

# Substitution of Fishmeal with Black Soldier Fly *Hermetia illucens* Linnaeus, 1758 Larvae in Finfish Aquaculture – A Review

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## Abstract

The reliance of aquaculture feed industry on fishmeal has been challenged by ecological, economic, and ethical limitations leading fish nutritionists to look for viable alternatives, and conventional animal-based protein sources showed varying degrees of success in this regard. However, a non-conventional protein source, black soldier fly *Hermetia illucens* Linnaeus, 1758 larvae (BSFL), received due attention as a focal point in fish nutrition research. Though many studies were conducted on *H. illucens* as a potential protein source for fish, a comprehensive review is not available. Therefore, this article aims to review the existing literature on the use of BSFL as a fishmeal replacer in aquaculture. The nutrient composition of BSFL varied with the developmental stage and the composition of growing medium. High crude protein levels (40.4–56.2 %) and high crude fat levels (4.8–24.8 %) made BSFL suitable for high protein and high energy diet. Early prepupa was the best stage for harvesting, considering its high crude protein content and dry matter yield and less chitin content. Up to 100 % inclusion levels of BSFL meal were tested in a wide range of fish species. Most studies revealed that up to 50 % of fishmeal replacement would be possible without negative effects on the fish. Notable adverse effects were found beyond 50 % of fishmeal replacement, mainly due to high chitin levels in the pupal stage and high crude fat levels. Harvesting BSFL before the pupal stage and de-fattening made it possible to replace 100 % of fishmeal without adverse effects on fish.

**Keywords:** growth, fish diet, fish farming, fish feed

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## Introduction

Aquaculture nutrition has long been plagued by its reliance on fishmeal as a major protein source. Increasing demand for aqua-feeds and the limited supply of fishmeal with its inherited problems of ecological, ethical, technical, and economic roots have rendered it unsustainable for use in aquaculture (Rumsey, 1993; Kudi et al., 2008; Li et al., 2015). With the choice of protein source making a significant impact on the growth and development of the fish, as well as the cost of production (Tacon and Metian, 2008; Asche et al., 2012; Barroso et al., 2014), a viable alternative to replacing fishmeal without compensating its advantages would be a priced finding. Plant-based protein sources gained substantial research interest due to low cost and unlimited availability, yet, had proven incompatibilities

due to unbalanced amino acid profiles mismatching with fish requirements and, presence of anti-nutritive factors (Gomes et al., 1995; Francoise and Sadasivam, 2009; Rimoldi et al., 2014; Caimi et al., 2020). Alternatively, animal-based proteins have shown more promise (Francoise and Sadasivam, 2009; Rimoldi et al., 2019), and among them, dipteran larvae meals received due attention. Black soldier fly *Hermetia illucens* Linnaeus, 1758 is one such dipteran whose larval meals have been well tested as a fishmeal replacer in many studies. With its high protein content and balanced amino acid profile suitable for fish, supported by the fly larvae to be used as a source of bio-degrading wastes (Diener et al., 2011; Huis et al., 2013), BSFL have pushed fish nutrition research into a new dimension. Even with such potential, however, comprehensive reviews of the existing literature on using BSFL as a source of fishmeal replacer in

aquaculture feeds are so far unavailable. The present review was, therefore, an effort to fill that gap.

## Taxonomy and Life Cycle of *Hermetia illucens*

*Hermetia illucens* (Diptera: Stratiomyidae) is an insect commonly known as black soldier fly (BSF), has an external morphology similar to the wasps. BSF has two wings and does not possess any stinging structures (Díclaro II and Kaufman, 2009). Its natural distribution includes Asia, Europe and the southeastern United States. Subsequently, their distribution expanded to other areas and today, they are predominantly found in equatorial tropics (Liu et al., 2017). The life stages of BSF are shown in Figure 1.

The life cycle of the BSF extends from weeks to months, depending on the temperature and the quality and quantity of the diet (Veldkamp et al., 2012). After successful mating, female deposits clusters of eggs on the edges of containers near waste dumps and decaying organic matter (Tomberlin and Sheppard, 2001). Eggs normally hatch within 4–10 days (Mullen and Durden, 2002). Newly hatched BSFL are creamy white, gradually turning into brownish and becoming completely black during the prepupal stage within two weeks (Gennard, 2012). At the prepupal stage, larvae cease feeding and migrate towards dry and dark places to prepare for the oncoming pupal stage of 14–16 days (Hardouin and Mahoux, 2003).

