Indian J. Fish., 67(4): 160-170, 2020
DOI: 10.21077/ijf.2020.67.4.100172-20

Review

Potential of black soldier fly (*Hermetia illucens*) as alternative protein source in salmonid feeds - A review

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ABSTRACT

The current constant pressure on fishmeal production for aquaculture feeds stresses the need to search for alternative sources of protein. A possible protein ingredient is insect meal, being more cost effective than fishmeal and soybean meal. Black soldier fly (*Hermetia illucens*) larvae meal is used in many studies with positive results regarding the bioproductive performances of salmonids. The balanced amino acid profile is comparable with the one of soybean meal. The crude protein and amino acid digestibility is high and similar to that of fishmeal. For rainbow trout (*Oncorhynchus mykiss*), the protein biological value of the larvae is qualitatively lower than that of fishmeal. Atlantic salmon fed with experimental diets with larvae meal showed high bioproductive performances and a better protein biological value compared with salmon fed with a standard fishmeal diet. However, the nutritional quality and chemical composition of the larvae can be drastically improved through the feeding media, which make it a very interesting candidate as a substitute for fishmeal. The aim of this review is to present the current stage of *H. illucens* use in salmonid feeds, detailing the chemical composition and protein quality. Furthermore, the effect of larvae meal based diets on salmonids is presented.

Keywords: Atlantic salmon**,** Larvae meal, Rainbow trout

Introduction

Worldwide aquaculture is faced with many pressing issues, but one of the most important ones is the inability of feed producers to support the ever-growing necessities of this sector. Aquaculture fish production will continue to rise, assuming that by 2030 it will reach the production levels of commercial fishing (Kobayashi *et al.*, 2015). The current production of fishmeal can only partially cover the demand from feed producers in aquaculture and animal farming. If the fishmeal production would increase, there would be dire consequences on marine ecosystems (Merino *et al.*, 2012). Marine fish populations used for fishmeal production have been declining, slightly stabilising only in recent years (FAO, 2018). Legal measures have been imposed on commercial fishing and fish processing (Garcia-Rivera *et al.*, 2015) to achieve a sustainable development of fisheries and aquaculture (Hollingsworth, 2018). Furthermore, to maintain this fragile balance, new alternative protein sources are examined for replacing fishmeal and soybean meal (Shepherd and Jackson, 2013; FAO, 2018). The urgency to use alternative protein sources in aquaculture feeds resides in the high market demand for feeds, complemented by the limited offer and high production costs of fishmeal (Bene *et al.*, 2015).

Considering these aspects, off late, attention is focused on insects as alternative protein sources (Rumpold and Schluter, 2013; Sanchez-Muros *et al.*, 2014; Makkar *et al.*, 2014; Nogales-Merida *et al.*, 2018). The main advantage that insects have on vegetal sources is that insect protein presents a better biological value and it has higher concentrations of essential limiting amino acids (AA) for fish, like methionine and leucine (Hall, 1992). A high number of insect species has been tested as alternative protein sources in aquaculture feeds, like grasshoppers, crickets, termites, silk worms, mosquitos, mealworms and others (Henry *et al.*, 2015; Gasco *et al.*, 2016). The black soldier fly (*Hermetia illucens*) is sometimes preferred to other insects because it has the ability to process a wide variety of waste. Studies regarding the black soldier fly show that the protein meal made from larvae and prepupae can partially substitute fishmeal in aquaculture feeds, due to its high-energy content and balanced AA profile (Hale, 1973; Newton *et al.*, 1977; De Marco *et al.*, 2015). *H. illucens* experiments concerning fish feeds started in the 80s and still continue (Bondari and Sheppard, 1981; Zhou *et al.*, 2016; Katya *et al.*, 2017; Hu *et al.*, 2017, Li *et al.*, 2017; Magalhaes *et al.*, 2017; Zarantoniello *et al.*, 2018). The life stages suitable for producing protein meals are late larva and early prepupa (Liu *et al.*, 2017).

