy 2010; Oliva-Teles et al. 2015; Tacon and Metian 2008). Moreover, these resources experienced periodic fluctuations in availability and pricing. Nowadays, their inclusion is reduced to the minimum amount able to cover the optimal content of amino acids and other nutrients needed for fish growth and flesh quality; sometimes, aquafeed industry produces fish diets that are completely free of these marine ingredients. Plant feedstuffs are widely used as an alternative to FM as they are available in large quantities when compared to FM (Gatlin et al. 2007; Naylor et al. 2009; Oliva-Teles et al. 2015). Nevertheless, in carnivorous fish species, the complete substitution of FM is still a challenge and its complete elimination is still associated with reduced performances and fish health (Desai et al. 2012; Krogdahl et al. 2010; Oliva-Teles et al. 2015). Moreover, in recent years, plant proteins for fish feeds face problems of increasing price and of competition with other sectors as human consumption, animal husbandry sector or biodiesel production (Moutinho et al. 2017; Pinotti et al. 2014). The use of terrestrial processed animal proteins (PAP) such as blood meal (BM), meat and bone meal (MBM), feather meal and poultry by-product meal (PBM) in aquaculture feeds is a common practice.

PAP have high protein and lipid content which make them very interesting for the formulation of cost-effective aquaculture feeds. PAP are a natural source of several nutrients such as amino acids (lysine, sulphur amino acids, histidine and arginine) or phosphorus. They are relatively free from any anti-nutritional factor, result to be highly palatable to most fish species, and their inclusion in aquafeeds complement very well certain plant protein ingredients (Bureau 2006). Nevertheless, the inclusion levels are limited by fish species, poor digestibility, deficiency of some essential amino acids (EAA) and general nutritional quality that highly depend on the raw material composition, freshness and processing conditions (Bureau et al. 1999, 2000; Goda et al. 2007). The use of PAP is highly depending on the considered region of the world. For instance, following the bovine spongiform encephalopathy emergency, European Union (EU) prohibited the use of PAP (EC No 999/2001). In 2013, this ban was partially lifted (EC No 56/2013), and the use of PAP from non-ruminant animals (poultry and pigs) (category 3) was reintroduced in fish feeds. Recently, the European Commission approved the use of PAP from seven insect species in aquafeed with the Regulation 2017/893/EC.

In the following paragraphs, different alternative protein sources investigated in field trials on cultured fish species as well as their effects on growth, nutrient digestibility, fillet quality traits and sensorial perception are reported and commented.

1.2 Insect Meals

The potential use of insect meal in fish diets has recently attracted much attention (Barroso et al. 2014; Henry et al. 2015). Carnivorous fish already count insects as part of their natural diet (Henry et al. 2015). It seems therefore reasonable to consider insect meals as raw material in fish feeds. Following the European Food Safety Authority (EFSA) scientific opinion on the use of insects as food and feed, the Standing Committee on Plants, Animals, Food and Feed has approved recently the draft of the Regulation amending Annexes I and IV to Regulation (EC) No 999/2001 and of the Council and Annexes X and XV to Commission Regulation (EC) No 142/2011 as regards the provisions on processed animal protein. The use of insect-derived PAP in aquafeeds in Europe is allowed since July 2017 (Commission Regulation (EU) 2017/893 of 24 May 2017).

In EU, the authorized insect meal is only those obtained from: (i) *Hermetia illucens* (HI, Black Soldier Fly) and *Musca domestica* (MD); (ii) *Tenebrio molitor* (TM, Yellow Mealworm) and *Alphitobius diaperinus* (Lesser Mealworm); (iii) *Acheta domesticus* (House cricket), *Gryllodes sigillatus* (Banded cricket) and *Gryllus assimilis* (Field Cricket). Nevertheless, in countries other than EU, rules could be different and other insects are considered as very interesting for fish nutrition (Barroso et al. 2014; Henry et al. 2015; Makkar et al. 2014).

When considering insects and FM ingredients for fish feeds, not only aspects such as energy, protein and EAA, fat or mineral content and many other chemical data (Tables 1.1, 1.2 and 1.3) have to be considered but also the raw material availability. In this sense, only few insect species so far have the potential to be produced in large scale and thus have received much attention as aquaculture feeds namely TM, HI and MD.

The chemical composition and the nutritional value of insect larvae meals (Table 1.1) largely depend on the treatment (i.e. drying methodologies, defatting procedures) and on the substrate used to rear them (Henry et al. 2015). In particular, while the protein content does not vary to a large extent due to the rearing substrate, the lipid fraction is the most susceptible to changes, both from a quantitative and qualitative fatty acid (FA) profile point of view (Henry et al. 2015; Makkar et al. 2014). As far as gross energy is considered, insect larvae meals have contents greater than 21 MJ kg/dry matter (DM). The high insect larvae fat content (15–50%) can sometimes cause problems. In fact, their inclusion as protein source automatically brings also a high fat content that can generate problems both for feed formulation but also for storage and pellet stability. For these reasons, insect producers consider defatting process using various methods (physical or chemical extractions). In this case, the percentage of protein (and consequently of EAA) is greatly increased and the extracted oils may be used for other purposes such as feed inclusion (Schiafone et al. 2017) or biodiesel production (Henry et al. 2015; Li et al. 2016; Surendra et al. 2016). As far as EAA are concerned (Tables 1.1, 1.2 and 1.3), the profiles of HI and MD are considered close to FM profiles while the one of TM closer to that of SBM.

Table 1.1 Nutrient composition and nutritive value of most promising insect larvae meals compared to fishmeal (FM) and soybean meal (SBM). Values are reported as mean of values found in the cited references (in parenthesis minimum and maximum values). The following chemical values are shown here: dry matter (DM), crude fibre, crude protein, lysine, methionine and cysteine, methionine and cysteine, tryptophan, threonine, leucine, isoleucine, valine, histidine, arginine and the sum of phenylalanine and tyrosine