### Nutrient content of black soldier fly larvae

The composition of the black soldier fly larvae (BSFL) largely depends on the composition of the substrate

(Spranghers et al., 2017). The dry matter (DM) content of BSF larvae gradually increases from day-old larvae to the prepupal stage to an average of 35–45 %, which remains stable throughout the pupal stage and drops to 20–25 % when the larvae reach the adult stage (Newton et al., 2005; Liu et al., 2017; Gangadhar et al., 2018). The crude protein (CP) content of BSFL exceptionally varies throughout the life cycle and remains around 40 % during the larval stage (Li et al., 2015; Gangadhar et al., 2018). Body crude fat (CF) content also fluctuates depending on the nature of the diet consumed (Gangadhar et al., 2018). However, from day-old larvae to prepupal stage, the CF value remains at an average of 24–28 % (Li et al., 2015). A variety of essential amino acids and fatty acids are synthesised in every growth stage and the amounts are much higher in the early larval stages (Li et al., 2015). Significantly higher calcium and phosphorus levels (3000 mg.100 g<sup>-1</sup> and 620 mg.100 g<sup>-1</sup>, respectively) are also found in larval stages compared to adults. Furthermore, chitin content gradually increases with age and becomes much higher when it develops to the prepupal stage (Bosch et al., 2014; Maurer et al., 2015). Moreover, other minerals such as sodium, iron and zinc levels are 100 mg, 200 mg and 60 mg.100 g<sup>-1</sup>, respectively. BSFL are also rich in vitamins, especially vitamin E (7 mg.100 g<sup>-1</sup>) (Liu et al., 2017; Jansen, 2018).

### Changes in body composition of BSFL throughout the life cycle

Owing to drastic changes in life stages during a short period, the body composition of BSFL also varies during each phase. A study by Liu et al. (2017) described the use of commercial broiler chicken feed as a growth substrate for BSFL to show the dynamic changes in their body composition. Percentage CP



Fig. 1. Life stages of black soldier fly *Hermetia illucens*, A: Adult; B: Eggs; C: Larvae; D: Pupae.

levels in BSFL ranged between 38.4 to 56.5 % where the highest levels were in one day old larvae, and the least was in 12 days old larvae (Fig. 2). The CP content of individual larva shows a gradual increasing trend, where the lowest content was in the one-day-old early larval stage (0.006 mg per larva), and the highest (28.228 mg per larva) was in the early pupa stage. The CP levels decreased at late pupa (25.798 mg) and adult (15.149 mg per individual) stages. Considering percentage CP levels and biomass, it is likely to have the best output at the mature larval stage (14<sup>th</sup> day of development) and early-prepupa.

Variations in CF content have also been reported during the life cycle. Decreasing CF content from 16 % to 5 % was reported during the development of eggs to one-day-old larvae (Fig. 2). An increase in CF content was observed during certain stages of larval development, followed by a plateauing trend at the late prepupal stage. Increased levels of CF were reported in adult flies. Evidence suggests that mature larval stages and early prepupal stages contain the highest CF contents of 18.091 mg and 18.760 mg, respectively (Liu et al., 2017). The decline in CF content was reported during later larval stages. Adult phases showed an increase in CF content, ensuring fulfilment of the energy demand for reproduction of the species.

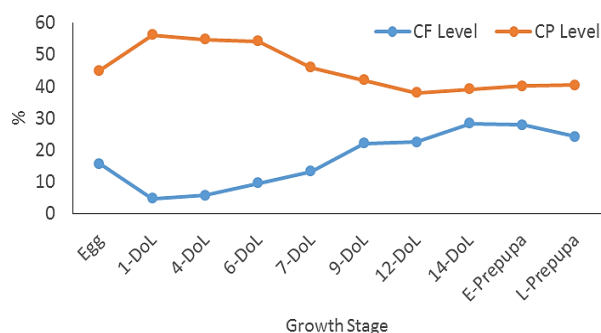


Fig. 2. Changes in crude protein (CP) and crude fat (CF) percentages of *Hermetia illucens* larvae throughout the life cycle. CP level increased in one-day old larvae. From the 7<sup>th</sup> day of larval development, a reduction in CP content was observed until 12<sup>th</sup> day, followed by less variation in the next development stages. A decreasing trend of CF content was reported during early larval development, followed by a steady increase up to 22 % on the 9<sup>th</sup> day. The value remained around 28 % until late-prepupa stage and reduced to 8 % at early-pupa stage. However, the CF level increased up to 30–32 % during the adult stage. DoL stands for day old larvae. Source: Liu et al. (2017).

The dry biomass increased to a range of 65–70 mg at the early prepupa stage. From the fourth day onwards, there is an increase of biomass along with the larval development, where a rapid increment was observed between day 9 and day 12. Afterwards, a decline in dry biomass was noticed until death.

However, biomass accumulation was about 4000-fold in 14–16 days.

The early prepupa stage was revealed as one of the critical stages in the life cycle of BSF due to high CP content and dry matter (Spranghers et al., 2017; Gangadhar et al., 2018; Cardinaletti et al., 2019). The cuticle begins to develop beyond the early prepupae stage, and this growth stage was found to be ideal for harvesting and used for the preparation of aquaculture feed.