Some fish species do not require a high protein level in their nutrition, while salmonid aquaculture relies heavily on fishmeal and soybean meal, as ingredients in feeds. Hence, substituting the protein source, even partially, is more complicated. Two of the economically important salmonid species that are great fishmeal consumers are the rainbow trout (*Oncorhynchus mykiss*) (Hardy, 2002) and the Atlantic salmon (*Salmo salar*). Considering the positive experimental results, *H. illucens* is gaining more and more popularity in research regarding aquaculture feeds. The aim of this paper is to present and interpret a series of helpful information regarding *H. illucens* as an alternative source of protein in salmonid feeds.

Chemical composition of the *H. illucens* larvae and prepupae meal

Most research presents chemical composition of the larvae, prepupae or larvae meal used in different experiments. However, the chemical composition, as the nutritional quality, varies widely, mostly depending on the feeding media of the insects.

The dry matter (DM) from larvae and prepupae can be usually found between 32-40% (Diener *et al.*, 2009; Tschirner and Simon, 2015; Spranghers *et al.*, 2016), but there are reports that suggest a wider range, from 23 to 44% (Hale, 1973; Liland *et al.*, 2017). Larvae meal can have a higher DM concentration (94.18%), compared with fishmeal (92.3%) and soybean meal (90.9%). Commonly, the relative humidity of larvae meal is around 10% (Sheppard *et al.*, 2002; Arango Gutierrez *et al.*, 2004; Oonincx *et al.*, 2015).

The crude protein (CP) content of larvae is more than a third of the DM *i.e.,* 34-43.9% (Newton *et al.*, 1977; Sheppard *et al.*, 1994; St-Hilaire *et al.*, 2007a; Diener *et al.*, 2009; de Marco *et al.*, 2015). The CP level in larvae meal is slightly lower than that of fishmeal and comparable with that of soybean meal, in some cases exceeding it by 5% (Renna *et al.*, 2017) (Table 1). Nonetheless, it is still a high enough concentration to determine its experimental use in salmonid feeds. Some authors consider the evaluation of CP as irrelevant, because it considers the nitrogen from non-protein nitrogenous substances (NPS), not only the nitrogen from AA (Janssen *et al.*, 2017). The statement is supported by the work of Liland *et al.* (2017), which determine a CP content of 33.7-42.3% of DM in *H. illucens* larvae, but with a NPS level of 9.4-13.1% of DM, leading to a lower true protein content. Still, most studies regarding insect use in fish nutrition present the CP content, being easier to determine. The chemical composition of the larvae, prepupae and larvae meal compared with the one of soybean meal and fishmeal are presented in Table 1.

The use of larvae meals in salmonid feed has a slightly negative effect on its digestibility. The digestibility of the organic matter in diets decreases with the inclusion of larvae meal and subsequently, with the decrease of fishmeal percentage in the feed. The apparent digestibility coefficient (ADC) of dry matter is 75%, while CP and AA have an ADC of 87-93%, for rainbow trout (Dumas *et al.*, 2018). The digestibility of CP from diets with larvae meal is lower with 3% than that of standard diets for Atlantic salmon, while the apparent digestibility of AA is lower by 1% (Belghit *et al.*, 2018a).

The prepupae have a different chemical composition than the larvae, mainly because the chitin content increases in this life stage. After correcting the chitin fraction, a CP content of 28-42% can be observed in the DM of prepupae

Table 1. Chemical composition of *H. illucens* larvae, prepupae and larvae meal, compared with fishmeal and soybean meal

H. illucens ingredient	Dry matter (DM) (% of wet weight)	Crude protein (% of DM)	Chitin (% of DM)	Crude fat (% of DM)	Nitrogen-free extracts (% of DM)	Ash (% of DM)	Reference
Larvae	34-41	34-42	3.7-3.8	-	-	-	Diener *et al.* (2009)
Larvae	-	36.2	-	18	36.5	9.3	Barroso *et al.* (2014)
Larvae	17.9-30.02	37.2-57.6	-	3.4-38.6	-	4.8-22.9	Tschirner and Simon (2015)
Larvae	-	37.5-55.2	-	15.8-22.9	16.3-35.6	10.5-12.7	Barroso *et al.* (2017)
Larvae meal	-	37	-	19	-	17	Arango Gutierrez *et al.* (2004)
Larvae meal	-	36	-	34	-	-	de Marco *et al.* (2015)
Larvae meal	94.18	55.34	5	17.19	14.57	7.12	Renna *et al.* (2017)
Larvae meal	-	47.6	9.6	11.8	15.1	15.9	Kroeckel *et al.* (2012)
Prepupae	-	44	-	33	-	15.5	St-Hilaire (2007a)
Prepupae	-	40.7	-	15.6	24	19.7	Barroso *et al.* (2014)
Soybean meal	90.9	50.65	-	2.85	36.21	6.47	Zduńczyk *et al.* (2014)
Fishmeal	92.3	67.9	-	9.7	-	15.9	Overland *et al.* (2009)

