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REVIEW ARTICLE

Black soldier fly (*Hermetia illucens*) larvae as an ecological, immune booster and economical feedstuff for aquaculture

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ABSTRACT

Black soldier fly larva can renovate biological trashes into valuable nutrients, for instance, proteins, lipids, and chitin, which decrease ecological encumbrance happening due to organic wastes accumulation. Even though rapid demand for proteinaceous food is predictable, insects got less attention in the animal feed business primarily due to technical and monetary hurdles. Moreover, many times research highlighted the consumer and producer preferences for insects' meal potential in livestock feeding. This review is anticipated to elucidate the prominence of black soldier fly larvae meal as a substitute to conventional feedstuffs including soybean and fishmeal and soybean oil ensuring productive, cost proficient, environmentally friendly, least land necessitating, least pathogenic risk, immunity-boosting, purely organic and everlasting source of non-conventional protein feedstuff for aquatic habitats.

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Introduction

Aquaculture is the most rapidly developing commerce globally. Approximately half of the global food demand is met by fish production and it will increase up to 70% until 2030 (Obiero et al., 2019). Aquaculture has been increasing widely since the last decade (FAO, 2016). Aquaculture comprises a variety of species and farming approaches, succeeding in various communal, financial, nutritive, and eco-friendly aftermaths (Gephart et al., 2020). The evolution of worldwide aquaculture upsurges the ultimatum for aqua feedstuffs. Feeds delivers nutrient to fish for quicker development, existence and support their vigorous lifespan. It represents the key of the entire operative budget in aquaculture approximately 60%

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(Pauly & Zeller, 2017). The global manufacturing of aquaculture feed was about 0.04 billion tons in 2018 (Mo et al., 2018). There is a necessity of alternative protein resources for fish feeding because fish and soybean are also the staple food of human. Moreover, poisonous gas emissions and land erosions occur due to soybean cultivation (Cottrell et al., 2020). Almost 12% of the 0.171 billion tons of entire fish production was used to produce fishmeal and fish oil in 2016 (Gerber et al., 2013). The monitoring scheme varies commonly amongst countries globally about the use of insect feeding to animals and consumer preferences and acceptance about insect utilization (Lahteenmaki-Uutela et al., 2017). In 2001, (EC 999/2001) because of Bovine Spongiform Encephalopathy (BSE), processed animal proteins (PAPs) were excluded due to contagious BSE persepective (European Parliament and Council, 2009). In the following year (European Parliament and the Council of the EU, 2002), the list of feedstuffs intended for animal feeding were outlined along with their maximum tolerable inclusion levels (Verstraete, 2013).

On 16 January 2013, Annexes I and IV to EC Regulation No: 999/2001 was amended along with directive 2002/32/EC and EC Regulation No: 56/2013 acknowledged the use of non-ruminant originated processed animal proteins to be permissible in aquaculture (European Parliament, 2013). Nonetheless, insects were not specifically enclosed in the raw material directory of the European Commission. So, on 24 May 2017, EC amended annexes X, XIV and XV to EU Regulation No: 142/2011 and Annexes I and IV to EC regulation No: 999/2001 and EU Regulation No: 2017/893 was publicized whose Annex II specifically stated the farmed insect derived proteins to be utilized for the aquaculture, farmed animals other than fur animals and pet animals (European Commission, 2017) and it allowed use of three species of Crickets including *Gryllus assimilis, Acheta domesticus* and *Gryllodes sigillatus*; two species of Mealworms including *Alphitobius diaperinus* and *Tenebrio molitor*; common house fly (*Musca domestica*) and black soldier fly (*Hermetia illucens*). Moreover, in this regulation the fat extracted from these insects was allowed to be fed to every sort of animal. Annex III to Regulation No: 767/2009 forbids the use of feces and digestive tract content although these ingredients are used in other countries for insect production. Rendering to EC Regulation No: 1069/2009 broadcasted on 21 October 2009, insects are well-thought-out as 'farmed animals' and therefore, for their feeding the use of dung, kitchen waste comprising meat and fish, are not permissible (European Parliament and Council, 2010).

The present review illustrates the importance of black soldier fly larva in aquaculture feeding as an economical, more environmentally friendly, immunizing and productive ingredient.