### Effect of growth substrate on the body composition of BSFL

Several studies were conducted to determine the effect of different growth substrates on the body composition of BSFL. According to Liu et al. (2017), dynamic changes in the nutrient composition were observed in BSF grown in broiler chicken feed. CP content was 38 % in 12-day old larvae. The mean value for CP was at 43 % in the prepupa stage. CF content increased during larval development and was reported to remain at a range of 22–28 %. Spranghers et al. (2017) studied the effect of different growth substrates such as chicken feed, vegetable waste and restaurant waste on BSFL body composition. The results showed that CP content was higher in larvae grown on restaurant waste (43 %) and lowest in vegetable waste (39 %). CF content was highest in restaurant waste and vegetable waste-based growth substrates (36–38 %). However, the chitin content of BSFL was between 5.7 % and 6.7 %. According to Jucker et al. (2017), CP content in BSFL grown on fruits, vegetables and the fruit-vegetable mixture was 12–18 %. The CF content was reported to be 21 % in larvae grown on the fruit-based substrate and 3 % in larvae grown on vegetables. The ash content of larvae was 4 % in fruit and vegetable-based substrates. Furthermore, Newton et al. (2005) reported that CP content in larvae grown in poultry and swine manure was 43 %. However, variations in CF content were reported among substrates (35 % in poultry manure and 27 % in swine manure). The ash content in both fruit-based and vegetable-based substrates was 16 %. Accordingly, it was evident that the growth substrate has a direct impact on the proximate composition of BSFL. The selection of growth substrate depends on the purpose of rearing BSFL, such as rearing BSFL on substrates based on restaurant waste, can be beneficial for biodiesel production. Similarly, substrates based on poultry and swine manure can be ideal for harvesting larvae with higher CP content for animal feed production.

### Incorporation of BSFL in aquaculture feeds

Many studies have been conducted on the inclusion of BSFL in finfish aquaculture (Table 1). Different inclusion levels of BSFL have been observed in

Table 1. Effect of different inclusion rates of black soldier fly larval (BSFL) meal on different fish species.

Fish species	BSFL inclusion rate (fishmeal replacement)	Effect	Reference
African catfish <i>Clarias gariepinus</i> (Burchell, 1822) fingerling	Meal, up to 30 %	No negative effects on growth performances until 20 % inclusion	Talamuk (2016)
Atlantic salmon <i>Salmo salar</i> Linnaeus, 1758 post-smolt, pre-smolt	Meal, up to 100 %	No negative effects on growth performances until 85 % inclusion. Altered fatty acid profile and increased dry matter content	Lock et al. (2016); Belghit et al. (2018; 2019b; 2019a); Li et al. (2019); Stenberg et al. (2019)
Barramundi <i>Lates calcarifer</i> (Bloch, 1790) juvenile	Meal, up to 100 %	No negative effects on growth performances until 50 % inclusion. Altered essential amino acid content	Katya et al. (2017)
Blue tilapia <i>Oreochromis aureus</i> (Steindachner, 1864) fingerling	Chopped and frozen larvae, up to 100 %	No negative effects on growth performances until 100 % replacement	Bondari and Sheppard (1981)
Channel catfish <i>Ictalurus punctatus</i> (Rafinesque, 1818) broodstock	Meal, up to 100 %	No negative effects on growth performances until 100 % replacement	Bondari and Sheppard (1981)
European seabass <i>Dicentrarchus labrax</i> (Linnaeus, 1758) juvenile	Meal, up to 45 %	No negative effects on growth performances until 45 % inclusion	Sánchez-Muros et al. (2014); López (2015); Magalhães et al. (2017); Abdel-Tawwab et al. (2020)
Grouper-hybrid <i>Epinephelus fuscoguttatus</i> (Forsskål, 1775) ♀ x <i>Epinephelus lanceolatus</i> ♂ (Bloch, 1790) juvenile	Meal, up to 30 %	No negative effects on growth performances until 30 % inclusion	Zulkifli et al. (2019)
Hong Kong catfish <i>Clarias fuscus</i> (Lacepède, 1803) juvenile	Meal, up to 100 %	No negative effects on growth performances until 66 % inclusion	Stankus (2013)
Japanese seabass <i>Lateolabrax japonicus</i> (Cuvier, 1828) juvenile	Meal, up to 64 %	No negative effects on growth performances until 64 % inclusion	Wang et al. (2019)
Jian carp <i>Cyprinus carpio</i> Linnaeus, 1758 fry, juvenile	Meal, up to 100 %	No negative effects on growth performances until 100 % replacement. Elevated saturated fatty acid content	Li et al. (2017); Zhou et al. (2018)
Nile Tilapia <i>Oreochromis niloticus</i> (Linnaeus, 1758) fry, fingerling	Meal and whole larvae (dried), up to 100 %	No negative effects on growth performances until 50 % inclusion. Body crude fat content and saturated fatty acid contents increased	Rana et al. (2015); Muin et al. (2017); Teye-Gaga (2017); Devic et al. (2018)

Table 1. Continued.