DM - Dry matter

(Diener *et al.*, 2009) and the level can increase further, to 47.6% (Kroeckel *et al.*, 2012). Caligiani *et al.* (2018) determined the protein forms in prepupae, establishing the following structure: albumins 23%, globulins 14%, prolamins 9%, glutelins 31% and residual insoluble proteins and chitin 23%, for approximately 90% of the protein nitrogen from prepupae. Albumins and glutelins total 54% of the proteins existent in prepupae (Caligiani *et al.*, 2018).

Chitin is the main polysaccharide in *H. illucens* and a major non-protein nitrogen source that increases the CP level. It is a polymer of N-acetylglucosamine and glucosamine, being the fundamental component of the exoskeleton of the insect. The level of chitin greatly increases after the larval stage, in the prepupal, pupal and imago stages. Chitin amounts to 5.41% of DM in larvae (Finke, 2013), with a 5% content in larvae meal (Renna *et al.*, 2017) and 8.7% of DM in prepupae (Cullere *et al.*, 2016). It is one of the major factors negatively influencing the protein digestibility in fish (Marono *et al.*, 2015). Some fish species do not possess enzymatic equipment for processing chitin (Rust, 2003). Nevertheless, in low levels, up to 2% of diet DM, it does not affect the protein digestibility in rainbow trout (Renna *et al.*, 2017). Otherwise, chitin does produce positive effects. It can reduce cholesterol levels, by facilitating its absorption (Ushakova *et al.*, 2016; Magalhaes *et al.*, 2017). Furthermore, chitin contains chitosan (Muller *et al.*, 2017), a bioactive compound with antimicrobial, regenerative and anti-inflammatory properties (Muanprasat and Chatsudthipong, 2017).

Larvae also contain different types of enzymes, helping them in the digestion of various media: trypsin, chymotrypsin, cellulase, lignin-modifying enzymes and chitinase (Muller *et al.*, 2017). Larval extracts have been proven to degrade bacterial biofilms produced by *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas fluorescens* and *Bacillus subtilis*. The presence of some antimicrobial peptides like defensins, cecropins, atacins and diptericins has been observed in larvae (Muller *et al.*, 2017).

The larvae are an important source of energy. Some studies report a gross energy level of larvae meal between 21.1 and 23.8 MJ kg^{-1} (Kroeckel *et al.*, 2012; de Marco *et al.*, 2015), exceeding the gross energy values of fishmeal (20.47 MJ kg^{-1}) and soybean meal (19.7-21.1 MJ kg^{-1}). When larvae meal is used in aquaculture feeds, replacing fishmeal by 25 to 50%, the gross energy of the diet is 5.97 and 6.25 MJ kg^{-1}, respectively (Sealey *et al.,* 2011). One hundred gram of live larvae have 0.83 MJ gross energy (Payne *et al.*, 2015), while the metabolisable energy of one larva is 0.00068 MJ, or 163 calories (Finke, 2013).

Nutritional quality of the larvae and prepupae protein meal

The nutritional value of the larvae meal is dependent on the essential and non-essential AA profiles. The AA concentrations of larvae meal are comparable with those of fishmeal and soybean meal (Sánchez-Muros *et al.*, 2014). Because of this, *H. illucens* proteins are considered a possible substitute for the two classical protein sources (St-Hilaire *et al.*, 2007 a; Sealey *et al.*, 2011). The AA content and profile can vary not only due to the feeding media of the larvae, but also because of the processing/killing method of the insect (Leni *et al.*, 2019). The AA concentrations in larvae meal meet the nutritional requirements of salmonids, for all age groups (NRC, 2011), but care should be taken regarding the percentage of larvae meal when formulating the feeds. However, the essential AA concentrations in larvae meal have lower values than those of fishmeal and soybean meal. Usually, three essential AAs are considered as limiting factors for salmonids: methionine, lysine and arginine. Sometimes, a fourth limiting essential AA is mentioned, *i.e.,* threonine.