Black Soldier Fly (BSF) as Eco-Friendly Feed Ingredient

Feed and food demand puts a key environmental impact worldwide that could be increased up to 80% by 2050 due to the nonexistence of high-tech modification and alleviation processes (Wiseman et al., 2019). Livestock is considered to be the most environmentally detrimental of all the evolutionary happenings with immense primary and secondary influences to global warming (75% of total agricultural emissions) (Springmann et al., 2018). Owing to less feed efficacies, poor intestinal fermentation in cattle, and higher gaseous emanation in feces there is extensive ecological humiliation as land deprivation, harm to natural habitats, greenhouse effects, and stress on aquatic and land species (Sakadevan & Nguyen, 2017). Restaurant, bakery and kitchen waste is getting increased consideration for its management due to its undesirable ecological, social and health influences (Teigiserova et al., 2020). Undeniably, the EU expected this Sort of waste material volumes to about 100 million tons per year in the European Union countries recommending that the forecast for 2020 is 140 million tons (Guo et al., 2021). According to the European Union Waste Framework Directive (2008) efficient utilization of such sort of organic wastes must be the foremost stratagem to decline the ecological hazards in Europe.

Keeping this in view, the environmental responsiveness of insects as food and feed has been studied widely with orientation to alternatively sustainable, ecologically and economically sufficient protein sources (Gahukar, 2016). Biotransformation of BSF larvae and contribution to the greenhouse emissions could be changed according to the diversity and availability of the substrates (Rahmi et al., 2020). The feeding of bio-wastes to BSF has direct effect on the larvae growth performance, waste conversion ratio, and nutritional contents of BSF larvae (Raksasat et al., 2020). In comparative research conducted about putrefaction of organic wastes by BSF larvae versus aerobic bacteria by Perednia et al. (2017), it was reported that larvae consumed the waste material within one week with 28% of CO_2 emission while bacterial decomposers utilized it within 45 days with 50% CO_2 discharge in the environment. It was reported that the BSF larvae converted about 45% of the biomass fed into valuable feedstuff enriched with protein, fats and chitin. In research conducted about BSF larvae growth on restaurant waste and vegetable/fruit waste, it was determined that CH_4 and N_2O emissions were very low as compared to the conventional protein feedstuffs (Bosch et al., 2019). According to Bosch et al. (2019), the impact of BSF larvae on environmental pollution was relatively very low as compared to the soybean and fishmeal when BSF was grown on manure and food waste. Rearing of BSF larvae by using bovine manure and other metropolitan waste emits lower greenhouse gases rather than using beet pulp as a substrate for larvae growth. Similarly, larvae have grown on municipal wastes and has emitted 2.1 kg of CO_2 and 0.05 m^2 land has been used per kg of larval protein (Salomone et al., 2017). Moreover, it was reported that there is no significant conservational influence when animal feedstuffs like sorghum, DDGs, beet pulp, poultry feed is being used for BSF rearing. It was scientifically evaluated in a research trial where BSF larvae was grown on manure containing an antibiotic, tetracycline residues (Caligiani et al., 2018) and insecticides (Lalander et al., 2016) and it significantly degraded these residues, thus it could be stated that BSF production assists to eliminate the ecological hazards and public health concerns.

BSF as Immunity Booster Ingredient

BSF larvae have immune regulating properties due to some of antimicrobial peptides and chitin present in them. In a study, broiler chicks were confronted orally with *Salmonella* and *Escherichia coli* and fed with mealworm as a substitute to antibiotics, as a result, the cecal contents of *Salmonella* and *E. coli* were decreased along with the rising level of serum IgG and IgA and it was indorsed due to the effectiveness of chitin content of insects acting as probiotic (Islam & Yang, 2017). In a study conducted on chick manure, house fly larvae reduced the levels of *E. coli*, *Salmonella enteritidis* and *Campylobacter jejuni* (Nordentoft et al., 2017). Similarly, black soldier fly larvae have been reported to reduce *E. coli O157:H7* and *S. enterica* serovar *enteritidis* in poultry manure (Erickson et al., 2004). Research on broilers show that feeding BSF larvae initiates immune response against *Salmonella enterica* serovar *Gallinarum,* a typhoid fever causing strain in chicks. (Provost et al., 2011). Moreover, using 0.2% chitosan obtained from BSF larvae reduced the number of *S. enterica* serovar *Typhimurium* in broilers (Menconi et al., 2014).