Fish species	BSFL inclusion rate (fishmeal replacement)	Effect	Reference
Rainbow trout <i>Oncorhynchus mykiss</i> (Walbaum, 1792) fry, juvenile	Meal, up to 75 %	No negative effects on growth performances until 30 % inclusion	St-Hilaire et al. (2007); Sealey et al. (2011); Stamer et al. (2014); Borgogno et al. (2017); Renna et al. (2017); Bruni et al. (2018); Dumas et al. (2018); Mancini et al. (2018); Terova et al. (2019); Cardinaletti et al. (2019); Huyben et al. (2019); Józefiak et al. (2019); Rimoldi et al. (2019)
Siberian sturgeon <i>Acipenser baerii</i> Brandt, 1869 juvenile	Meal, up to 100 %	No negative effects on growth performances until 25 % inclusion	Caimi et al. (2020)
Turbot <i>Psetta maxima</i> (Linnaeus, 1758) juvenile	Meal, up to 76 %	No negative effects on growth performances until 33 % inclusion	Kroeckel et al. (2012)
Yellow catfish <i>Pelteobagrus fulvidraco</i> (Richardson, 1846) juvenile	Meal, up to 100 %	No negative effects on growth performances until 25 % inclusion	Hu et al. (2017); Xiao et al. (2018)
Zebra fish <i>Danio rerio</i> (Hamilton, 1822) whole life cycle	Meal, up to 100 %	No negative effects on growth performances until 25% inclusion	Vargas et al. (2018); Zarantoniello et al. (2019)

studies, with some claiming complete replacement of fishmeal. Such studies have focused on the effect of the BSFL diet in different life stages of finfish species. Most studies demonstrated little or absence of negative effects on the growth of finfish species.

### Incorporation of BSFL meal on finfish species with different feeding habits

Numerous studies were conducted focusing on the growth of omnivorous, carnivorous and herbivorous finfish species to determine the effect of BSFL meal. Growth performance indices were used to investigate the impact of BSFL meal. Such indices include weight gain/daily gain (WG/DG), specific growth rate (SGR), weight gain ratio (WGR), feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV), condition factor and Thermal-unit growth coefficient (TGC). Organosomatic indices (OSI) such as gonadosomatic index (GSI), hepatosomatic index (HSI), viscerosomatic index (VSI), spleen index (SI) and kidney index (KI) were used to investigate the effect of BSFL meal.

### Effect of BSFL meal on omnivorous finfish species

A thirteen-week feeding experiment in African catfish

*Clarias gariepinus* (Burchell, 1822) fingerlings revealed that growth was positively affected with 10 % and 20 % BSFL diet groups while 30 % incorporation had a negative effect. Moreover, it was clearly mentioned that the poor growth in 30 % incorporation was due to the high-fat content followed by reduced feed intake and digestibility. It is possible to replace fishmeal up to 30 % with BSFL meal without significant alterations in FCR, PER, average DG and SGR (Talamuk, 2016). African catfish are omnivorous and mostly feed on detritus, insects, macrophytes and zooplanktons (Dadebo et al., 2014). The distribution of taste buds, mucous cells and micro ridges on epithelial tissues, papilliform teeth found on upper and lower jaws are several key adaptations for their wider range of food preferences (Gamal et al., 2012). *Clarias gariepinus* have been grouped under carnivorous finfish species due to adaptations such as the formation of a pyloric stomach with the absence of gastric glands to facilitate an alkaline mediated digestion and J-shaped stomach comprised of thick tunica muscularis developed with circular muscles for efficient digestion (Ekele et al., 2014). Such adaptations seem to be advantageous for *C. gariepinus* to utilise BSF larval meal effectively.

According to Rana et al. (2015), mono-sex tilapia *Oreochromis niloticus* (Linnaeus, 1758) fry was tested

over 90 days using 25 % and 50 % BSFL meal incorporated diets and dehydrated BSF larvae. Lower FCR levels were reported at 50 % inclusion rate and poor growth with 100 % dehydrated larvae. Poor WG was accompanied by poor digestibility in the presence of chitin associated with BSF larvae. The study of Muin et al. (2017) done by incorporating 25 %, 50 %, 75 % and 100 % BSFL meal in *O. niloticus* fingerlings diets showed similar results with Rana et al. (2015). Fish fed with 50 % BSFL meal showed the highest SGR and best FCR values. Ideal values of WG and PER were reported at 50 % inclusion rate (Muin et al., 2017). A previous study by Teye-Gaga (2017) indicated the possibility of 75 % BSFL meal incorporation without any adverse effects on SGR, CF and FCR. Although the growth rates of *O. niloticus* increased with the inclusion of BSF meal, 50 % was recommended as the ideal level.

Bondari and Sheppard (1981) conducted a 10-weeks study by incorporating 25 %, 50 %, 75 % and 100 % chopped and frozen BSF larvae into the diets of blue tilapia *Oreochromis aureus* (Steindachner, 1864) fingerlings. This study revealed that feed intake was remarkably reduced at the beginning and gradually increased with time. The study concluded by recommending BSFL meal as a potential feed ingredient that can be used combined with other feeds or as the sole ingredient. As described by Sklan et al. (2004), lipase activity is much higher with animal proteins in blue tilapia. In addition to this, the small intestine of *O. aureus* consists of a series of nested loops, lined with villi that facilitate increased absorption due to larger surface area. Furthermore, the study demonstrated that high protein diets had altered the site of nitrogen absorption. In line with the findings of Bondari and Sheppard (1981), the possibility of 100 % BSFL incorporation was evident with *O. niloticus* (Devic et al., 2018). However, lower chitin levels associated with early larval stages of *H. illucens* may be the possible reason.