	Unit	<i>Tenebrio molitor</i> ^a	<i>Hermetia illucens</i> ^b	<i>Musca domestica</i> ^c	FM ^d	SBM ^e
Dry Matter (DM)	% as fed	42.2 (37.1–57.6)	91.3 (90.0–92.5)	92.4 (90.0–94.7)	92.1 (90.0–94.4)	87.9 (85.0–92.1)
Crude fibre	% DM	5.9 (5.0–6.9)	7.0	14.7 (1.6–29.7)	–	6.7 (3.5–10.1)
Crude protein	% DM	51.5 (44.1–60.3)	49.1 (35.5–72.5)	49.9 (37.5–63.8)	75.6 (70.2–80.7)	51.4 (48.3–54.5)
Lysine	% protein	4.5 (1.7–6.1)	6.4 (5.6–8.0)	6.1 (4.4–8.2)	6.1 (5.5–7.5)	6.1 (5.7–6.6)
Methionine	% protein	1.5 (1.2–2.0)	1.8 (1.4–2.4)	2.3 (1.3–3.7)	2.2 (2.0–2.6)	1.4 (1.2–1.6)
Methionine + Cystine	% protein	2.3 (1.8–2.9)	2.2 (1.5–3.1)	3.0 (1.7–4.7)	2.9 (2.6–3.2)	2.9 (2.5–3.3)
Tryptophan	% protein	0.9 (0.0–1.8)	0.8 (0.5–1.1)	1.8 (1.4–3.2)	0.8 (0.7–0.9)	1.3 (1.2–1.4)
Threonine	% protein	3.6 (2.7–4.4)	3.6 (1.3–4.8)	3.8 (2.0–7.6)	3.1 (2.9–4.3)	3.9 (3.5–4.3)
Leucine	% protein	7.6 (4.5–10.6)	7.3 (6.6–8.4)	5.7 (4.5–6.4)	5.9 (5.2–7.3)	7.5 (6.8–8.0)
Isoleucine	% protein	4.1 (2.6–5.0)	4.7 (4.0–5.6)	2.9 (1.7–3.7)	3.7 (3.3–4.4)	4.6 (4.3–5.0)
Valine	% protein	5.5 (3.7–6.6)	6.9 (5.6–9.1)	3.3 (1.3–4.9)	4.2 (3.9–4.8)	4.8 (4.3–5.4)
Histidine	% protein	3.0 (2.1–3.6)	3.1 (2.3–4.5)	3.0 (1.0–5.1)	1.8 (1.7–1.9)	2.6 (2.4–2.9)
Arginine	% protein	4.5 (3.6–5.6)	5.4 (4.8–6.1)	4.9 (3.7–5.8)	4.6 (4.0–6.0)	7.4 (6.8–8.1)
Phenylalanine + tyrosine	% protein	10.7 (8.6–12.1)	11.2 (9.6–13.3)	9.8 (6.2–17.3)	5.5 (5.2–6.5)	8.5 (7.7–9.4)

^aData from Barroso et al. (2014), Feedpedia: <http://www.feedpedia.org>, Gasco et al. (2016), Marono et al. (2015), Sánchez-Muros et al. (2015), Siemianowska et al. (2013)

^bData from Diener et al. (2009), Feedpedia: <http://www.feedpedia.org>, Józefiak et al. (2016), Makkar et al. (2014), Maurer et al. (2016), Marono et al. (2015), Sánchez-Muros et al. (2015), Tschirner and Simon (2015)

^cData from Aniebo et al. (2008), Aniebo and Owen (2010), Barroso et al. (2014), Feedpedia: <http://www.feedpedia.org>, Fasakin et al. (2003), Józefiak et al. (2016), Makkar et al. (2014), Sánchez-Muros et al. (2015), Sogbesan et al. (2006), Tschirner and Simon (2015), Zuidhof et al. (2003)

^dData from: Feedpedia: <http://www.feedpedia.org>, Guilleme et al. (2001)

^eData from: Feedpedia: <http://www.feedpedia.org>, Guilleme et al. (2001)

Table 1.2 Nutrient composition and nutritive value of most promising insect larvae meals compared to fishmeal (FM) and soybean meal (SBM). Values are reported as mean of values found in the cited references (in parenthesis minimum and maximum values). The following chemical values are shown here: ether extract, saturated fatty acids (FA), monosaturated FA, n6-polyunsaturated FA and n3-polyunsaturated FA

	Unit	<i>Tenebrio molitor</i> ^a	<i>Hermetia illucens</i> ^b	<i>Musca domestica</i> ^c	FM ^d	SBM ^e
Ether extract	% DM	30.2 (16.6–43.1)	20.0 (3.4–38.6)	15.8 (6.3–31.3)	8.1 (2.0–12.0)	2.1 (2.0–2.2)
Saturated fatty acids (FA)	% Total FA	26.4 (22.2–35.1)	33.3	41.3 (33.1–49.2)	19.5 (18.9–19.7)	15.1 (14.9–15.2)
Monosaturated FA	% Total FA	41.7 (35.1–51.5)	43.4	38.9	50.8 (50.5–52.1)	21.1 (20.5–21.7)
n6 Polyunsaturated FA	% Total FA	25.5 (11.5–34.5)	15.0	18.4	1.8 (1.8–2.0)	56.1 (55.9–56.3)
n3 Polyunsaturated FA	% Total FA	1.1 (0.8–1.4)	8.3	–	8.1 (2.0–12.0)	2.1 (2.0–2.2)

^aData from Barroso et al. (2014), Feedpedia: <http://www.feedipedia.org>, Gasco et al. (2016), Marono et al. (2015), Sánchez-Muros et al. (2015), Stemianowska et al. (2013)

^bData from Diener et al. (2009), Feedpedia: <http://www.feedipedia.org>, Józefiak et al. (2016), Makkar et al. (2014), Maurer et al. (2016), Marono et al. (2015), Sánchez-Muros et al. (2015), Tschirner and Simon (2015)

^cData from Aniebo et al. (2008), Aniebo and Owen (2010), Barroso et al. (2014), Feedpedia: <http://www.feedipedia.org>, Fasakin et al. (2003), Józefiak et al. (2016), Makkar et al. (2014), Sánchez-Muros et al. (2015), Sogbesan et al. (2006), Tschirner and Simon (2015), Zuidhof et al. (2003)

^dData from: Feedpedia: <http://www.feedipedia.org>, Guillaume et al. (2001)

^eData from: Feedpedia: <http://www.feedipedia.org>, Guillaume et al. (2001)

Table 1.3 Nutrient composition and nutritive value of most promising insect larvae meals compared to fishmeal (FM) and soybean meal (SBM). Values are reported as mean of values found in the cited references (in parenthesis minimum and maximum values). The following values are shown here: minerals (ash), calcium, phosphorus, sodium, potassium, magnesium and gross energy

