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Pre-pupae (larvae) of black soldier fly-a potential alternate protein source for aquaculture feeds

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Black soldier fly, Hermetia illucens, National Arboretum, Washington, D.C.

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Feed represents a large proportion of expenditure in fish farming and current farming practices mean that the main limiting factor in terms of feed is the use of fish meal and fish oil (Tacon and Metian, 2008). Fish meal is a major source of protein used in the formulation of feed for different fish species to increase feed efficiency and growth, as it helps to enhance feed palatability; nutrient uptake, digestion and absorption. The balanced amino acid composition of fish meal complements other animal and vegetable proteins to provide synergistic effects that promote faster growth (Mile and Chapman, 2006). Fishmeal has low fibre content and is also a valuable source of vitamins B1, B2, B6 and B12, in addition to calcium, phosphorous, magnesium, potassium, trace elements, attractants and fatty acids (Sheen et al., 2014; Dawood et al., 2015).

Approximately 20 million tonnes of the total marine catches go into the production of fish meal (Natale et al., 2013), equivalent to 36% of the total wild capture fishery production. However, total output from capture fisheries have not increased since the1980s (FAO, 2006) leading to unstable supply, higher demand and increasing cost of fish meal, suggesting that the current practice of using fish meal and fish oil in aquaculture is environmentally and economically unsustainable.

Aquaculture production in developing countries is expected to be the worst impacted (El-Sayed, 1999, Tacon et al., 2009, Welch et al., 2010). The majority of finfish aquaculture production currently revolves around omnivorous and carnivorous species such as carp and other cyprinids (Welch et al., 2010). Hence, current fish farming practices involving the use of fish meal must be optimised if the sector is to continue its rate of growth.

Optimisation of fish meal and fish oil use may be achieved through the use of alternative sources of protein (Hardy, 2010; FAO, 2014) which are cost effective and rich in protein (Lim et al., 2011). As a whole, plant and animal derived sources of protein have shown good ability to replace portions of fish meal in the diets. However, the accessibility of these protein sources is limited in developing countries in which a large proportion of aquaculture production is occurring, since most fish meal alternatives are incorporated into human consumption (Sathe, 1968).

Studies have shown that there is potential for insect meals to be incorporated into aquaculture diets, reducing the reliance on fish meal (Kroeckel et al., 2012). Insect meals have also been shown to allow for enriching through dietary intake, possibly for enrichment of polyunsaturated fatty acid levels. Insects form a natural food source for some aquatic animals. This and the presence of chitinase in them makes insect meal a logical alternative to fish meal. Of particular interest has been the use of black soldier fly larvae in fish diets.

The black soldier fly

The black soldier fly (*Hermetia illucens* Linnaeus 1758) is a member of the Stratiomyidae family. The adult fly is wasp-like and 15-20 mm long (Hardouin et al., 2003). Primarily black, the female’s abdomen is reddish at the apex and has two translucent spots on the second abdominal segment. The male’s abdomen is somewhat bronze in color.

H. illucens is native to the tropical, sub-tropical and warm temperate zones of America, but during World War II they spread into Europe, Asia, including India, and even to Australia. The development of international transportation since the 1940s has resulted in its naturalisation in many regions of the world (Leclercq, 1997). It is now widespread in tropical and warmer temperate regions (Diener et al., 2011), breeding in compost, manure and outdoor toilets. The flies can be seen in bright sunlit areas, resting on nearby structures or vegetation.

They are generally considered a beneficial insect and non-pests. The adult fly does not have mouthparts and doesn’t even feed during its short lifespan. They do not bite or sting, feed only as larvae, and are not associated with disease transmission. Black solider flies make breeding areas of houseflies less desirable. The fly is often associated with the outdoors and livestock, usually being found around decaying organic matter such as animal waste or plant material. Adult flies are easily distinguished by their long antennae (Gennard, 2012). Black soldier flies are an extremely resistant species capable of dealing with demanding environmental conditions, such as drought, food shortage or oxygen deficiency (Diener et al., 2011).

