



### Exploring the Role of Insects as Sustainable Feed in Aquaculture Nutrition and Enhancing Antioxidant Capacity, Growth and Immune Response

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#### Abstract

As the global demand for aquaculture products continues to rise, sustainable and efficient feed sources are crucial for the industry's growth. Insects have emerged as a promising alternative due to their high nutritional content, low environmental impact, and potential to alleviate overreliance on conventional feed ingredients. This abstract delves into the role of insects as sustainable feed in aquaculture, elucidating their benefits and challenges. Insects offer a rich source of fats, minerals, proteins, and vitamins that meet the dietary requirements of various aquatic species. Their rapid growth rates and ability to thrive on organic waste contribute to their eco-friendly nature, reducing the environmental burden associated with aqua-feed production. Notably, insects can be reared on various organic substrates, minimizing competition for arable land and reducing the demands on wild fish hoards utilized for fishmeal construction. However, integrating insects into aqua-feed presents certain hurdles. Standardized production methods, quality control, and regulatory frameworks need to be established to ensure consistent and safe insect-derived feed. Additionally, understanding the potential impacts of insect-based diets on fish health, growth, and product quality is vital. It is conclusion that the insects hold immense potential as a sustainable feed source for aquaculture, addressing nutritional demands while minimizing environmental impacts and addressing the challenges through collaborative researcher research, and eco-friendly aquaculture sector.

#### Introduction

Aquaculture or Fish farming is essential for supplying the rising demand for seafood on a global scale. However, the sustainability of aquaculture practices has become a growing concern. One major aspect contributing to sustainability is the feed used in fish farming (Freccia, et al., 2020). Traditional fish feed sources, such as fishmeal and soybean meal; often rely on unsustainable practices, including overfishing and deforestation. Therefore, finding alternative, sustainable feed sources is imperative for the long-term viability of aquaculture (Röthig et al., 2023). To fulfill the rising demand for seafood around the world, aquaculture the growing of aquatic organisms like fish, shellfish, and aquatic plants has become an essential sector. As the world population continues to grow, sustainable food production practices are crucial to ensure food security and protect the environment (Maulu et al., 2022; Alfiko et al., 2022). One significant

aspect of sustainability in aquaculture is the feed used for the farmed organisms. Firstly, it contributes to overfishing, depleting wild fish populations and disrupting marine ecosystems (Llagostera et al., 2019; Röthig et al., 2023). Secondly, the production of fishmeal and fish oil often involves industrial fishing methods that harm non-target species and damage marine habitats (Llagostera et al., 2019). Lastly, the reliance on wildcaught fish for feed places a burden on global fish stocks and undermines efforts to maintain healthy ocean ecosystems (Freccia, et al., 2020).

To address these challenges, there is a growing need for sustainable feed sources in aquaculture (Arru et al., 2019). Sustainable feed refers to alternative ingredients and production methods that minimize environmental impact, reduce reliance on wild fish stocks, and promote the responsible use of resources (Röthig et al., 2023). By transitioning to sustainable feed, aquaculture can mitigate its ecological impression, ensure long-term viability, and contribute to a more sustainable food system. Sustainable feed options in aquaculture encompass a range of possibilities (Freccia, et al., 2020; Röthig et al., 2023). These include the utilization of plant-based ingredients, such as soybean meal and algae, which reduce the reliance on wild fish as feed inputs (Alfiko et al., 2022). Additionally, researchers and farmers are exploring alternative protein sources, such as insects, microorganisms, and single-cell proteins that can offer efficient and ecofriendly feed options (Mulazzani et al., 2021).

Insects have emerged as a promising solution to the sustainable feed challenge in aquaculture. While insects might not be the first thing that comes to mind when thinking about fish feed, they offer several unique advantages (Röthig et al., 2023). Insects are highly nutritious, rich in proteins, essential amino acids, and micronutrients necessary for fish growth and development (Llagostera et al., 2019). Moreover, insects can be reared using organic waste streams, dropping the environmental impression of feed production. As a result, insects have gained awareness as a sustainable and environmentally friendly feed alternative for aquaculture (Mousavi et al., 2020). In current years, insects have gained significant attention as a sustainable and viable alternative for aquaculture feed. These small creatures offer a range of benefits that make them an attractive option for sustainable feed production (Freccia, et al., 2020). Insects are highly nutritious and possess a rich profile of proteins, essential amino acids, and micronutrients (Shah et al., 2022). Many insect species have protein content comparable to or even higher than traditional feed sources like fishmeal. Furthermore, insects are often packed with vitamins, minerals, and fatty acids necessary for the growth and development of farmed organisms (Binggian et al., 2023). One of the key advantages of using insects as feed is their low environmental impact (Nogales-Mérida et al., 2019). Insect farming requires less land, water, and energy compared to traditional feed sources like soybean or fishmeal production. Furthermore, pest can be nurtured on untreated waste materials such as agricultural byproducts, food scraps, or manure (Freccia, et al., 2020; Hua 2021). This ability to up cycle waste streams reduces the environmental burden associated with feed production and contributes to a circular