Li et al. (2017) conducted a study by replacing 25 %, 50 %, 75 % and 100 % fishmeal for juvenile Jian carp *Cyprinus carpio* Linnaeus, 1758 for 59 days. The study suggested that 100 % replacement of FM can be done using BSFL meal, without any adverse effects on growth. *Cyprinus carpio* fry has been tested for *H. illucens* meal incorporated diets by Li et al. (2017) for 56 days and observed no differences in growth between dietary groups. A study performed by Zhou et al. (2018) also recommended maximum incorporation of 100 % BSFL meal in the diets of *C. carpio*.

The activity of protease enzymes and lipases are much higher in carnivorous finfish followed by omnivorous and herbivorous. However, as revealed by several studies (Furné et al., 2005; Castro et al., 2013; Zhao et al., 2020) the activities of trypsin and lipases in Jian carps were similar to carnivorous species. Furthermore, the study revealed an increase in

intestinal fold height with the feeding of earthworms, ensuring efficient nutrient absorption. The enhanced digestive ability of insect-based meals is associated with efficient enzymatic activity and intestinal morphology in *C. carpio*.

Li et al. (2017) reported that the crude lipid content of Jian carp was lower in a fully defatted BSFL meal-based diet. Moreover, histological examinations confirmed that the accumulation of lipids in the hepatopancreas was also reduced. Furthermore, histological examinations revealed that gut histology was altered with the incorporation of partially defatted BSFL meal. Though partially defatted BSFL meal on rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) showed no effect on gut histology (Renna et al., 2017), Li et al. (2017) observed some morphological changes in the anterior intestine of Jian carp fed with 8 % fully defatted BSFL meal. Moreover, the defatted BSFL meal contains less cholesterol than fishmeal, leading to lower cholesterol levels in the blood serum of Jian carp. Furthermore, BSFL meal does not show any undesirable effects on the hepatopancreas and immune system of the Jian carp.

BSFL meal is considered a better alternative to fishmeal in the ornamental fish trade. A feeding study on zebrafish *Danio rerio* (Hamilton, 1822) with 100 % incorporation rate of BSFL meal revealed favourable results in growth. BSF prepupae incorporated diets showed no significant alterations in standard length, dry weight and SGR compared to 100 % commercial diet (Vargas et al., 2018). A study conducted for the same fish species over 6 months with the incorporation of 25 % and 50 % BSFL meal diets suggested that 25 % was the ideal inclusion rate (Zarantoniello et al., 2019). As demonstrated by Vargas et al. (2018) by molecular markers, the BSF prepupae meal can be used as a complete fishmeal replacer in *D. rerio* without any harmful effects on growth.

### Effect of BSFL meal on carnivorous finfish species

A study conducted on Atlantic salmon *Salmo salar* Linnaeus, 1758 revealed that 85 % of BSFL meal diet group does not affect the daily feed intake (FI), FCR, CF and PER. Although growth indices remained unchanged, the GSI and HSI were higher at 85 % inclusion rate (Belghit et al., 2018). In line with that, a feeding trial on *S. salar* post-smolt with 33 %, 66 % and 100 % BSFL meal inclusion rates showed no effect on growth performances (Belghit et al., 2019a). Belghit et al. (2019b) revealed no difference in growth observed in a feeding study conducted over 56 days for the same species. However, a reduction in feed intake was observed at 25 % and 50 % inclusion rates. Moreover, an experiment done by replacing up to 100 % fishmeal showed no sacrifice in growth for *S. salar* (Lock et al., 2016). The presence of digestive enzymes in pyloric caeca in Atlantic salmon facilitates better

digestion of proteins, lipids and carbohydrates (Løkka and Koppang, 2016). This might be the possible reason for enhanced growth performances with BSF larval meal incorporated diets. Moreover, the meal was prepared with larvae harvested at 13 days of development to avoid maturing of BSFL into prepupae. Belghit et al. (2019a) provided further evidence in favour of BSFL meal in *S. salar* farming. The authors mentioned that the early larval stage of BSF (around 08 days old) was used to prepare experimental diets, ensuring lower levels of chitin content. According to Kawasaki et al. (2019), chitin content in prepupae meal is twice the larval meal. However, Li et al. (2019) observed increased uptake of fatty acids in Atlantic salmon fed with full fat BSF larval meal. Lock et al. (2016) and Dumas et al. (2018) revealed that lauric acid content is much higher in BSFL meal fed to fish. Moreover, lipid droplet accumulation within the enterocytes, known as "hyper-vacuolisation" is caused mainly due to poor quality protein sources. The hyper-vacuolisation causes lipid mal-absorption, leading to a condition termed floating faeces". The study of Li et al. (2019) revealed that hyper-vacuolisation in proximal intestinal enterocytes were reduced in Atlantic salmon fed with non-defatted BSFL meal.