A phenolic compound, melanin, is a bio-active component that is used as a feed additive to prevent stress and tumors in farmed animals (Cordero & Casadevall, 2020). Melanin that imparts black color to black soldier fly is eumelanin and it has a wide range of antibacterial and antifungal action (Ushakova et al., 2017). In a study, the extract of BSF larvae was evaluated for antibacterial action by the agar diffusion method. The concentration of 320 mg/ml showed a potent ($P<0.05$) bactericidal action against *Salmonella* and *E. coli* due to the presence of chitin and 49.5% lauric acid, a strong antibacterial saturated fatty acid (Harlystiarini et al., 2019). Similarly, methanol extract of BSF larvae has shown a reduction in growth and proliferation of gram-negative bacteria including *Neisseria gonorrhoeae, Klebsiella pneumoniae, Shigella spp.* (Choi et al., 2012). Not only BSF larvae show activity against gram-negative bacteria but also against gram-positive bacteria. A new defensin-like peptide 4 (DLP4) was harvested from the hemolymph of BSF larvae and it was purified by chromatography and resulted to have strong antibacterial activity against MRSA (methicillin resistant *staphylococcus aureus*) (Park et al., 2015). Similar to this, cecropin-like peptide 1 (CLP1) was extracted and purified from the hemolymph of BSF larvae and evaluated to have good inhibitory activity against gram-negative bacteria (Park & Yoe, 2017). Anti- *H. pylori* peptides were extracted from the hemolymph of BSF larvae and were used to combat metronidazole resistance against these bacteria (Alvarez et al., 2019). In a study, two antimicrobial peptides extracted from BSF larvae termed as Hidefensin-1, Hidiptericin-1 decreased the growth of *Streptococcus pneumoniae* and *Escherichia coli* by bacterial membrane lysing (Xu et al., 2020). The lyophilized BSF larvae methanol extract was evaluated to have good action against *Staphylococcus aureus* and *pseudomonas aeruginosa*. After HPLC, the extract showed a strong activity against methicillin resistant *staphylococcus aureus* (MRSA) (Park et al., 2014). Hexanedioic acid or apidic acid from BSF has shown growth inhibition effects against *MRSA*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Shigella dysenteriae* in dose dependent manner (Choi & Jiang, 2014). Recombinant attacin-like peptide obtained from BSF has shown bacteriostatic activity against *MRSA* and *E. coli* (Shin & Park, 2019).

Based upon above-mentioned studies it is clear that BSF larvae have many sorts of antimicrobial peptides that prevent the animals from diseases and averts the use of antibiotics in feed and certainly discourage antibiotic resistance.

BSF Production and Larvae Defattening Techniques

For the production of BSF larvae, there are some optimum parameters that should be controlled for their proper BSF breeding and larva hatching. The nutritional profile varies with respect to the substrate used for larvae rearing. For instance, in a study aiming to research breeding techniques of BSF, it was shown that the best larvae density was 2 larva/gram of the feed and optimum oviposition was 8500 individuals/m^3 under a maximum of sunlight exposure. The prepupae provided with soybean meal, euphorbia leaves supplemented with colza oil were enriched in C18:2n-6 fatty acid, a fatty acid essential for the growth of tilapia fish. The prepupae provided with soybean meal and fish offal were enriched in C20:5n-3, a fatty acid healthy for humans. Moreover, for optimum production, a temperature of 26.7±0.49°C and light duration of not less than 12 hours is required. The cost of larva production using soybean and fish offal was 1.83 USD per kg of the larvae (Gougbedji et al., 2021). According to Bekker et al. (2021), the effects of moisture content in the substrates was evaluated on the growth rate of the larvae and the results showed highest growth efficiency (0.62) at 45% moisture and lowest (0.52) at 75% moisture content. BSFL larvae have approximately 30% fat as dry matter basis. High fat level causes the product prone to lipid peroxidation making unpleasant texture and flavor. Moreover, it may cause smearing and blocking of the pelleting machines. A simple and competent method to defat BSF larvae is the mechanical extraction by a screw press as used in oil extractions of nuts and seeds. At 100°C the fat is squeezed from the larvae by screw press giving cake and liquid. Further processing separates protein meal from the cake and BSF larvae fat from the press liquid (Matthäus et al., 2019). Another way to extract fat from the BSF larvae is microwave assisted solvent extraction method. In this method, first of all larvae cell disruption is performed by a rotor-stator homogenizer. Then, the insect slurry is dried in a thermal dryer. Then the dried insect powders (about 20 g) are placed in holder with hexane solvent. This lipid extraction process is performed using a microwave equipment. The liquid part consisting of lipids and solvent, and residual defatted insect powders are physically divided after lipid extraction by centrifugation (Feng et al., 2019). According to 2-methyloxolane (2-MeO) has better lipid extraction capability, improved bioactivity in the BSF oil, and comparatively superior protein quality in the defatted flour as compared to n-hexane and hence it is the best solvent for BSF larvae fractionation (Ravi et al., 2019). In another study, organic solvents were compared with inorganic solvent and the results showed that organic acid increase the lipid extraction yield as 45% in case of organic solvent that was 35% in case of inorganic solvents. Organic lactic acid increased the lipid purity from 75% to 85% with 60% of protein purity (Soetemans et al., 2019). Many protease enzymes are used for lipid extraction to fast the process in solvent extraction method. Among them, protamex is the more effective enzyme during pretreatment increasing the fat fraction 2.2 times more as compared to when no enzyme was used (Su et al., 2009). Another technique for larvae fat extraction is by using CO_2 supercritical extraction method. İn this method, 10–18 mm sized crushed larval powder is delivered to carbon dioxide supercritical extraction machine at 350 bar pressure for 6 hours and it produces a powder with maximum 5% of fat (Kim et al., 2019). Another way to extract fat from larvae is by wet mode fractionation (WMF). In this method, BSF larvae are steam blenched to obtain pulpy texture. After that BSF larvae juice and press cake is obtained. The BSF juice part is homogenized and incubated with enzymes and subsequent centrifugation gives four phases (lipid, cream, aqueous and solid residuals) having more aqueous and solid fraction. Moreover, the press cake contains 60 % of the chitin in it (Ravi et al., 2021).