	Unit	<i>Tenebrio molitor</i> ^a	<i>Hermetia illucens</i> ^b	<i>Musca domestica</i> ^c	FM ^d	SBM ^e
Minerals (ash)	% DM	3.8 (1.0–6.5)	13.6 (4.3–28.4)	11.4 (5.0–23.1)	16.6 (12.0–23.3)	6.9 (6.8–7.0)
Calcium	g/kg DM	2.7 (0.3–6.2)	75.6 (50.0–86.3)	4.7 (3.1–8.0)	36.3 (15.4–78.3)	3.9 (2.3–6.3)
Phosphorus	g/kg DM	7.8 (4.4–14.2)	9.0 (6.4–15.0)	16.0 (9.7–24.0)	25.9 (19.0–40.4)	6.9 (5.8–8.6)
Sodium	g/kg DM	0.9	1.3	5.2 (2.8–8.6)	10.0 (5.9–14.4)	0.1 (0.0–0.8)
Potassium	g/kg DM	8.9 (8.5–9.3)	6.9	5.7 (1.0–12.7)	10.2 (5.9–14.4)	23.7 (21.8–26.0)
Magnesium	g/kg DM	2.3 (2.0–2.8)	3.9	3.4 (0.7–11.5)	2.5 (1.6–3.1)	3.1 (2.4–3.6)
Gross energy	MJ/kg DM	26.2 (24.4–28.7)	22.8 (21.2–24.4)	21.7 (19.3–24.4)	21.4 (19.6–23.8)	19.9 (19.8–20.0)

^aData from Barroso et al. (2014), Feedpedia: <http://www.feedipedia.org>; Gasco et al. (2016), Marono et al. (2015), Sánchez-Muros et al. (2015), Siemianowska et al. (2013)

^bData from Diener et al. (2009), Feedpedia: <http://www.feedipedia.org>, Józefiak et al. (2016), Makkar et al. (2014), Maurer et al. (2016), Marono et al. (2015), Sánchez-Muros et al. (2015), Tschirner and Simon (2015)

^cData from Aniebo et al. (2008), Aniebo and Owen (2010), Barroso et al. (2014), Feedpedia: <http://www.feedipedia.org>, Fasakin et al. (2003), Józefiak et al. (2016), Makkar et al. (2014), Sanchez-Muros et al. (2015), Sogbesan et al. (2006), Tschirner and Simon (2015), Zuidhof et al. (2003)

^dData from: Feedpedia: <http://www.feedipedia.org>, Guillaume et al. (2001)

^eData from: Feedpedia: <http://www.feedipedia.org>, Guillaume et al. (2001)

Deficiencies in lysine or methionine (Barroso et al. 2014; Henry et al. 2015) are reported.

TM larvae meals evaluated so far have a protein content varying from 44 to 60%. The lipid fraction (about 16.6 to 43% DM) is characterized by high levels of oleic (42.18% fatty acid—FA), linoleic (24.70% FA) and palmitic (18.42% FA) acids. The low ash content (about 3.8% DM) is very interesting for the aquaculture sector even if TM larvae are usually low in calcium. Nevertheless, it has been highlighted that their calcium content can be modified through the rearing substrate (Anderson 2000; Klasing et al. 2000), increasing the level of this important mineral in meals.

The protein content of HI larvae meals evaluated in different research varied between 35.30 and 72.50% of DM. This high variation can be justified by the availability on the market of several different defatted HI meals. The processing and extraction of part of the lipid fraction from HI larvae generates protein meals having lipid content that varied from 3.4 to 38.6% of DM. The FA profile of HI meals used to be characterized by high values of lauric acid as, independently from the used substrate, larvae neo-synthesized and accumulated this FA (Sprangers et al. 2016). HI meals are rich in ash, calcium and phosphorus (Makkar et al. 2014; Tschirner and Simon 2015). MD larvae meals have a protein and lipid content ranging from 37.5 to 63.8% of DM and from 6.28 to 31.30% of DM, respectively (Aniebo and Owen 2010; Barroso et al. 2014; Józefiak et al. 2016; Makkar et al. 2014; Sánchez-Muros et al. 2014; Sogbesan et al. 2006; Zuidhof et al. 2003). The main reported FA are palmitic (32.37% FA), oleic (21.96% FA), linolenic (19.70% FA) and palmitoleic (17.10% FA) acids. The calcium content (about 4.7 g/kg DM) is higher than that of the TM larva but lower than that of HI (Makkar et al. 2014).

When compared to conventional protein sources (FM or soybean meal—SBM), the insect larvae protein content is lower than that of the FM (66–72%) but similar or higher than SBM (44–50%). With regard to the lipid content, insect larvae used to have higher values than conventional sources. Larvae contain between 8 and 36% of nitrogen-free extracts (sugars, starch, chitin and fibrous fractions) (Barroso et al. 2014).

As far as the lipid profile is concerned, insect larvae are poor in highly unsaturated fatty acids. The main difference between insect meals and FM is the content of eicosapentaenoic acid (EPA, C20:5 n3) and docosahexaenoic acid (DHA, C22:6 n3) which are present in the marine and freshwater products, but are absent in land-based products (including SBM) and insects. Usually, increasing levels of insect meal inclusion lead to a dramatic change of the FA profile in fish with a decrease in EPA and DHA and a decrease in the n3/n6 fatty acid ratio (Belforti et al. 2015; Gasco et al. 2016; St-Hilaire et al. 2007a, b). Nevertheless, it is possible to increase the insect unsaturated FA content manipulating the rearing substrate (Belforti et al. 2014; St-Hilaire et al. 2007a). In particular, while the fatty acids of HI pre-pupae reared on cow manure were high in saturated fatty acids (SFA) and very low in poly-unsaturated FA n3 (0.2%), when larvae were fed manure cattle enriched with fish by-products, this content increased up to 4% (St-Hilaire et al. 2007a).

Any change in the profile or the lipid composition of the diet directly affects the lipid-volatile component, then the aroma and flavour of fish (Turchini et al. 2007), and can therefore dramatically modify the perception of the product by the consumer.

The inclusion of insect meals could thus influence the sensory properties of fish products even if the information available, till now on this aspect, did not highlight any negative effect. For instance, the results of a panel test (aroma and texture) using fish (catfish and tilapia) fed chopped HI larvae alone or in combination with commercial diets indicated that fish were scored and ranked similarly with regard to control diets (Bondari and Sheppard 1981). In the same way, no significant differences were detected by panellists comparing fish fed with diets containing HI meals and fish fed with FM-based diets (Borgogno et al. 2017; Lock et al. 2016; Sealey et al. 2011a). The influence of dietary inclusion of insect meals on fillet or whole body composition (WBC) lead to controversial results. Some authors reported decreased values of DM and ether extract (EE) with the inclusion of insect meals (Belforti et al. 2015; Dong et al. 2013; Kroeckel et al. 2012; Ogunji et al. 2008a). Concerning crude protein (CP) content, only two trials reported changes (Belforti et al. 2015; Ng et al. 2001) while others did not find any influence of insect meal inclusion on this value (Gasco et al. 2016; Kroeckel et al. 2012; Ogunji et al. 2008a; Sealey et al. 2011a).