A feeding experiment for juvenile barramundi *Lates calcarifer* (Bloch, 1790) over 8 weeks by Katya et al. (2017) described no significant effect on the average WG and SGR among 25 %, 50 % BSFL incorporated diets and the control diet. The study also reported that WG and SGR were considerably low in 75 % and 100 % larval meal incorporated diets. FCR was reportedly higher in fish fed with 100 % BSFL meal. However, no remarkable difference was observed in the control diet and 25 %, 50 % and 75 % larval incorporated diets. This study showed that up to 75 % fishmeal replacement is possible without any sacrifice in growth. Due to the carnivorous nature of *L. calcarifer*, the availability of pyloric caeca with less abundance of secretory glands and the presence of a pyloric sphincter facilitates longer retention of feed for prolonged digestion (Purushothaman et al., 2016).

A study conducted over 62 days on European sea bass *Dicentrarchus labrax* (Linnaeus, 1758) juveniles showed no adverse effects on growth up to 19.5 % replacement of FM (López, 2015). Incompatible with that, 20 days feeding experiment disclosed no significant difference in growth, FCR and PER with up to 45 % incorporation of BSFL meal. Moreover, a depletion in PER was observed at 45 % BSFL meal incorporation (Magalhães et al., 2017). As described by Abdel-Tawwab et al. (2020), no remarkable differences were seen in growth up to 50 % incorporation of BSFL meal. Since European sea bass juveniles are carnivorous, their protein requirement ranged between 45 % and 50 % (Peres and Oliveira-Teles, 2006). The higher abundance of taste buds in *D. labrax* may transform themselves into selective feeders (Abbate et al., 2012). Wang et al. (2019)

observed a remarkably higher feed intake in juvenile Japanese seabass *Lateolabrax japonicus* (Cuvier, 1828) fed with defatted BSFL meal incorporated diets over fishmeal and found no adverse effects on growth up to 64 % incorporation.

Caimi et al. (2020) examined up to a 100 % inclusion of fully defatted BSFL meal in Siberian sturgeon *Acipenser baerii* Brandt, 1869 juvenile diets. However, 100 % BSFL meal incorporated diet was not accepted by the juveniles and only 25 % inclusion rate was found to be ideal.

A 12-week study performed with BSFL meal incorporated diets for juvenile grouper fish *Epinephelus fuscoguttatus* (Forsskål, 1775) x *Epinephelus lanceolatus* (Bloch, 1790) suggested 30 % inclusion rate for better SGR, FCR and CF values (Zulkifli et al., 2019). Katya et al. (2017), described the overall growth performances of barramundi *L. calcarifer* juveniles were retarded at inclusion rates above 50 %. Even though this species is a carnivore, growth performances were not remarkably higher, because BSF prepupae have been used in the preparation of meal, leading to higher chitin content. Zhang et al., (2014) reported that chitin in diets could lead to poor accessibility of enzymes into substrates and reducing retention time of faecal matter. The results of Magalhães et al. (2017) followed the results of Katya et al. (2017), where European sea bass *D. labrax* was used as the experimental fish species. Additionally, they reported that cholesterol content was much lower with BSFL meal incorporation. This was mainly due to the presence of chitin, which ensures the presence of chitosan that has the ability to reduce cholesterol. However, as described by Karapanagiotidis et al. (2014) and Talamuk (2016), the nutrient composition was not changed by the inclusion of BSFL meal in gilthead sea bream *Sparus aurata* Linnaeus, 1758 and *C. gariepinus*, respectively.

As described by Sealey et al. (2011), no retardations in the growth were observed in carnivorous *O. mykiss*, even at 100 % incorporation rate of BSF prepupae meal. Balancing dietary amino acid, which can be limited at higher chitin content, may lead to such a positive effect on growth. Although contradictory results were reported by Sealey et al. (2011), most of the feeding studies conducted for *O. mykiss* using prepupae meal ended up with retarded growth at inclusion levels above 50 % (St-Hilaire et al., 2007; Stamer et al., 2014; Cardinaletti et al., 2019). These results were similar to the findings of Kroeckel et al. (2012) and Karapanagiotidis et al. (2014) for carnivorous *S. aurata* and *Psetta maxima* (Linnaeus, 1758). According to Sealey et al. (2011), significant growth changes were observed in *O. mykiss* fingerlings reared by feeding BSFL based diet. Weight gain (WG) values were low in 25 % and 50 % incorporated BSFL diets compared to the control diet. However, FCR values of 25 % and 50 % incorporated BSFL diets were closer to 1. Moreover,