with feed production and contributes to a circular economy approach (Alfiko et al., 2022). By utilizing insects as a feed source, aquaculture can decrease its dependency on wild-caught fish for fishmeal and fish oil production (Hua 2021). This shift helps alleviate the pressure on global fish stocks and promotes the conservation of marine ecosystems. Insects can be raised sustainably in controlled environments, minimizing the impact on natural habitats and allowing for more efficient use of resources (Röthig et al., 2023).

Insect farming has demonstrated excellent scalability potential. Insects reproduce rapidly and have high conversion rates, meaning they can efficiently convert feed into biomass (Shah et al., 2021). Their short life cycles allow for quick and continuous production cycles. Additionally, insect farming can be practiced in various scales, from small-scale operations to large commercial facilities, making it adaptable to different aquaculture setups (Barroso et al., 2014). As a sustainable feed source, insects offer a promising solution to the environmental and ethical challenges faced by aquaculture. While still a developing field, the use of insects in feed production has shown significant potential and is gaining momentum in the industry (Llagostera et al., 2019; Röthig et al., 2023). Researchers, farmers, and entrepreneurs are exploring innovative methods to rear insects efficiently and economically, contributing to the development of a sustainable insectbased feed sector. Traditional fish feed sources, such as fishmeal and soybean meal, pose significant environmental challenges (Freccia, et al., 2020; Röthig et al., 2023). The production of fishmeal relies heavily on the global fishery industry, contributing to overfishing and depletion of wild fish stocks. This not only disrupts marine ecosystems but also threatens the livelihoods of coastal communities reliant on fishing. Additionally, the cultivation of soybean meal often involves large-scale deforestation, leading to habitat loss, biodiversity decline, and increased greenhouse gas emissions (Furuya 2010; Freccia, et al., 2020).

Insects offer several ecological advantages as a feed source for aquaculture. Firstly, pests can be nurture using untreated waste materials, such as food scraps or agricultural byproducts. This reduces the environmental burden associated with waste disposal while simultaneously converting waste into valuable protein and nutrient-rich feed (Shah et al., 2021 and 2022). Furthermore, insect farming requires minimal land, water, and energy compared to traditional feed production methods (Shah et al., 2021). The lower resource requirements and reduced carbon impression make insects a sustainable and environmentally friendly option. Freccia, et al., (2020) the utilize of insects as nourish in aquaculture reduces the industry's reliance

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on wild-caught fish for fishmeal and fish oil production (Tubin et al., 2023). By shifting to insect-based feed, aquaculture can alleviate the pressure on wild fish stocks, allowing them to recover and maintain healthy population levels. This contributes to the conservation of marine ecosystems and helps preserve biodiversity (Freccia, et al., 2020; Röthig et al., 2023). Insects, being a renewable and readily available resource, can provide a more sustainable and scalable feed option for the aquaculture industry (Alfiko et al., 2022). While numerous published papers and reports have explored the utilization of insects as protein resource in fish feeds, the existing knowledge in this field remains fragmented (Hua 2021). Although the experimental results are promising, there is a need to gather and compile comprehensive information from various sources. Therefore, the purpose of this review is to conduct a thorough survey and compile a comprehensive overview of the information available in Figure 1. This review are consists of the following sections. Section 1, Introduction; Section 2, Nutritional aspects of insect meal and their effect on aquaculture; Section 3, Comparing the nutrient profiles of insects to traditional fish feed sources,; Section 4, The potential benefits for fish health and growth; Section 5, Conclusion.