Significance of BSF in Aquaculture

Insects are the most valuable feedstuffs for fish feeding and amongst the insects, the nutritional profile of the black soldier fly larva is very close to and sometimes better than fishmeal. Moreover, insects are easy to house and grow, cause an insignificant environmental influence and have high protein ratio as compared to conventional feedstuffs (Llagostera et al., 2019). Many studies signify the importance of the BSF larvae in fish farming. In a recent study, the fish meal was replaced fully and partially by BSF prepupae for climbing perch *Anabas testudineus*. The results showed that final weight and crude protein of fillets were similar to the control group while crude fat and protein efficiency ratio was better in the 100 percent replacement group (Vongvichith et al., 2020). In a 56-day trial, soybean meal was replaced with defatted BSF larvae meal and fed to juvenile grass carp and the results showed no significant difference in feed efficiency, productivity. However, triglyceride content and total antioxidant status was better in 100% BSF fed group as compared to soybean fed group (Lu et al., 2020). Defatted BSF larvae meal was replaced with fish meal and fed to Japanese seabass (*Lateolabrax japonicus*) without any significant effect on final weight, hepatic and gastric histopathology, antioxidant status and immunity. Moreover, deposition of lipids in the liver was reduced in BSF fed groups as compared to the control (Wang et al., 2019). Fish meal was replaced with BSF larvae meal and fed to juvenile rainbow trout (*Oncorhynchus mykiss*) for intestinal microbial population effect and results indicated the proliferation of lactic acid bacteria, *Megasphaera, Pectinatus, Selenomonas, Zymophilus, actinobacteria* and *Bacillaceae* (Huyben et al., 2019). Similarly, fish meal replacement was performed with BSF larvae meal for juvenile Jian carp and it was reported to have significant effect on body weight gain (Zhou et al., 2018). Partially defatted BSF larvae meal was replaced with fish meal and fed to rainbow trout (*Oncorhynchus mykiss*) without any significant difference on the growth rate, villus and crypt depth and dorsal fillet quality and higher apparent digestibility coefficient of dry matter and crude protein were seen for BSF fed groups as compared to fish meal group (Renna et al., 2017). Partially defatted BSF larvae meal was fed to rainbow trout diet as a replacement to fish meal and the results showed no difference in growth and performance while higher concentration of Hydroxyproline in BSF fed groups was seen as compared to the control group (Dumas et al., 2018). According to (Muin et al., 2017), fish meal can be substituted up to 50% with BSF larvae meal without affecting growth performance and efficient feed utilization. Fish meal and soybean meal were substituted with BSF larvae and fed to Nile tilapia without having any adverse effect on fish meat quality parameters, growth and feed efficiency (Devic et al., 2018). BSF full-fat larvae meal was used as a replacement to fishmeal and fed to zebrafish and the result showed a better lipid profile in the meat of the fish (Zarantoniello et al., 2018). Many studies have shown that 50% substitution of the fish meal with BSF larvae meal is the best conciliation between ingredient bioavailability and appropriate fish development and production (Zarantoniello et al., 2020; Oteri et al., 2021). Fish meal was replaced with BSF prepupae meal and fed to European seabass (*Dicentrarchus labrax*) juveniles and results showed that there is no significant difference in weight gain, feed utilization and plasma metabolic parameters. Moreover, the apparent digestibility coefficient of histidine, arginine and valine was more in BSF fed groups as compared to the control (Magalhães et al., 2017). Recently, European seabass (*D. labrax*) was confronted with *Vibrio alginolyticus* and fed with BSF larvae meal as a partial substitution to fishmeal and the result showed a linear significant decrease in tissue and blood malondialdehyde level and increase in superoxide dismutase, catalase and glutathione peroxidase enzyme activities. Moreover, the serum lysozymes and phagocytic actions was increased in BSF larvae fed groups as compared to the control group (Abdel-Latif et al., 2021). Fish meal was replaced with BSF larvae meal in the diet