Methionine has variable concentrations in the protein of *H. illucens*, depending heavily on the feeding media (Table 2). Being the first limiting essential AA, the protein chemical score of larvae is calculated based on its concentration, reaching values between 70-80. In salmonids, methionine amounts to 70% of the total sulfur AA (Rodehutscord *et al.*, 1997). On an average, the concentration of methionine in larvae is higher than the one in soybean meal, but much lower than that of fishmeal and does not meet the nutritional requirements of salmonids for methionine. It has only approximately 93% of the methionine requirements of the rainbow trout, that can decrease to 80% in some cases and 76% of the nutritional requirement of Atlantic salmon (Table 2). Despite this, the potential to increase methionine levels in larvae by improving the feeding media exists, a suitable feeding medium being vegetal waste (Renna *et al.*, 2017). However, vegetal waste has a lower concentration of lysine, which is the second limiting AA for rainbow trout (Gaylord and Barrows, 2009).

On average, larvae meal has a much lower concentration of lysine than fishmeal and soybean meal, with 48.54 and 25.15%, respectively. Even if the values are lower, they exceed the nutritional requirements of rainbow trout and Atlantic salmon with 30 and 23-37% (depending on age category), respectively (Table 2). A suitable feeding medium for a high lysine concentration in the larvae seems to be cattle feeds (Dumas *et al.*, 2018). Rainbow trout does not manifest the lysine-arginine antagonism, found in many fish species (Chiu *et al.*, 1987; Kim *et al.*, 1992).

Table 2. Essential amino acid contents of *H. illucens*, fishmeal, soybean meal and the nutritional requirements of rainbow trout and Atlantic salmon (% of dry matter)

H. illucens ingredient	Essential amino acids content in *Hermetia illucens*										Reference
	Arg	His	Iso	Leu	Lys	Met	Phe	Thr	Try	Val	
Larvae	2.09	1.42	1.81	2.86	2.58	0.69	1.56	1.71	-	2.47	Tschirner and Simon (2015)
	1.8-2.6	0.92-1.12	1.48-1.68	2.48-2.76	2.16-2.48	0.52-0.68	1.20-1.72	1.48-1.64	-	2.16-2.40	Liland *et al.* (2017)
	2.60	2.01	2.01	3.23	3.10	0.25	2.60	1.89	-	2.81	Müller *et al.* (2017)
Larvae meal	1.94	1.13	1.72	2.4	2.23	0.90	1.44	1.52	-	2.20	de Marco *et al.* (2015)
	2.15	1.21	1.82	2.87	2.10	1.16	1.66	1.71	-	2.71	Renna *et al.* (2017)
	2.29	1.5	1.87	3.23	2.71	0.66	1.63	1.70	0.55	2.56	Cummins *et al.* (2017)
	2.70	1.63	2.40	3.67	2.52	0.85	2.18	2.18	-	3.45	Schiavone *et al.*(2017)
	2.19	1.06	1.92	3.00	2.79	0.63	1.79	1.67	0.54	-	Dumas *et al.* (2018)
Prepupae	2.65	1.18	2.03	3.10	2.62	0.74	2.00	1.78	-	2.79	St-Hilaire *et al.* (2007a)
	1.78	0.76	1.83	2.66	2.05	0.77	1.83	1.58	-	2.99	Sealey *et al.* (2011)
	1.99-2.03	1.24-1.38	1.72-1.91	2.80-3.06	2.26-2.57	0.71-0.87	1.63-1.87	1.54-1.68	0.54-0.67	2.41-2.82	Spranghers *et al.* (2016)
	1.96	1.17	1.47	2.70	2.30	0.60	1.30	1.49	-	2.40	Caligiani *et al.* (2018)
	1.89	0.82	1.89	2.72	2.12	0.79	1.82	1.60	-	3.06	Sealey *et al.* (2011)
Fishmeal	3.44	1.42	2.64	3.25	4.80	1.62	2.59	2.91	0.84	3.10	Cummins *et al.* (2017)
Soybean meal	3.80	1.20	2.40	3.90	3.30	0.70	2.60	1.90	0.60	2.40	Yamamoto *et al.* (2010)
AA requirement for rainbow trout	1.4	0.5-0.6	0.7-1.4	1.1-1.4	1.9	0.8-0.9	0.7	1.10	0.2-0.9	0.8-1.6	NRC (2011)
AA requirement for Atlantic salmon	1.60-2.00	0.70-0.80	0.80	1.40	1.80-2.00	1.00-1.10	1.20	0.80	0.20	1.30	NRC (1993)