Life cycle

The duration of the life cycle ranges between several weeks to several months, depending on ambient temperature, and the quality and quantity of the diet (Veldkamp et al., 2012). In natural areas, black solider flies lay their eggs on moist organic material while in urbanised areas the BSF lays eggs in dumpsters or compost, which provide similar odors and nutritional needs to naturally occurring organic matter (Diclaro and Kaufman, 2009). Mating usually occurs two days after adult emergence from the pupal case. The male fly intercepts a passing female in mid-air and they descend in copula. Soon afterwards females begin to deposit egg masses near the edges of decaying organic matter. Male flies utilize lekking sites (gathering of males for competitive mating display), where they await female flies (Tomberlin and Sheppard, 2003).

The lifecycle of the black solider fly begin with about 500 eggs laid in a cluster that hatch within four days to three weeks (Mullen and Durden, 2002). About 1 mm long, the elongate-oval egg is pale yellow or cream colored when newly laid, but darkens with time. When newly hatched, the larvae are creamy white and about 1.8 mm long, slightly flattened, with a tiny, yellowish to black head. The skin is tough and leathery. Under optimal conditions, larvae take two weeks to reach the pre-pupal stage, but this period can increase to five months if food is limited (Furman et al., 1959; Myers et al., 2008; Gennard, 2012). On reaching the pre-pupal stage, the larva empties its digestive tract and stops feeding and moving (Hardouin et al., 2003).

Mature larvae are flattened and have a reddish-brown, elongated and slightly flattened body (Mullen and Durden, 2002). They can reach 27 mm in length, 6 mm in width and weigh up to 220 mg in the last larval stage. The larvae can feed quickly, consuming from 25 to 500 mg of fresh matter per day, on a wide range of decaying organic materials, such as rotting fruits and vegetables, coffee bean pulp, distillers’ grains, fish offal, corpses (used for forensic purposes) and animal manure (van Huis et al., 2013; Diener et al., 2011; Hardouin et al., 2003). Gayatri and Madhuri (2013) observed the fly hovering on a compost bin containing vegetable kitchen waste including stalks and peels of vegetables, eggshells, raw and processed leftover vegetarian as well as non-vegetarian food. The flies were observed on the upper strata of the bin and their larvae were found buried in the middle layers of the compost. The pre-pupae then migrate in search of a dry and protected pupation site (Diener et al., 2011).

The lifespan of an adult fly is about eight days (Mullen and Durden, 2002; Hardouin et al., 2003). These adults spend their short lifespan finding a mate and laying eggs (Mullen and Durden, 2002). The females mate two days after emerging and oviposit into dry cracks and crevices adjacent to a feed source (Diener et al., 2011). The adults do not feed and rely on the fats stored from the larval stage (Diclaro and Kaufman, 2009).The life-history traits of adults differ (Liu et al., 2008), depending on the quantity and quality of the food supplied to them as larvae (Tomberlin et al., 2002).The development of the ovaries and number of ovarioles in insects is genetically determined in most species. Nevertheless, the

Table 1. Mineral and amino acid composition of BSF larvae.

Mineral	Mean value	Amino acid	Mean protein
Calcium	75.6 g/kg	Alanine	7.7%
Phosphorus	9.0 g/kg	Arginine	5.6%
Potassium	6.9 g/kg	Aspartic acid	11.0%
Sodium	1.3 g/kg	Cysteine	0.1 %
Magnesium	3.9 g/kg	Glutamic acid	10.9 %
Iron	1.37 g/kg	Glycine	5.7 %
Manganese	246 mg/kg	Histidine	3.0 %
Zinc	108 mg/kg	Isoleucine	5.1 %
Copper	6 mg/kg	Leucine	7.9 %
		Lysine	6.6 %
		Methionine	2.1 %
		Phenylalanine	5.2 %
		Proline	6.6 %
		Serine	3.1 %
		Threonine	3.7 %
		Tryptophan	0.5 %
		Tyrosine	6.9 %
		Valine	8.2 %

Source: Arango Gutierrez et al., 2004; Newton et al., 1977; Sealey et al., 2011; St-Hilaire et al., 2007a.

number of ovarioles and their size can also vary depending on the quantity and quality of food consumed and stored during their life cycle (Magnarelli et al., 1982; Engelman, 1984).