### Nutritional Aspects of Insect Meal and Their Effect on Aquaculture

Three bug species that have been thoroughly investigated will be the focus of our exploratory analysis: Black soldier fly (*Hermetia illucens*), Housefly (*Musca domestica*), and mealworm (*Tenebrio molitor*). Within the context of European aquaculture, especially in the cultivation of European sea bass, gilthead sea bream, and rainbow trout, these species have demonstrated remarkable promise and attracted major interest.

For the successful incorporation of option component in animal feed, a comprehensive understanding of their dietary characteristics (biological, chemical, and physical) is crucial. This information plays a paramount role in assessing their potential as substitutes or complementary components in animal diets (Gasco et al., 2016). An exhaustive evaluation of these ingredients within the animals' diet is essential, taking into account factors such as sustainability throughout the entire production chain, economic effects, and factors including digestibility, performance, nutrient balance, carcass features, and sustainability (Ogunji et al., 2008). The immense

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#### Potential benefits for fish health and growth

- 1. Enhanced growth performance accompanied by a decrease in survival rate.
- Enhanced development performance and optimized feed utilization, accompanied by increased antioxidant enzyme activity, contribute to reduced lipid oxidation in the fillet.
- 3. Elevated growth performance and restored gut microbiota equilibrium.



Collected blood and meat for further analysis



Figure 1. Role of insects as sustainable feed in aquaculture nutrition



potential of utilizing insects in both animal and human food arises from the staggering diversity of over 1 million known species (Shah et al., 2022). This abundance opens up countless possibilities and alternatives for their application. However, it is imperative to conduct extensive research. Identification of a species with potential should be followed by the development of strategies encompassing production, reproduction, genetic evolution, and processing (Ogunji et al., 2008). By doing so, we can harness the full benefits of this resource while ensuring its viability and compatibility within our food systems (Gasco et al., 2016). The amino acid content, bromatological composition, and fatty acid profile of diverse insect diets were compiled and categorized in Table 1 and 2.

The quantity of ethereal extract (EE) in insect meal can vary, which has an impact on the crude energy (kcal kg1) of the diets, the energy-to-protein ratio, and the amount of ethereal extract found in the corpses (Ogunji et al., 2008). Some studies have also highlighted elevated levels of ethereal extract in insect meal, leading to limitations in their inclusion in diets (Ogunji et al., 2008; Gasco et al., 2016). High ethereal extract values increase the risk of fat oxidation (rancidity), thereby reducing the product's shelf life. To mitigate this issue, the addition of antioxidant additives in insect meals is suggested. A short-term solution involves refining preprocessing and manufacturing methods to extract excess lipids from the meals, which can then be used as a lipid source in feeds or other industries. This approach has already been employed in the production of terrestrial animal by-product meals (Nogales-Mérida et al., 2019). Several studies have revealed significant variations in ethereal extract (EE) and crude protein (CP) content within the same insect species. For instance, one study establish 40% CP and 25% EE in tenebrio, which is lower than values reported in other studies showing crude protein levels exceeding 50% but with lower EE levels than the current research. Insects can serve as valuable sources of both protein and energy (Belforti et al., 2015; Gasco et al., 2016). Insects often have greater EE values during the larval stage as they build up energy for metamorphosis. Moreover, their fatty acid profiles show considerable variability, suggesting that the insects' fatty acid (FA) profile can be modulated through dietary manipulation (Gasco et al., 2016). This may be a viable method for obtaining important fatty acids from inferior materials, such as EPA (eicosapentaenoic acid, 20:5n-3) and DHA (docosahexaenoic acid, 22:6n-3) (Ogunji et al., 2008).

Roasted insect meal establish to be an excellent protein source, with impressive protein content (~66.84%), surpassing typical fish diet protein components include viscera meal, fishmeal, meat and bone meal, and soybean meal (Barroso et al., 2014). In contrast, lower-quality ingredients like feather meal and blood meal require processing to enhance their digestibility (Barroso et al., 2014; Shah et al., 2022). This highlights the potential of insect-based feed to provide superior protein nutrition for fish and other animals, offering a sustainable alternative to traditional protein sources. Regarding the nutritional profile, it is crucial to conduct thorough research on utilizing organic residues for insect production, particularly when using food sources with elevated levels of mycotoxins (Gasco et al., 2016). When insects consume these mycotoxins, it can lead to production issues and pose a significant concern as they are bioaccumulative (Gasco et al., 2014). Consequently, this can adversely affect the value of insect food and subsequently impact animal performance. Therefore, it becomes essential to carefully assess the potential risks associated with mycotoxin-contaminated organic residues in insect farming to ensure the overall success and safety of the process (Kroeckel et al., 2012).