AA - Amino acids; Arg - Arginine; His - Histidine; Iso - Isoleucine; Leu - Leucine; Lys - Lysine; Met - Methionine; Phe - Phenylalanine; Thr - Threonine; Try - Tryptophan; Val - Valine

Arginine is the third limiting essential AA for rainbow trout (Gaylord and Barrows, 2009) and it can be found in lower levels in larvae meal than in fishmeal and soybean meal. Nevertheless, the arginine content of the meal can be improved by different processing methods. If the larvae meal is highly defatted, the difference between the arginine concentration in fishmeal and larvae meal is negligible (Schiavone *et al.*, 2017). Soybean meal has a much higher concentration of arginine than both fishmeal and larvae meal. The arginine levels in larvae meals usually exceed the nutritional requirements of salmonids (Table 2).

Some authors consider threonine as the fourth essential limiting AA for rainbow trout (Gaylord and Barrows, 2009). It has lower concentrations in larvae meal than in fishmeal and soybean meal, but it meets the nutritional requirements of salmonids (Table 2). It can exceed the levels found in soybean meals if the larvae are reared on cereal byproducts (Schiavone *et al.*, 2017).

The other essential AA (histidine, leucine, isoleucine, phenylalanine, tryptophan and valine) can be found in larvae meal in concentrations that meet the nutritional requirements of salmonids, usually lower than the ones of fishmeal and soybean meal, with some exceptions (Table 2). Larvae meal presents a concentration of leucine two times higher than the nutritional requirements of Atlantic salmon and rainbow trout. Tryptophan has the highest apparent digestibility coefficient (ADC) of the 10 essential AA (996%), while leucine has the lowest ADC (4%), in the case of rainbow trout. The apparent digestibility of AA from larvae meal is similar to that of fishmeal AA, for Atlantic salmon (Lock *et al.*, 2015).

There needs to be a constant reminder that all essential AA concentrations can be improved by the processing method and the nature of the feeding media. Most studies use larvae reared on organic waste, like vegetal waste, kitchen waste, sludge, cereal byproducts and others, to highlight the double benefit of using *H. illucens* larvae: the entomo-remediation properties and the high protein biomass resulted. When commercial larvae or larvae reared in media with a higher protein content (pet feeds, fish offal and chicken feeds.) are tested for the nutritional value, the essential AA concentrations, as well as other nutrient concentrations, are greatly improved, making *H. illucens* even more suitable for aquaculture feeds. Of course, the current great challenge is to make a medium for feeding larvae with the lowest cost possible, but also respecting the EU and other regulations for insects and insect products used in animal feeds (EU, 2017).

The non-essential AA concentrations are higher than those of essential AA, aspartic and glutamic acids being abundant (Liland *et al.*, 2017). As essential AA, non-essential AA have lower concentrations in larvae meal than in fishmeal or soybean meal (Table 3), with a few exceptions.

Proline concentrations of larvae meal exceed those of fishmeal and soybean meal (Table 3). Alanine and cysteine can have higher levels in larvae meal than in soybean meal. The optimum ratio of essential to non-essential AA for rainbow trout is between 49:51 and 66:34, for a high nitrogen retention level (Green and Hardy, 2002). Some attention should be directed at the pairs of sulfur AA and aromatic AA. Cysteine can result from methionine, being a one-way process. Sometimes, methionine is substituted with cysteine in rainbow trout feeds, but methionine should not go lower than 70% of the total sulfur AA content. For rainbow trout fry, a sulfur AA concentration of 1.8-2% of diet DM is recommended (Lall and Anderson, 2005). Tyrosine can replace phenylalanine up to 50%, but aromatic AA should amount to 1.5% of diet DM (Lall and Anderson, 2005). Tyrosine has a higher level in larvae meal than in fishmeal or soybean meal (Table 3).