of juvenile white shrimps and results showed that there was no significant difference in weight gain and feed conversion as compared to the control group (Cummins et al., 2017). Partial replacement of fishmeal was done with BSF larvae meal and fed to rainbow trout juveniles without decreasing growth performance, feed conversion rate and product quality (Stadtlander et al., 2017). In another study, rainbow trout were fed with BSF larvae meal as compared to the fishmeal in control. The physical and chemical analysis of the dorsal fillets were performed after freezing them for 120 days. The results showed that higher levels of replacement did not alter the pH, color, shear force and water holding capacity while the monounsaturated fatty acids in the flesh were lower as compared to the control (Secci et al., 2019). Juveniles of *Argyrosomus regius* were fed with BSF larvae meal as replacement to fish meal and the results showed that saturated fatty acids and polyunsaturated fatty acids, lauric acid and plasma glucose levels were increased with respect to the increasing level of BSF larvae meal supplementation in the experimental diets as compared to the control (Guerreiro et al., 2020). In a 65-day feeding trial of BSF larvae meal as replacement to fish meal in yellow catfish showed increased growth performances and serum lysozyme action enhancing immune response as compared to the control group (Xiao et al., 2018). In another study, juveniles of African catfish (*Clarias gariepinus*) were fed with BSF larvae as a substitution to fishmeal and the results showed that the group supplemented with 50% of the BSF meal have highest body weight gain, better growth rate and nutrient utilization indexes, improved superoxide dismutase and catalase activity contributing to valuable antioxidant status as compared to the control group (Fawole et al., 2020). Nile tilapia (*Oreochromis niloticus*) juveniles were fed with BSF larvae meal as replacement to fishmeal for 3 months and the results showed that the growth parameters and feed efficiency were not significantly different ($P>0.05$) amongst all of the experimental groups. Moreover, the lysozyme and peroxidase activity were enhanced in BSF fed groups as compared to the control group (Tippayadara et al., 2021). In 42 days feeding trial of BSF larvae meal as replacement to fishmeal in juvenile barramundi (*Lates calcarifer*), the results showed no significant difference on growth but the immunity and intraperitoneal fat were improved in BSF fed group as compared to the control (Chaklader et al., 2020). There are many more studies which indicate the positive influence of BSF larvae meal in aquaculture without influencing growth and health performance.

Conclusion

Based on the above-mentioned studies, black solider fly larva is an effective replacement for fish meal and soybean meal for feeding aquaculture without any negative impact on production. The nutritional profile of BSF larvae varies with respect to organic wastes being used. Growing BSF larvae on vegetables, fruits, kitchen wastes and leftover poultry wasted feeds produce larvae having an excellent nutritional profile. Moreover, the essential amino acids contents are also good in BSF larvae as compared to expensive soybean and fish meal. Generally, the protein and fat content of BSF larvae is very equivalent to soybean and fish meal. Substitution of conventional protein ingredients don't have any negative effects on the production and product quality if used in a limited percentage. With respect to economics and environmental impact, BSF larvae are very cheaper to grow because they only need biowastes, and very little land and labor are required for BSF farming. It helps to reduce the impact of organic wastes on land and convert them to organic fertilizers. BSF larvae do not release any poisonous gases in the environment as compared to soybean. There is no uncertainty to conclude that BSF larvae production for aquaculture has the potential to decrease the feed costs and it provides supernatural, environmentally friendly, and immunity boosting feed to aquatic habitats.

Compliance With Ethical Standards

Authors' Contributions

Author SRAS has planned and written the manuscript and author ISÇ has technically studied the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

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