Species	Stage	Proxima	te analy	sis (% dry i	matter)	Amir	no acid (%	% total)	Fatty acid (% total)			
		NFE %	EE %	ASH %	CP %	LYS	THR	MET	Satura.	Polyuns.		
Hermetia illucens	Larvae	36.5	18.0	9.3	36.2	7.6	5.3	15.0	67.1	15.9		
Tenebrio molitor	Larvae	8.0	30.1	3.5	58.4	6.0	4.4	0.6	22.2	31.5		
Musca domestica	Larvae	15.3	31.3	6.5	46.9	8.3	4.8	3.0	32.6	7.6		
References	Jayanegara et al., 2017; Makkar et al. 2014; Pieterse & Pretorius, 2013; Moreki et al., 2012; Khan, 2018; De											
	Marco et al., 2015; Cullere et al., 2016; Gasco et al., 2016; Shah et al., 2022; Ogunji et al., 2008; Nogales-											
	Mérida et al., 2019; Cullere et al., 2016											

	Macro	minerals [	Milligran	n per kilog	ram	Micro	ominerals	[Milligra	m per kilo		
Species		(				(mg/kg)]		References			
_	Ca	Р	Na	К	Mg	Zn	Fe	Cu	Mn	S	
Hermetia illucens	21.40	11.50	1.30	13.50	3.90	13.10	20.40	11.20	23.20	27.04	Pieterse Pretorius, 2013; Hussein et al., 2017; Röthig et al., 2023
Musca domestica	4.90	10.90	5.40	12.70	2.30	10.39	47.50	32.40	42.50	ND	Karapanagiotidis et al., 2014; Piccolo et al., 2017; Arru et al., 2019
Tenebrio molitor	2.10	10.60	2.10	11.20	3.00	138.2	71.50	19.40	05.70	ND	Barroso et al., 2019; Gasco et al., 2020

#### Nutritional Aspects of Black Soldier Fly Larvae and Their Effect on Aquaculture

Various researchers have documented divergent nutrient compositions in feeds derived from Black Soldier Fly (BSF) larvae, as depicted in Table 1 and 2. According to Shah et al., (2022) On average, BSF larvae exhibit a composition of Dry Matter (DM) at 27.40%, Crude Protein (CP) at 56.10%, Crude Fiber (CF) at 23.20%, ash at 9.85%, Calcium (Ca) at 2.14%, Phosphorus (P) at 1.15%, Magnesium (Mg) at 0.39%, Potassium (K) at 1.35%, Zinc (Zn) at 13.10 mg/kg, Copper (Cu) at 11.20 mg/kg, Manganese (Mn) at 23.20 mg/kg, and Iron (Fe) at 20.40 mg/kg, all based on DM content. These larvae, commonly referred to as BSFL, eating black soldier fly larvae food, BSF pre-pupae food, or BSF worm food, can be utilized in their natural state or processed through drying, chopping, and grinding. The DM content in fresh BSFL is notably higher, ranging from 34.9% to 44.9%, rendering them more cost-effective and convenient compared to alternative fresh foodstuffs.

On average, BSFL encompass approximately CP, 41.1% to 43.6%; Ether Extract (EE),15.0% to 34.8%; CF, 7.0% to 10%; Gross Energy (GE) at 5,278.49 kcal/kg, and ash content varying from 14.6% to 28.4%, all based on DM (De Marco et al., 2015; Jayanegara et al., 2017). The calcium content of BSFL larvae ranges from 5% to 8%, while phosphorus content lies within the range of 0.6% to 1.5%. Moreover, the mineral profile includes Copper (Cu) at 6.0 mg/kg, Iron (Fe) from 0.14% to 14%, Manganese (Mn) at 246 mg/kg, Magnesium (Mg) at 0.39%, trace Sodium (Na) at 0.13%, Potassium (K) at 0.69%, and Zinc (Zn) at 108 mg/kg (De Marco et al., 2015; Cullere et al., 2016).