A problem often reported using insect meals in fish feeds is their high chitin content. Chitin is a primary component of the insect exoskeletons and it is considered as poorly digestible by fish, due to a reduced enzyme activity (Gasco et al. 2016; Henry et al. 2015; Rust 2002; Sánchez-Muros et al. 2014). The presence of chitinase, chitobiase and lysozyme has been reported in several species (Gasco et al. 2016; Henry et al. 2015). Nevertheless, because of the complex matrix in which chitin is encompassed, the enzyme activity seems to be limited, reducing thus the overall nutrient digestibility (Belforti et al. 2015; Henry et al. 2015; Sealey et al. 2011a).

Insect meals producers can reduce the content of chitin through extraction process (Belluco et al. 2013; Sánchez-Muros et al. 2014) or its digestibility can be increased through dietary enzyme inclusion (Henry et al. 2015) but these technologies are still not fully applied and studies need to be implemented. For instance, the inclusion of exogenous enzymes (carbohydrases or proteases) in diets for European sea bass did not improve the protein and fibre digestibility (Gasco et al. 2016).

It has to be underlined that low levels of chitin have been reported to have immune stimulants (Esteban et al. 2001; Henry et al. 2015, 2018; Hoffman et al. 1997; Lin et al. 2012), bacteriostatic (Vidanarachchi et al. 2010) or antifungal and antimicrobial properties (Faruck et al. 2016; Khoushab and Yamabhai 2010). Insects also contain antimicrobial peptides that have been proved to be active against Gram-positive and Gram-negative bacteria and to have antifungal properties. Great attention is then paid to their possible use as natural antibiotic or antifungal (Yi et al. 2014; Żyłowska et al. 2011).

Positive results on the fish immune status and resistance to diseases were observed with the supplementation of 2.5% of MD meal in black carp (*Mylopharyngodon piceus*) (Ming et al. 2013). Higher survival rates in fish fed with insect meals have been reported when compared to fish with fed other protein sources (Atse et al. 2014).

While several papers reported the possible negative effects of plant proteins on histology of liver and gastrointestinal tract (Oliva-Teles et al. 2015), very few information is available on the consequences of the dietary inclusion of insect meals. First investigations on these aspects are promising with no statistical differences detected for histology or morphometry parameters between fish fed with insect diets and control diets (Lock et al. 2016; Renna et al. 2017).

Results on the use of insect in aquaculture species are dramatically impacted by the type of used larvae, its condition (fresh or dried, whole, ground, defatted) or the method of nutrient isolation and processing (sun drying, thermal treatments, lipid extraction methodologies) and, of course, the fish species object of the experimentation.

As far as whole or cut, live or frozen larvae are concerned, they were mainly tested on warm water fishes and detailed results can be found in Henry and coworkers (Henry et al. 2015).

1.2.1 *Tenebrio Molitor*

Several experiments have shown that TM meal could be used in partial or total substitution of FM or other conventional protein sources. The level of TM meal inclusion ranged from 8 to 50% substituting up to 100% of FM. Performances results are unequal and bad performances are usually assigned to deficiency of some nutrients when high levels of inclusion were performed. In African catfish fingerlings, no significant differences were found up to 40% of FM substitution while a significant reduction in all parameters was observed when 60% or more of the FM component was replaced by TM (more than 26% of inclusion) (Ng et al. 2001). Roncarati and coworkers performed a pre-fattening trial substituting 50% of FM in common catfish (*Ameiurus melas*) fingerlings diets (Roncarati et al. 2015). Lower final body weight was found in fish fed with TM diet compared to control diet without TM even if results were still considered as acceptable.

Sánchez-Muros and coworkers investigated the nutritive value of a full-fat TM larvae meal as partial protein replacement of FM and SBM in Nile tilapia (*Oreochromis niloticus*) fingerlings (Sánchez-Muros et al. 2015). The dietary inclusion up to 430 g/kg of TM meal worsened performance parameters. Differences on feeding rate (FR), feed conversion rate (FCR), protein efficiency rate (PER) and specific growth rate (SGR) were observed with diets containing increasing levels of TM inclusion (Belforti et al. 2015). Recently, Gasco and colleagues evaluated the effects of dietary inclusion of a full-fat TM larvae meal on European sea bass (*Dicentrarchus labrax* L.) juveniles (Gasco et al. 2016).

Dietary TM inclusion level of 50% led to a worsening of final body weight, weight gain (WG), SGR and FR. Piccolo and coworkers found that TM larvae meal can replace FM up to 25% of inclusion in the diet for *Sparus aurata* without negative effects on weight gain, CP and ether extract digestibility, and marketable indexes after 163 days of feeding (Piccolo et al. 2017). On the contrary, when TM larvae meal was included at 50%, nutrients digestibility and dressed yield were penalized. Moreover in blackspot sea bream, the use of TM meal as an alternative dietary protein source did not show detrimental effects on fish growth performance even if its effects on fillet quality should be considered (Iaconisi et al. 2017).

1.2.2 *Hermetia Illucens*

Hermetia illucens larvae have been the subject of study for their exceptional ability to grow on organic waste, giving a value of greater sustainability to the obtained meal. As highlighted for TM, the meal preparation methods affect substantially the trial results. In general, the replacement of FM meal with HI meal is higher when a defatted meal is used.

On channel catfish (*Ictalurus punctatus*) decreased WG was observed using diets containing 10% of HI meal inclusion (Bondari and Sheppard 1987) in cage culture, while no statistical differences were reported (Newton et al. 2005) including up to 30% of HI prepupae meal in total FM and partial SBM substitution. Using dried full-fat prepupae meal in rainbow trout diets, St-Hilaire and coworkers were able to obtain inclusion levels of 15% without adverse effect on WG, feed intake (FI) and FCR (St-Hilaire et al. 2007b). Moreover, this diet allowed a 38% reduction in FO (i.e. from 13 to 8%). Highest levels of inclusion (30%) worsened all parameters. Later, Sealey and coworkers evaluated the growth and sensory parameters of trout fed diets having increasing levels (25 and 50%) of FM substituted with normal (NHI) or fish offal-enriched black soldier fly (EHI) prepupae meal (Sealey et al. 2011a). Growth of fish fed with the EHI diets was not significantly different from those fed with the FM-based control diet, while the growth of fish fed with the NHI diets was significantly reduced. The fatty acid profile was influenced by dietary treatments but fish fed with EHI highlighted good EPA and DHA contents. No differences were highlighted in a blind sensory comparison of fish fed with the FM control diet as compared to fish fed with the EHI or NHI diets.