Bioproductive efficiency of *H. illucens* used in salmonid feeds

Considering the chemical composition and nutritional quality of the *H. illucens* larvae, various experiments have been carried out to determine the possibility of replacing fishmeal in aquaculture feeds. Most experiments on salmonids were conducted after 2015, mainly for rainbow trout and Atlantic salmon (Lock *et al.*, 2015), but there are a few prior studies (St-Hilaire *et al.*, 2007a; Sealey *et al.*, 2011). Earlier results showed that fishmeal could be substituted with larvae meal in low ratios, usually below 25%, in rainbow trout diets. Beyond this threshold, the growth was hindered and the fillet quality suffered. The larvae rearing technology evolved and diets with larvae meal could be administered without affecting the growth of rainbow trout. However, the fillet quality still suffered (Renna *et al.*, 2017; Bruni *et al.*, 2018). In diets for Atlantic salmon, current studies present better results. Larvae meal can totally-substitute fishmeal, without negative effects, in the post-smolt stage of Atlantic salmon (Belghit *et al.*, 2019).

The chemical composition of trout fillet varies depending on the chemical composition of the larvae, percentage of inclusion in feeds and processing method (Mancini *et al.*, 2017). Thus, trout fed partially defatted larvae meal has a higher crude fat percentage than both trout fed standard feeds (Renna *et al.*, 2017) and trout fed normal (defatted) larvae meal diets (Sealey *et al.*, 2011). Partially defatted larvae meal may substitute fishmeal upto 50% in trout diets, without adverse effects on growth performances, Fulton's condition factor, somatic indices physical quality or viscera morphology (Renna *et al.*, 2017; Bruni *et al.*, 2018). Nonetheless, negative effects on the fatty acid levels and crude fat content of trout fillet are recorded when replacing fishmeal with more than 20% partially defatted larvae meal (Renna *et al.*, 2017). Dumas *et al.* (2018) noticed a hindered development in trout fed diets containing more than 13% larvae meal.

Prepupae meals were also tested. The growth of young rainbow trout (22.62 mean body weight) fed with diets where fishmeal was replaced by prepupae meal in ratios of 25 and 50% was hindered (St-Hilaire *et al.*, 2007a).

Table 3. Non-essential amino acid content in *H. illucens* (% of dry matter)

H. illucens ingredient	Non-essential amino acids content in *H. illucens*									Reference
	Asp	Ser	Glu	Gln	Pro	Gli	Ala	Cys	Tyr	
Larvae	3.16-3.76	1.60-1.96	4.12-5.12	-	2.00-2.44	1.72-2.12	2.48-2.76	-	1.44-2.28	Liland *et al.*(2017)
	4.32	1.72t	5.12	-	2.60	2.26	2.60	0.21	2.52	Müller *et al.* (2017)
Larvae meal	3.22	1.84	3.85	-	3.73	1.91	3.03	1.38	2.16	de Marco *et al.* (2015)
	3.70	2.04	4.86	-	3.04	2.32	3.43	0.05	2.65	Renna *et al.* (2017)
	3.72	2.03	4.87	-	3.06	2.35	3.45	0.01	2.64	Schiavone *et al.* (2017)
Prepupae	3.72	1.68	3.78	-	2.39	2.28	3.02	-	3.08	St-Hilaire *et al.* (2007a)
	4.09	1.37	-	4.42	-	1.72	2.45	-	2.22	Sealey *et al.* (2011)
	3.36-3.78	1.50-1.66	3.98-4.58	-	2.14-2.51	2.22-2.52	2.42-2.78	0.21-0.25	-	Spranghers *et al.* (2016)
	3.60	1.76	4.20	-	1.87	2.67	3.80	-	2.60	Caligiani *et al.* (2018)
	4.14	1.42	-	4.17	-	1.80	2.55	-	2.57	Sealey *et al.* (2011)
Soybean meal	5.70	2.60	10.10	-	2.50	2.30	2.20	0.70	1.90	Yamamoto *et al.* (2010)
Fish meal	6.17	2.88	8.82	-	2.78	4.50	4.20	0.70	2.17	Overland *et al.* (2009)