HSI remained equal for both treatments. The study of Stamer et al. (2014) was an extension of Sealey et al. (2011) with an additional BSFL meal inclusion rate of 75 %. Results showed that the replacement of FM by 75 % of BSFL was ineffective due to a 15 % body weight loss compared to 0 % inclusion. However, 50 % and 75 % inclusion levels showed higher HSI values. A 78-day feeding study with 0 %, 25 % and 50 % FM substitution using partially defatted BSFL meal was examined for juvenile rainbow trout (Renna et al., 2017). WG, SGR, FCR, PER, CF, HSI and VSI does not significantly vary among the treatments. The results reported by Bruni et al. (2018) were based on an extended study of Renna et al. (2017), showing that HSI and VSI were not affected by the diets and up to 50 %. St-Hilaire et al. (2007), described that out of 25 % and 50 % replacements, the lowest WG were observed in 50 %. FCR values were higher than 1 for all treatments, while 50 % incorporation of BSFL diet showed the highest FCR value. A 3-month feeding trial conducted by Dumas et al. (2018) using 6.6 %, 12.2 % and 24.6 % BSFL meal incorporation rates revealed that FCR values increased with the inclusion of BSFL meal except for 24.6 %. Therefore, the study suggested that up to 12.2 % inclusion rate is possible without any disturbance to the growth. *Oncorhynchus mykiss* juveniles, fed with 0 %, 25 % and 50 % BSFL diet groups, showed a gradual decline in SGR and gradual increment in FCR values (Cardinaletti et al., 2019). Moreover, in another study, up to 30 % incorporation of BSFL meal revealed no significant alterations among SGR and FCR. Hence, the FCR values were below one up to 30 % of BSFL meal inclusion is recommended for *O. mykiss* (Terova et al., 2019). However, incorporation of BSF prepupae full-fat meal showed no complications in the growth of juveniles, only up to 20 %. This study evidenced that higher fat and chitin levels might lead to poor growth associated with full-fat BSF prepupae meals (Józefiak et al., 2019).

A study conducted for *S. aurata* juveniles concluded BSF prepupae meal as a better alternative for FM due to remarkably higher feed consumption even at 100 % FM replacement (Karapanagiotidis et al., 2014).

A 10-week study conducted for channel catfish *Ictalurus punctatus* (Rafinesque, 1818) using 25 %, 50 %, 75 % and 100 % chopped and frozen BSFL incorporated diets showed no significant alterations in weight and length gain. However, length and weight gains were found to vary among male and female fish. The study by Bondari and Sheppard, (1981) was the first to investigate the effect of BSFL on fish growth showing the requirement of further studies on different stages of different fish species. As described by Bailey and Harrison (1948), terrestrial and aquatic insects are prominent dietary sources for the wild populations of *I. punctatus*. Midges Chironomidae Erichson, 1841 and blackflies Simuliidae Newman, 1834 belong to the most commonly utilised insects in the wild.

A 56-day feeding experiment was performed by replacing 0 %, 17 %, 33 %, 49 %, 64 % and 76 % of fishmeal in juvenile turbot *P. maxima*. SGR values were lower in diets of 49 % and above inclusion rates of BSFL. FCR was also notably increased beyond 33 %. Elevated levels of chitin and fat contents were revealed as the possible causes for poor feed intake (Kroeckel et al., 2012). According to Han et al. (1999), poor lipid digestibility and absorption were governed by high chitin contents.

Hu et al. (2017) described a significant reduction in WGR and feed conversion beyond 20 % BSFL meal incorporation in juvenile yellow catfish *Pelteobagrus fulvidraco* (Richardson, 1846). However, contradictory results were reported by Xiao et al. (2018) for the same species. WGR, SGR and PER were remarkably higher in BSFL diet groups compared to control diets. Lower FCR values were reported in 13 %, 25 %, 37 % and 48 % BSFL diet groups. PPV values were also higher in said diet groups. However, when the incorporation rates of BSFL meal exceeded 68 %, growth was adversely affected. In juvenile Hong Kong catfish *Clarias fuscus* (Lacepède, 1803), 66 % replacement of fish meal with BSFL meal caused minimum adverse effects on growth (Stankus, 2013).

No studies were conducted to test the effectiveness of BSFL meal on herbivorous fish, highlighting the need to fill this knowledge gap using further experimentation. Also, with only one exception by Zarantoniello et al. (2019), BSFL incorporation into fish feeds is mainly focused on one life stage of selected fish species, not for the whole lifecycle.

In some cases, the ideal level of BSFL inclusion within a species appears to vary according to the study. Vargas et al. (2018) recommended 100 % inclusion in *D. rerio* diet, while Zarantoniello et al. (2019) suggested 25 % inclusion. St-Hilaire et al. (2007) recommended up to a 25 % inclusion rate for juvenile *O. mykiss*, while 50 % inclusion was recommended by Stamer et al., 2014. Even though these differences in inclusion levels may be justified by the growth stages of the fish species and BSFL, further studies are needed to verify the effective utilisation of BSFL in fish. Changes in gut microflora may also be a possible reason, as described by Huyben et al. (2019). An increased abundance of chitinase secreting *Bacillacea* was found in the gut of *O. mykiss* fed with BSF meal. It may be interesting to follow these gut microbiological studies and gut histopathology to unravel the utilisation potential of BSFL meal by different fish species.

### **Effect of BSFL meal incorporated diets on body composition of finfish**

Some studies focused on whether the BSFL inclusion would change the body composition in selected fish species. Increased dry matter content in the muscles of *S. salar* fed with BSFL meal was reported by Belghit



et al. (2018). This is in contrast with the reduction of dry matter content in *D. rerio* fed with 100 % BSFL meal incorporated diets (Vargas et al., 2018). Dry weight loss in *C. carpio* (Li et al., 2017), *O. mykiss* (St-Hilaire et al., 2007) and *P. fulvidraco* (Xiao et al., 2018) was reported after feeding a diet incorporated with full-fat BSFL meal.