On juvenile turbot (*Psetta maxima*), Kroeckel and coworkers tested partially defatted HI prepupae meal and found a general worsening of performances at the inclusion levels higher than 33% (Kroeckel et al. 2012). Moreover, authors found a decrease of FI with increasing HI meal incorporation, due to low palatability. The authors suggested that the presence of chitin might have influenced the FI, 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).

In Atlantic salmon, the FM replacement by two different HI larvae meals, varying in their protein and fat contents, led to controversial results (Lock et al. 2016).

A FM-based control diet and a vegetable protein-based diet were tested against two HI larvae diets (18.5 and 37.5 of HI inclusion in substitution of 25 and 50% of FM) for Siberian sturgeon (*Acipenser baerii*) juveniles (Gasco et al. 2017). Preliminary results indicated that the inclusion of HI significantly affected fish performances and condition factor. Generally, up to 25% of FM substitution, fish performed as well as FM- and vegetable protein-based diets.

Results from a trial using a partially defatted HI larvae meal as potential feed ingredient in rainbow trout diet showed that survival, growth performance, condition factor, somatic indexes and dorsal fillet physical quality parameters were not affected by diet (Renna et al. 2017). The use of HI larvae meal induced a decrease of valuable polyunsaturated fatty acids (PUFA) in trout dorsal fillet even if the differences were only reported at the highest level of HI inclusion. The HI worsened the lipids health indexes of the same muscle.

1.2.3 *Musca Domestica*

In Africa, sustainable local production of insects is conceivable considering the environmental conditions. Moreover, the rise of imported fish feed price has pushed the research for more sustainable source of protein for several livestock production systems, including fish (Makkar et al. 2014). Due to their ubiquitous nature and the short time needed from eggs to suitable larvae for feed purposes, MD have been extensively evaluated in fish diets, primarily with warm water fish species (Henry et al. 2015).

Dietary inclusion levels performed ranged from 7.5 to 100%. Even if performance results were not always positive, MD meals are considered to be sustainable and economically interesting (Adewolu et al. 2010; Aniebo et al. 2009; Fasakin et al. 2003; Makkar et al. 2014; Olele 2011; Sogbesan et al. 2006), as the cheapest and more easily accessible for farmers. Moreover, often maggots are reared on manure helping in the control of the nuisance dung (Sogbesan et al. 2006).

Results on fish are variable in relation to breeding conditions and larvae treatment. In particular, Fasakin and coworkers evaluated drying and processing methods (hydrolysed, defatted, full-fat, sun-dried and oven-dried maggots), on growth and utilization of African catfish (*Clarias gariepinus*) diets highlighting how these influenced the nutrient composition of obtained meal (Fasakin et al. 2003). The authors stated that fish performed better when fed with diets containing defatted MD larvae meals than full-fat MD larvae meal. In diets for *Clarias gariepinus* fingerlings, Idowu and coworkers substituted whole MD larvae meal to FM and SBM without noticeable differences up to 25% of MD inclusion with 50% of replacement (Idowu et al. 2003).

Positive results in terms of growth performances have also been reported (Sogbesan et al. 2006) in hybrid catfish diets, when the replacement of the FM using oven-dried maggot meal reached 25% (dietary inclusion level of 7.5%).

A whole MD larvae meal (oven-dried) has also been studied in the diet of rainbow trout but with little success, as an inclusion of 9.2% (25% of FM substitution) resulted in a decrease of production parameters and to a deterioration in fish quality (lipid FA profile) (St-Hilaire et al. 2007b).

The fish performance, the concentration of plasma glucose, the cortisol and blood characteristics of *Oreochromis niloticus* fingerlings fed with increasing levels of MD meal in diets were evaluated (Ogunji et al. 2008b). The inclusion of MD larvae meal in the diet did not impair fish growth and performance. At the same time, no adverse or stress effect on the haematology and homeostasis was observed. Moreover, no significant difference was observed in liver glycogen reserve and in hepatic catalase, activity did not differ significantly. The authors reported elevated glutathione S-transferases activities when fish received higher dietary magmeal concentration.

Adewolu and colleagues evaluated an animal protein mixture containing MD larva meal in diets for *Clarias gariepinus* fingerlings (Adewolu et al. 2010). The inclusion up to 50% of animal protein mixture did not influence the performances parameters. However, in fish fed with highest FM substitution levels, these indicators were significantly lower.

Dong and coworkers investigated the effect of dietary supplementation with MD maggot meal or SBM on the growth performance and antioxidant responses of gibel carp and dark barbel catfish (Dong et al. 2013). Interestingly, even if MD inclusion lead to a worsening of some performances when compared to the control diet, fish fed with MD diets exhibited equal or better results compared to fish fed with SBM diets. Moreover, the MD supplementation enhanced the antioxidant capacity in gibel carp. Positive results have also been reported (Ming et al. 2013) on the black carp, where the replacement of the FP has reached 25%.

Feeding African catfish (*Heterobranchus longifilis*) larvae with non-isonitrogenous nor isoenergetics diets containing different protein sources, Atse and coworkers reported similar or better performances in fish fed with MD diets if compared to fish fed with *Artemia salina* or fish by-products diets even if the CP was lower (Atse et al. 2014). If compared to other protein sources based diets, (blood, brain or SBM) MD always showed better results. When diets were fortified with minerals, vitamins and amino acids premix, MD-fed fish reported the highest performances. The survival rate was also always higher in fish fed with MD compared to other groups.

Recently, Lin and colleagues reported that MD meal can be included up to 30% without negative effects on growth and feed utilization of barramundi and no major influence on body composition (Lin and Mui 2016). When oxidative status and immune responses are considered, the inclusion rate of 10% corresponding to a FM substitution of about 25% is recommended.