Asp - Aspartic acid; Ser - Serine; Glu - Glutamic acid; Gln - Glutamine; Pro - Proline; Gli - Glycine; Ala - Alanine; Cys - Cysteine; Tyr - Tyrosine

The food conversion ratio (FCR) was affected by the substitution of fishmeal, reaching 1.47 for the diet with 50% replacement, while for the standard diet, FCR was 1.18 (St-Hilaire *et al.*, 2007a). The results were confirmed later, but this time the growth was hindered only in higher replacement ratios (Stamer *et al.*, 2014). However, it is important to mention that larvae meal was used in diets for bigger trout. Another negative effect of diets with larvae/prepupae meal is the low content of essential fatty acids in the meal and consequently, in the trout fillet. This is of great concern, because one of the main reasons for fish consumption is the n-3 and n-6 fatty acids content. Some solutions have been proposed, like improving the feeding media of the larvae. Larvae reared in a medium enriched with fish offal present a better fatty acid profile and content (St-Hilaire *et al.*, 2007b).

There are also positive experimental results. No growth deficiencies were observed for rainbow trout (145 g mean body weight) fed experimental diets with prepupae meal (Sealey *et al.*, 2011). Larvae meal was reported to replace half of the fish meal in diets for rainbow trout, without significantly influencing its development. Moreover, in the same study, the FCR values for the control and experimental lots were the same, 0.8 and the average daily gain was slightly better in the experimental lot than that of the control lot, 1.45 and 1.43%, respectively (Stadtlander *et al.*, 2017). Trout fed diets with prepupae meal present lower intramuscular and intraperitoneal fat deposits than fish fed a standard diet (Sealey *et al.*, 2011). Some of the inclusion levels of *H. illucens* meal in the experimental diets of salmonids with good results are presented in Table 4.

Even though Atlantic salmon reared in salt water suffers significant growth deficiencies when administered diets with a complete replacement of fishmeal with insect larvae meal (Lock *et al.*, 2015), larvae meal can replace 50% of fishmeal without effects on growth. Interestingly, opposed to the situation observed for rainbow trout, the FCR modified, inversely proportional to the increase of larvae meal in the diets, resulting in lower values for larvae meal experimental diets than for the standard (control) diet, 1.24-1.14 and 1.24, respectively, for salmon. This is due to the higher lauric acid content found in larvae, which acts as a source of energy (Lock *et al.*, 2015). The high lauric acid content of diets based on larvae meals reduces the fat deposits of salmon liver without any other observed effects (Belghit *et al.*, 2018b). A good FCR value was recorded for salmon with an initial body weight of 46-49 g, farmed in freshwater, fed with diets including larvae meal and larvae oil. The FCR values of the experimental lots were only slightly higher compared with the one from the control lot, 0.79-0.85 and 0.77, respectively (Belghit *et al.*, 2018a). The percentage of arsenic and mercury decreased by 85 and 50% in salmon fed diets with larvae meal. No adverse effects were observed regarding the growth performances, apparent digestibility and chemical composition of salmon fed experimental diets with larvae meal and oil (Belghit *et al.*, 2018a).

Regarding the fillet palatability, sensory analyses show that there is no difference in the taste of trout fed standard feed and trout fed experimental diets with larvae meal (Stamer *et al.*, 2014), or prepupae meal (Sealey *et al.*, 2011). There are also results that suggest the taste of rainbow trout fillet is influenced by the larvae meal, the taste being characterised not in negative terms, but perceived as different from the taste of trout fed standard diets (Borgogno *et al.*, 2017). Sometimes, a metallic taste can be depicted for trout fed experimental diets with larvae meal. A possible explanation is that the larvae have the predisposition to bioaccumulate heavy metals, especially cadmium and lead (Li *et al.*, 2019).