Larva as bio-converter

Rearing *H. illucens* has been proposed as an efficient way to dispose of organic wastes, by converting them into a protein- and fat-rich biomass suitable for various purposes, including animal feeding for all livestock species, biodiesel and chitin production (van Huis et al., 2013; Diener et al., 2011; Li et al., 2011). They have been used to reduce animal manure in commercial swine and poultry facilities in western countries, but in India the practice is not common. Black soldier fly larvae can convert around 58% of the dry matter within an organic source into high quality animal feedstuff (Sheppard et al. 1994). There is a good opportunity to utilise these flies for bioconversion considering the fact that approximately 1.3 billion tonnes of food is wasted from the food produced each year (Gustavsson et al., 2011). The larvae work on organic waste material faster than worms used in vermicomposting. A colony of 2,000 larvae can consume about a kg of house hold food waste per day. They have large and powerful chewing mouthparts and hence are able to consume organic compounds before they have time to decompose, thereby immediately eliminating odor. Additionally, the larvae modify the microflora of manure, potentially reducing harmful bacteria such as *Escherichia coli* 0157:H7 and *Salmonella enterica* (van Huis et al., 2013). It has been reported that the larvae contain natural antibiotics (Newton et al., 2008). In addition to the larvae, the residue or castings which are obtained during larval rearing under controlled conditions can be used for soil amendment.

Rearing of the fly and larvae

Several methods have been designed for rearing black solider flies on substrates such as pig manure (Newton et al., 2005), poultry manure (Sheppard et al., 1994), and food wastes (Barry, 2004). The migrating behavior of the prepupae is used for self-collection (Diener et al., 2011). Optimal conditions for larval rearing include a narrow range of temperature (29-31°C) and humidity (between 50 and 70%), as well as a range of suitable levels of texture, viscosity, and moisture content of the diet. It is also necessary to maintain a year-round breeding adult colony in a greenhouse with access to full natural light. The greenhouse must be a minimum of 66 m3 to allow for the aerial mating process (Barry, 2004). Optimal temperatures for mating and ovipositing range between 24-40°C and 27.5-37.5°C, respectively (Sheppard et al., 2002). Wide ranges of relative humidity are tolerated: e.g. 30-90% (Sheppard et al., 2002) and 50-90% (Barry, 2004). The greenhouse would need a container with a very attractive, moist medium to attract egg-laying female adults (Barry, 2004).

Gayatri and Madhuri (2013) opined that rearing of black soldier flies is easier in India as larvae flourish more in a tropical environment than in a colder one. Larvae have been found in poultry houses in Punjab (Ashuma et al., 2007). For the first time in India, Freshrooms Lifesciences, Cuddalore, Tamilnadu, claims to have developed climate controlled artificial medium for rearing black soldier fly larae on large scale (http:// freshroomslifesciences.com/).

Nutritional composition of larvae

The larva is odorless and dry, and can still be easily dried for longer storage and friability. It can be fed to pets, fish and even earthworms and red worms (Leclercq, 1997; Veldkamp et al., 2012). Nutrient analysis revealed that freshly harvested pre-pupae contain 55-65% of moisture, a good amount of crude protein (40-44% dry matter), lipid rich in omega 3 and omega 6 fatty acids and crude fiber (7%) among other nutrients. The high dry matter content of larvae (35-45%) makes them easier and less costly to dehydrate than other fresh by-products (Newton et al., 2008).