1.3 Poultry by-Product Meals

Poultry by-product meal (PBM) is a high protein source commonly used in domestic animal feeds. The Association of American Feed Control Officials defines PBM as the '*ground, rendered, clean parts of the carcass of slaughtered poultry such as necks, heads, feet, undeveloped eggs, gizzards and intestines (provided their content is removed), exclusive of feathers (except in such amounts as might occur unavoidably in good processing practices)*' (AAFCO 2010).

In recent years, mainly two PBMs are available in the market: feed grade and pet food grade. The former, less expensive, is usually considered as produced from low-quality by-product fractions and contains a higher level of ash and lower protein content (Aldrich 2006; Dozier and Dale 2005). The latter, due to its high price and quality, is mostly used in pet foods.

Poultry by-product meal quality and nutritional value (Tables 1.4 and 1.5) can change from one batch to another depending on the included materials and on the processing (time and temperature of the cooking process) applied for the production (Cruz-Suárez et al. 2007; Dale et al. 1993).

As for all the rendering processes, the PBM production involves the application of heat, the extraction of moisture and the separation of fat (Cruz-Suárez et al. 2007; Meeker and Hamilton 2006). The applied rendering process enables the destruction of pathogenic microorganisms and provided aseptic protein product free of potential biohazards and environmental threats (Hamilton et al. 2006). Technological developments in the production process have significantly improved the quality of PBM (Badillo Zapata et al. 2016; Cruz-Suárez et al. 2007; Sealey et al. 2011b). Nowadays, due to its high nutritional quality, large availability and palatability, PBM has considerable potential as feed ingredient for aquaculture providing substantial feed cost saving (Hernández et al. 2010).

The PBM protein content ranges from 51.6 to 81 (% DM); despite a relatively good amino acid profile, as far as fish nutrition is concerned, lysine and methionine are often reported as the first limiting EAA (Castillo-Lopez et al. 2016; Hertrampf and Piedad-Pascual 2000; Nengas et al. 1999; Rawles et al. 2006; Riche 2015; Rossi and Davis 2012). Its average gross energy content is similar to the one shown by insect meals and varies between 16 and 25 MJ/kg DM. The crude fibre content is very low (about 1%) while the level of ash varies between 5 and 30%. As far as fat content is concerned, publications reported values from 6.7 to 22.5%. PBM has very low content in precious n-3 FA (EPA, DHA) (NRC 1993; Sealey et al. 2011b). This can cause problems at high levels of inclusion, especially in juveniles or marine species. Particular attention during the diet formulation and the inclusion of appropriate quantities of FO in diets can overcome the problem but increase the feed price (Sealey et al. 2011b).

As far as ADC of nutrients is concerned, the majority of the trials were carried out with salmonids. The first one was that of Cho and Slinger who found quite low CP digestibility (about 70%) in rainbow trout (Cho and Slinger 1979). Research performed over the successive years showed different results (Bureau et al. 1999;

Table 1.4 Nutrient composition and nutritive value of poultry by-product meal (PBM) compared to fishmeal (FM) and soybean meal (SBM). Values are reported as mean of values found in the cited references (with minimum and maximum values). The following chemical data are shown here: dry matter (DM), crude protein and essential amino acids, ether extract and fibre

	Unit	PBM ^a	FM ^b	SBM ^c
Dry Matter (DM)	% as fed	93.7 (82.4–97.4)	92.1 (90.0–94.4)	87.9 (85.0–92.1)
Crude protein	% DM	66.1 (51.6–81.0)	75.6 (70.2–80.7)	51.4 (48.3–54.5)
Lysine	% protein	4.4 (3.3–8.2)	6.1 (5.5–7.5)	6.1 (5.7–6.6)
Methionine	% protein	1.4 (1.0–2.0)	2.2 (2.0–2.6)	1.4 (1.2–1.6)
Methionine + Cystine	% protein	–	2.9 (2.6–3.2)	2.9 (2.5–3.3)
Tryptophan	% protein	0.5 (0–0.8)	0.8 (0.7–0.9)	1.3 (1.2–1.4)
Threonine	% protein	2.8 (1.9–3.9)	3.1 (2.9–4.3)	3.9 (3.5–4.3)
Leucine	% protein	5.0 (3.9–9.7)	5.9 (5.2–7.3)	7.5 (6.8–8.0)
Isoleucine	% protein	2.7 (1.8–4.7)	3.7 (3.3–4.4)	4.6 (4.3–5.0)
Valine	% protein	3.1 (2.2–5.2)	4.2 (3.9–4.8)	4.8 (4.3–5.4)
Histidine	% protein	1.9 (1.2–5.6)	1.8 (1.7–1.9)	2.6 (2.4–2.9)
Arginine	% protein	5.1 (3.2–8.8)	4.6 (4.0–6.0)	7.4 (6.8–8.1)
Phenylalanine	% protein	2.8 (2.2–4.0)	5.5 (5.2–6.5)	8.5 (7.7–9.4)
Ether extract	% DM	13.8 (6.7–22.5)	8.1 (2.0–12.0)	2.1 (2.0–2.2)
Crude fibre	% DM	1.1 (0.5–2.1)	–	6.7 (3.5–10.1)

^aData from: Barreto-Curiel et al. (2016), Bureau et al. (1999), Castillo-Lopez et al. (2016), Cheng and Hardy (2002), Cruz-Suárez et al. (2007), de Carvalho et al. (2016), Dozier and Dale (2005), El-Haroun et al. (2009), Fasakin et al. (2005), Feedpedia: <http://www.feedipedia.org>, Goda et al. (2007), Guimarães et al. (2008), Hernandez et al. (2010), Hernandez et al. (2014), Li et al. (2009), Ma and Wang (2014), Nengas et al. (1999), Riche (2015), Sealey et al. (2011b), Shapawi et al. (2007), Subhadra et al. (2006), Sugiura et al. (1998), Wang et al. (2006), Wang et al. (2016), Yang et al. (2004)

^bData from: Feedpedia: <http://www.feedipedia.org>, Guillame et al. (2001)

^cData from: Feedpedia: <http://www.feedipedia.org>, Guillame et al. (2001)

Cheng and Hardy 2002; Dong et al. 1993; Guimarães et al. 2008; Hernández et al. 2010; Pfeffer et al. 1995; Sugiura et al. 1998). The ADC improvement obtained over the years reflects the improvement of PBM processing methodologies, but also supported the hypothesis that nutrients ADC is highly dependent on the PBM origin, quality and the faecal collection methodology.