Contradicting results were also reported in CP levels of BSFL meal fed fish species. Unaffected CP levels were reported in *S. salar* (Belghit et al., 2019a) and Jian carp (Li et al., 2017; Zhou et al., 2018), whereas elevated body CP levels were reported in *O. mykiss* (Mancini et al., 2018; Stamer et al., 2014) and *O. niloticus* (Muin et al., 2017). However, Dumas et al. (2018), reported decrease in body CP levels in *O. mykiss* after feeding them with BSFL meal incorporated diets.

Increased levels of essential amino acids, i.e. arginine, histidine, lysine and methionine, were found in *L. calcarifer* (Katya et al., 2017) fed with BSFL incorporated diets, while unchanged amino acid profiles were reported in *S. salar* (Belghit et al., 2019a). However, in Jian carp, amino acid profiles were highly varied with the diet (Li et al., 2017; Zhou et al., 2018).

BSFL inclusion in the diet made a variable degree of effects on CF content of the fish body. Unchanged CF contents in the muscles were reported in *S. salar* (Belghit et al., 2019a) and Jian carp (Li et al., 2017; Zhou et al., 2018). Increased CF levels were found in *O. niloticus* (Muin et al., 2017), whereas a significant reduction in CF was observed in *O. mykiss* (Sealey et al., 2011; St-Hilaire et al., 2007), *P. maxima* (Kroeckel et al., 2012) and *P. fulvidraco* (Xiao et al., 2018). Affected fat digestibility and low energy intake coupled with elevated chitin content were suggested for the said reduction in CF levels.

A significant increase in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) levels were reported in *S. salar* fed with BSFL incorporated diet (Belghit et al., 2019a). Another study revealed that SFA content in *S. salar* was doubled at 85 % BSFL inclusion levels (Belghit et al., 2019b). Findings of Zhou et al. (2018) on *C. carpio*, Devic et al. (2018) on *O. niloticus*, Mancini et al. (2018) on *O. mykiss* also suggested that SFA contents remarkably increase with the BSFL meal. Elevated lauric acid levels were also detected in *O. mykiss* fed with BSFL meal incorporated diets (Sealey et al., 2011; Stamer et al., 2014; Renna et al., 2017). However, as described by Lock et al. (2016), n-3/n-6 ratio in *S. salar* muscles was decreased with the inclusion of BSFL meal. Unsaturated fatty acid (UFA) contents were also reduced with high levels of BSFL meal incorporation in *D. rerio* (Zarantoniello et al., 2019). A similar decrease in PUFA levels was also reported by Mancini et al. (2018). These variations in the fatty acid profile and CF contents of the fish fed with BSFL may be governed by the nature of

substrate used for BSFL rearing and the fish feeding habits (Ewald, 2019, Ewald et al., 2020).

BSFL meal incorporated diets made no changes in the ash content in *S. salar* (Belghit et al., 2019a), while the levels increased in *O. mykiss* (Stamer et al., 2014). Micronutrients such as selenium contents were reduced and iron and manganese contents increased in *S. salar* muscles (Belghit et al., 2019a). The presence of high mineral contents in insect meals compared to FM was identified as the potential cause for high ash contents (Hu et al., 2017).

These findings clearly show that the fish meal replacement by BSFL larval meal differently affects the body composition of fish. Since the composition of the BSFL meal itself can depend upon the growth stage of BSFL and the substrate in which they are grown, their CP levels, CF levels, amino acid and fatty acid profiles, levels of chitin and minerals would subsequently alter. Also, due to the adaptations in the digestive systems of fish species under investigation and their growth stage, these changes can be further magnified. Hence, one would expect positive changes in fish body composition by positively altering these factors.

## Conclusion

The increasing cost of fish meal and global concern of sustainability in aquaculture has led to the development of alternative sources of feed for culture organisms. Insect based diets, especially black soldier fly larvae (BSFL) meal, has shown very promising results in aquaculture. Due to high levels of crude protein, BSFL has been proven as a good alternative for fish meal. Though it may be disadvantageous if not balanced in the diet, its high energy and fat content can act as an additional benefit. A balanced amino acid profile and fatty acid profile, together with high mineral contents, made it further suitable as a dietary component in fish feed. However, as the larva get older, chitin deposition act as a bottleneck for its use. Different inclusion levels of BSFL have shown promising results in a variety of aquaculture species, where up to 100 % replacement is possible without adverse effects. However, in many cases, when levels reach more than 50 %, negative effects on growth parameters are seen, probably due to high chitin content, high crude fat content, and inability of fish species and growth stage to utilise insect-based diets. The body composition of BSFL fed fish, in many cases, seem to be positively affected. However, there is a need for future studies to be conducted on several commercially important finfish species to identify the potential of BSFL as a suitable alternative for fishmeal.

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