The amount of fat is extremely variable and depends on the type of diet and on its fat content: Reported values are 15-25% of dry matter (larvae fed on poultry manure, Arango Gutierrez et al., 2004), 28% (swine manure, Newton et al., 2005), 35% (cattle manure, Newton et al., 1977), and 42-49% (oil-rich food waste, Barry, 2004).

Ash content varies from 11 to 28% of dry matter. The larvae are rich in calcium (5-8% dry matter) and phosphorus (0.6-1.5%) (Newton et al., 1977; St-Hilaire et al., 2007a; Arango Gutierrez et al., 2004; Yu et al., 2009). Many experts believe that the high calcium content of the larvae may halt or reverse the effects of metabolic bone disease. The amino acid profile is particularly rich in lysine (6-8% of the protein). Methionine, an essential amino acid is also found in the larvae (freshroomslifesciences.com).The fatty acid composition of the larvae depends on the fatty acid composition of the diet. The lipids of larvae fed cow manure contained 21% lauric acid, 16% palmitic acid, 32% oleic acid and 0.2% omega-3 fatty acids, while their proportions were 43%, 11%, 12% and 3%, respectively, in larvae fed 50% fish offal and 50% cow manure. Total lipid content also increased from 21% to

30% dry matter in the latter. Of the 30% lipid, 3% constituted polyunsaturated fatty acids (St-Hilaire et al., 2007a).This was in contrast to larvae reared on cow manure which had 21% total lipids with negligible amounts of long-chain unsaturated fatty acids (Sheppard et al., 1994).

Feeding trials with fish

Studies have shown that black soldier fly larval meal can replace a large proportion of the fish meal used in rainbow trout *(Oncorhynchus mykiss)* diets (St-Hilaire et al., 2007b; Sealey et al., 2011, Kroeckel et al., 2012). Dried ground pre-pupae reared on dairy cattle manure enriched with 25 to 50% trout offal replaced up to 50% of fish meal in trout diets for 8 weeks without significantly affecting fish growth or the sensory quality of trout fillets (Sealey et al., 2011). In a nine-week study, replacing 25% of the fish meal component of rainbow trout diets with pre-pupae meal, reared on pig manure, did not affect weight gain and feed conversion ratio (St-Hilaire et al., 2007a).

Chopped larvae grown on poultry manure fed to channel catfish *(Ictalurus punctatus)* alone or in combination with commercial diets resulted in a similar growth performance as the control diet. Aroma and textures of channel catfish fed larvae were acceptable to the consumer (Bondariand Sheppard, 1981). However, in a later study, replacement of 10% fish meal with 10% dried larvae resulted in slower growth of sub-adult channel catfish grown in cages over a 15-week period. However, the substitution did not reduce growth significantly when channel catfish were grown in culture tanks. Feeding 100% larvae did not provide enough dry matter or protein intake for channel catfish grown in tanks to allow sufficient growth. A comparison between menhaden fish meal and black soldier fly larval meal showed that the latter could be advantageous as a replacement for fish meal (Newton et al., 2005). A study wherein chopped larvae grown on poultry manure were fed to blue tilapia *(Oreochromis aureus)* either alone or in combination with commercial diets showed similar results as the control diets. Juvenile turbot *(Psetta maxima)* accepted diets containing 33% de-fatted larval meal without significant effects on feed intake and feed conversion. However, specific growth was reduced. Higher rates reduced intake and nutrient availability, with a further reduction in growth rate, possibly because of the presence of chitin (Kroeckel et al., 2012). Black soldier fly larval meal, reared on dried distillers' grains when fed to prawns *(Macrobrachium rosenbergii)*, resulted in a similar performance as regular prawn feed. The prawns fed larval meal were of a lighter colour (Tiu, 2012). Further studies are proposed to utilise this in the feeds for other cultivable fishes in larval, grow-out and brood-stock rearing.

Conclusion

The ability of black soldier fly larvae to convert low value organic waste products into a high value feedstuff accessible not only to carps, but also to carnivorous fish may limit the need for fish meal and fish oil in the aquaculture industry.

Larvae and pre-pupae of Hermetia illucens.

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