Several researches have shown the high potential of PBM in aquaculture feeds. The dietary inclusion level, as well as the FM or other conventional protein sources substitution, varies among fish species mainly due to the PBM quality and the diet formulation. Reduced performances with high levels of FM substitution are often associated with decreased protein digestibility or deficit in EAA or essential FA (Badillo Zapata et al. 2016; Gaylord and Rawles 2005; Parés-Sierra et al. 2014; Shapawi et al. 2007). Furthermore, increased attention has recently been paid to the lower taurine content of PBM compared to FM. Historically, taurine has not been considered as an EAA (El-Sayed 2014; Salze and Davis 2015). Nevertheless, recently it has been demonstrated that the ability to synthesize taurine widely varies

Table 1.5 Nutrient composition and nutritive value of poultry by-product meal (PBM) compared to fishmeal (FM) and soybean meal (SBM). Values are reported as mean of values found in the cited references (with minimum and maximum values). The following data are shown here: minerals (ash), calcium, phosphorus, sodium, potassium and gross energy

	Unit	PBM ^a	FM ^b	SBM ^c
Minerals (ash)	% DM	15.0 (5.1–29.7)	16.6 (12.0–23.3)	6.9 (6.8–7.0)
Calcium	% DM	5.1 (2.2–9.9)	36.3 (15.4–78.3)	3.9 (2.3–6.3)
Phosphorus	% DM	2.7 (1.6–5.0)	25.9 (19.0–40.4)	6.9 (5.8–8.6)
Sodium	% DM	0.6 (0.5–1.0)	10.0 (5.9–14.4)	0.1 (0.0–0.8)
Potassium	% DM	0.8 (0.4–1.8)	10.2 (5.9–14.4)	23.7(21.8–26.0)
Gross energy	kJ/kg DM	21.2 (16.2–24.9)	21.4 (19.6–23.8)	19.9 (19.8–20.0)

^aData from: Barreto-Curiel et al. (2016), Bureau et al. (1999), Castillo-Lopez et al. (2016), Cheng and Hardy (2002), Cruz-Suárez et al. (2007), de Carvalho et al. (2016), Dozier and Dale (2005), El-Haroun et al. (2009), Fasakin et al. (2005), Feedpedia: <http://www.feedipedia.org>; Goda et al. (2007), Guimarães et al. (2008), Hernandez et al. (2010), Hernandez et al. (2014), Li et al. (2009), Ma and Wang (2014), Nengas et al. (1999), Riche (2015), Sealey et al. (2011b), Shapawi et al. (2007), Subhadra et al. (2006), Sugiura et al. (1998), Wang et al. (2006), Wang et al. (2015), Wang et al. (2016), Yang et al. (2004)

^bData from Feedpedia: <http://www.feedipedia.org>, Guillame et al. (2001)

^cData from Feedpedia: <http://www.feedipedia.org>, Guillame et al. (2001)

among fish species because of the different activities of key enzymes in its biosynthesis pathway (El-Sayed 2014; Rossi and Davis 2012; Salze and Davis 2015).

High levels of FM replacement could lead to decrease the taurine content beyond a limit level causing decrease in performances (Rossi and Davis 2012). The dietary supplementation with taurine could allow highest levels of FM substitution, in particular, when plant-based proteins are also present in the diets formulation (Chatzifotis et al. 2008; Rossi and Davis 2012; Salze and Davis 2015).

The minimum level of FM needed in fish fed with PMB to support good performances is species dependent (Table 1.6). Rossi and Davis showed that 5% of FM is sufficient in Florida pompano when 15% of PBM is used (Rossi and Davis 2012). Compared to a control diet containing 35% FM, 10% of PBM, 15% of SBM and 10% of rapeseed meal, Ma and Wang found reduced performances already at 40% of FM substitution (further 15% of PMB dietary addition) in a trial performed with Golden pompano (*Trachinotus ovatus*) juveniles suggesting that levels higher than 21% of FM are needed (Ma and Wang 2014). Using a locally produced PBM (29.2% of inclusion), Nengas and colleagues reduced the level of FM up to 35% in *Sparus aurata* diets with slight (but not statistical) performances reduction (Nengas et al. 1999). Lower levels of FM requirements were found for Japanese sea bass (8%) (Wang et al. 2015), red drum (10%) (Kureshy et al. 2000), Malabar grouper (25% and 13% reported by Wang and coworkers and Li and colleagues, respectively, Li et al. 2009; Wang et al. 2008), and cuneate drum (18%) (Wang et al. 2006). Badillo-Zapata and colleagues found that the total replacement of FM with PBM in diets for *Totoaba macdonaldi* juveniles led to worsened performances and

Table 1.6 Maximum level of FM substitution (and PBM inclusion) with PBM reached without impairing any of the performance parameter evaluated

Fish Species	Max level of FM substitution	% PBM inclusion	% FM in control diet	Other protein source	CP and CL diet content	Observation	Reference
<i>Sparus aurata</i>	50	29.2	72.9	–	45–13	Locally produced PBM	Nengas et al. (1999)
<i>Morone chrysops</i> x <i>M. saxatilis</i>	100	30	30	SBM (30)	40–6	Decreased (but not statistically different) performances	Webster et al. (2000)
<i>Carassius auratus gibelio</i>	15	10.8	46.3	–			Yang et al. (2004)
<i>Oreochromis niloticus</i> x <i>O. mossambicus</i>	66	30.3	44	–		Best productivity values (WG, FCR, SGR, PER) but not statistical differences vs FM diet	Fasakin et al. (2005)
<i>Scophthalmus maeoticus</i>	25	21.2	77.3	–	55–15		Turker et al. (2005)
<i>M. chrysops</i> x <i>M. saxatilis</i>	35	17.2	25	PBM (7.73) SBM (25.9)	42.5–11	Further 9.47% PBM inclusion	Rawles et al. (2006)
<i>D. labrax</i>	50	17.5	35	BM (3)	42.5–12.5		Wang et al. (2006)
<i>Carassius auratus gibelio</i>	100	53	53	–	38–9.5	The optimal replacement level of FM by PBM was estimated by second-order polynomial regression to be 66.5% in protein	Yang et al. (2006)
<i>Psetta maeotica</i>	50	43.2	77.3	–			Yigit et al. (2006)
<i>Clarias gariepinus</i>	100	34.5	25	–	25.5–8.5	Trial performed comparing also other protein sources as FM substitute	Goda et al. (2007)
<i>Cromileptes altivelis</i>	50 and 100	36.1 and 74	69.4	–		Pet food grade PBM enable 100% FM replacement while feed-graded PBM allowed only 50% replacement	Shapawi et al. (2007)