Scientific literature offers some information regarding the biological value of proteins in the case of *H. illucens* used in diets for salmonids. When 50 and 75% of fishmeal is replaced by larvae meal in the diets of rainbow trout, the protein efficiency ratio (PER) is 1.70 and 1.27, respectively, while for the control lot it was 1.74 (Stamer *et al.*, 2014). Decrease in protein efficiency can be observed when the substitution of fishmeal with larvae meal increases. For Atlantic salmon fed diets with larvae meal replacing 85% of proteins from fish and soybean meals, the PER was higher than in the control lot, 2.86 and 2.77, respectively. Close values were obtained when, in addition, vegetable oil was replaced with insect oil (Belghit *et al.*, 2018a). The protein productive values (PPV) for diets with larvae meal are slightly lower than those of standard diets, 0.5

Table 4. Levels of inclusion of *H. illucens* meal in experimental diets of salmonids with positive results

Reference	Fish species	*H. illucens* ingredient	Recommended inclusion level of ingredient in the diet (%)
Sealey *et al*. (2011)	*Oncorhynchus mykiss*	Prepupae meal	<14.54
Lock *et al*. (2015)	*Salmo salar*	Larvae meal	10
Dumas *et al*. (2017)	*Oncorhynchus mykiss*	Larvae meal	<13
Renna *et al*. (2017)	*Oncorhynchus mykiss*	Partially defatted larvae meal	<20
Belghit *et al*. (2018a)	*Salmo salar*	Larvae meal	60
Belghit *et al*. (2019)	*Salmo salar*	Larvae meal	14.75

and 0.6, respectively, for rainbow trout (Stadtlander *et al.*, 2017). Belghit *et al.* (2018b) reported a PPV value of 0.3 for Atlantic salmon reared in floating cages fed diets with larvae meal replacing fishmeal upto 100%. There were no differences among the PPV values of the experimental lots and the control lot. Other results for Atlantic salmon fed diets with larvae meal show a PPV between 0.40 and 0.44, with the control value at 0.42 (Lock *et al.*, 2015). The essential amino acid index (EAAI) has the value of 1.46 for larvae and 1.55 for prepupae (data processed as per NRC 2011; Spranghers *et al.*, 2016; Cummins *et al.*, 2017).

The bacterial community of the digestive tract of trout fed diets with larvae meal is significantly more diverse, with changes in structure and composition (Bruni *et al.*, 2018). Furthermore, it has been pointed out that bacterial biodiversity of the digestive tract differs depending on the life stage and crude fat content of insects used in feeds. The results recorded for rainbow trout fed diets with larvae meal are the farthest from the values recorded for the control lot, fed normal diets (Huyben *et al.*, 2019). The histological traits of some organs are not affected by dietary inclusion of larvae meal, but there are negative effects on the oxidative stress indices. It is recommended to include upto only 20% larvae meal in rainbow trout feeds (Elia *et al.*, 2018). By ^{1}H NMR metabolomic approach, differences between plant, yeast, microalgae (*Spirulina platensis*) diets and larvae meals were demonstrated, with favourable results for the latter (Roques *et al.*, 2018). The larvae meal has positive effects on the gut health of Atlantic salmon by reducing excessive fat deposits in enterocytes from the pyloric caeca (Li *et al.*, 2019).

Conclusions

H. illucens larvae meal is an alternative source of proteins for traditional ingredients in salmonid feeds. The issue is highly studied, especially in the circumstances of constant fish meal supply and increasing demand for enhancing aquaculture production. Chemical composition and nutritional quality of the larvae are variable and depend to a large extent on the feeding media and processing method. Thus, there exists the possibility of modifying and controlling the chemical composition and nutritional quality of the larvae through the media used, in order to improve their suitability to the nutritional requirements of salmonids. The chemical composition and nutritional quality of the larvae make it suitable for salmonid feeds, especially from view point of protein biological value. Meanwhile, fish meal can only be partially replaced by *H. illucens* larvae or prepupae meals in rainbow trout diets. For Atlantic salmon, the total replacement of fish meal with larvae meal seems to be possible.

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Date of Receipt : 16.04.2020
Date of Acceptance : 24.09.2020