(continued)

Table 1.6 (continued)

Fish Species	Max level of FM substitution	% PBM inclusion	% FM in control diet	Other protein source	CP and CL diet content	Observation	Reference
<i>Epinephelus malabaricus</i>	50	11.5	50	–	53–9		Wang et al. (2008)
<i>Oncorhynchus mykiss</i>	30	30	40	CGM (28) BM (6–12)	49–23.5	PBM partially replaced FM and CGM (total replacement of BM)	El-Haroun et al. (2009)
<i>Epinephelus malabaricus</i>	50	22.8	50	BM (5.7)	53–9.6	Fish fed with the 75% PBM diet had a significantly lower FI than fish fed with FM or 25% PBM diets	Li et al. (2009)
<i>O. niloticus</i>	100	26.5	27	SBM (12) CGM (9)	35.5–6.8	High quality PBM	Hernández et al. (2010)
<i>O. mykiss</i>	100	63.8	68.6	–	48.3–18.7	High quality PBM	Sealey et al. (2011b)
<i>Rachycentron canadum</i>	60	30	50	SBM (11.3)	45–11	Improved PER and FER in fish fed with 15 and 22.65 PBM inclusion. A quadratic regression indicated at 30.75% FM the optimal replacement level for PER value.	Zhou et al. (2011)
<i>Trochinos carolinus</i>	67	9.8	15	SBM (50)	40–8	Fish growth performance reduced when FM was completely removed from the diets.	Rossi and Davis (2012)
<i>Oncorhynchus mykiss</i>	100	59	66	–	43–12.5	Fish fed with the 67% PBM diet had a significantly higher FBW than the rest of the treatments, whereas the 100% PBM had a significantly lower FBW than 67% PBM	Badillo et al. (2014)
<i>Lutjanus guttatus</i>	65	39.4	52.6	SQ (6) K (7.6)	51.5–16	Fish fed with the 87% PBM showed worsened (not always stat. different) values	Hernández et al. (2014)

(continued)

Table 1.6 (continued)

Fish Species	Max level of FM substitution	% PBM inclusion	% FM in control diet	Other protein source	CP and CL diet content	Observation	Reference
<i>O. mykiss</i>	73.5	44	66	–	43.5–12.5		Parés-Sierra et al. (2014)
<i>Trochinosus carolinus</i>	67	from 19.8 to 22.3	32.6	–	48.5–21	Five different types of PBM. FI reduced only with CC66 PBM type	Riche (2015)
<i>Lateolabrax japonicus</i>	80	38.7	40	SBM (20) RSM (8)	47.5–11.5	Improved performances up to 80 substitution vs FM diets	Wang et al. (2015)
<i>O. niloticus</i>	100	26	20	SBM (15) CGM (11)	30.2–11.5		Yones and Mewalli (2015)
<i>Totoaba macdonaldi</i>	67	45	65	–	51–8		Badillo-Zapata et al. (2016)
<i>Dicentrarchus labrax</i>	60	18.94	47.4	SBM (11) CG (11)	48–14		Srour et al. (2016)

FM: fishmeal; PBM: Poultry by-product meal; CGM: Corn gluten meal
 FM: Fishmeal; PBM: Poultry by-product meal; CGM: corn gluten meal; BM: blood meal; SQ: Squid meal; K: Krill meal; RSM: Rapessed meal; CG: Corn gelatin
 WG: Weight gain; FI: Feed intake; FR: feeding rate; PER: Protein efficiency ratio; FCR: Feed conversion ratio; IBW: Initial body weight (g); FBW: Final body weight (g); FER: Feed efficiency ratio
 CP: crude protein; CL: crude lipid

increased mortality and that PBM can be used in up to maximum 67% FM replacement (Badillo-Zapata et al. 2016).

Wang and coworkers obtained 80% of FM replacement with diets containing also other protein sources (SBM, rapeseed meal) showing a high capacity of *Lateolabrax japonicus* in utilizing PBP nutrients (Wang et al. 2015). Recently, it was theorized that low performances obtained with high levels of FM substitution by PBM could be due to Selenium deficiency as the content of this essential nutrient is lower in PBM than in FM (Wang et al. 2016). Webster and colleagues indicated that diets without FM can be fed to juveniles sunshine bass without major negative effects on performances (Webster et al. 2000). Similarly, Sealey and coworkers, using a high-quality PBM, were able to substitute completely FM in rainbow trout diets (Sealey et al. 2011b). In addition, Barreto-Curiel and colleagues positively replaced 100% of FM in rainbow trout using a PBM mixed with acid fish silage (Barreto-Curiel et al. 2016).

As far as WBC is concerned, modifications can occur when alternative protein meals are used as FM replacers (Gatlin et al. 2007). The major effects of PBM inclusion on WBC have been reported for the CP and crude lipid content. Goda and colleagues found a reduction of the CP content in fish fed with 100% PBM as FM replacer and these results were supported by the findings of Zhou and coworkers (Goda et al. 2007; Zhou et al. 2011). Conversely, slightly higher CP values were found in fish fed with PBM than those fed with FM-based control diet (Shapawi et al. 2007; Yang et al. 2006). An increase of crude lipid WBC content was reported in rainbow (Alexis et al. 1985; Steffens 1994), in European eel (Gallagher and Degani 1988), in chinook salmon (Fowler 1991), in gilthead seabream (Nengas et al. 1999), in Nile tilapia fingerlings (Hernandez et al. 2010) and in spotted rose snapper (Hernandez et al. 2014).

The whole body fat increases in fish fed with diets having PBM were often justified by high fat content in PBM. Nevertheless, several research reported no differences in WBC even at high levels of PBM inclusion (El-Haroun et al. 2009; Ma and Wang 2014; Riche 2015; Yones and Metwalli 2015). These contradictory results support the theory that differences in utilization and transformation capacity for PBM exist among fish species, and are related to the quality and quantity of PBM used in diets formulation.

PBM has been widely studied and the improvement of quality due to better processing technologies allows high levels of FM replacement. This may allow reduction of feed cost formulation and increase in profitability. Nevertheless, as the technological process of PAP production was revised (EC No. 94/449; temperature over 133 °C, pressure, 3 bar by steam for 20 min; maximum particle size, 50 mm), this could compromise nutritional quality and modify the reference values obtained so far. Therefore, it is necessary to further evaluate these ingredients and research is highly needed (Moutinho et al. 2017).

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