The Fatty Acid (FA) synthesis in BSFL is contingent upon dietary FA synthesis. Larvae fed on cow dung display a composition rich in oleic acid (32%), lauric acid (21%), palmitic acid (16%) and omega fatty acids (30.2%). The FA ratio in BSFL offered to fish encompassed 43% lauric acid, 11% oleic acid, 12% omega fatty acids, and 3% palmitic acid, with cow dung accounting for 50% of the diet (Makkar et al., 2014). The overall lipid content improved from 21% to 30% DM. Rearing BSFL on cow fertilizer led to a remarkable 22% increase in fish weight within 24 hours, indicating significant enhancements in polyunsaturated Fatty Acids (FAs), particularly eicosapentaenoic acid and docosahexaenoic acids (Jayanegara et al., 2017).

The Black Soldier Fly (BSF) is classified within the Diptera order, making it a pivotal subject of research. Its potential as an alternative to fishmeal and Plant Meals has been extensively explored. Remarkable advantages of the Diptera group, particularly this fly species, lies in its amino acid composition closely resembling that of fishmeal (Bruni et al., 2018) Notably, *H. illucens*, unlike other Diptera, and doesn't act as a disease vector due to its non-feeding behavior in adulthood. Impressively, even the larvae play a role in reducing microflora within manure, consequently mitigating harmful bacteria populations (Makkar et al., 2014). The BSF larvae boast substantial protein levels, accounting for 40-44% of crude protein content. Additionally, the lipid content, offering considerable value, varies based on their dietary intake. Incorporating fish offal into their diet enhances the presence of vital fatty acids (FAs) like n-3, which significantly favors fish growth (St-Hilaire et al., 2007; Makkar et al., 2014). This intricate relationship between BSF diet and its nutritional profile underscores its potential significance in sustainable agriculture and aquaculture practices.

In this area, the fish species known as the rainbow trout (Oncorhynchus mykiss) is one that is frequently investigated. In an experiment by Sealey et al. (2011), prepupae of H. illucens fed on trout offal was used in place of up to 50% of fishmeal (FM), creating an enhanced diet. Surprisingly, the performance results of this substitution were parallel to those of the untreated diet. Surprisingly, here were no discernible variation in development or sensory characteristics between the enhanced diet and the control group. The findings from a diet concentrated on non-enriched BSF were noticeably worse than those from the control. On a different subject, Renna et al. (2017) replaced 25% and 50% of FM, respectively, with inclusions of 20% and 40% by integrating defatted BSF larvae into the diet. The only differences between the two experimental diets as a result of this change were those in apparent digestibility (ADC) and fatty acid composition. The increased chitin content in the former case is what caused the ADC of CP in the 40% inclusion diet to be lesser than that of the 20% inclusion fast. In the latter case, when the BSF content rose, the levels of polyunsaturated fatty acids (PUFAs), which include DHA and EPA, reduced. However, no additional significant variations were seen in the animals' growth, feed conversion ratio (FCR), specific growth ratio (SGR), or morphological characteristics. Lastly, Bruni et al. (2018) revisited the preceding study. The diet comprising 20% BSF inclusion yielded notably increased biodiversity within the microbiota, a benefit to fish due to the microbiota's role in enhancing digestibility and providing protection against pathogens, aligning with the conclusion of Gomez et al. (2013).

Karapanagiotidis et al. (2014) conducted a study employing gilthead sea bream (Sparus *aurata*) juveniles, wherein dried BSF prepupae meal replaced 10%, 20%, or 30% of fishmeal (FM). Notably, diets incorporating BSF exhibited an adverse impact on food intake due to reduced palatability, leading to diminished growth. In a different study, Kroeckel et al. (2012) integrated up to 76% of defatted *H. illucens* meal in the meals of turbot (*Psetta maxima*). Intriguingly, when the addition surpassed 33.0% (equivalent to replacing 35.9% of FM), a noteworthy decline in final fish weight, as well as reductions in exact growth rate (EGR) and protein efficiency ratio (PER), coincided with rising the BSF content. Simultaneously, the feed conversion ratio (FCR) exhibited an increment. Lock et al. (2016) demonstrated significant progress in Atlantic salmon (Salmo salar) by accomplishing a commendable 50% replacement of fishmeal (FM). In experimental diets substituting 25%, 50%, or even up to 100% of FM, the reed conversion ratio (FCR) exhibited a reduction compared to the control. Strikingly, despite this substitution, all groups achieved an identical weight gain, resulting in net growth equivalent to the FM-fed fish, even at the complete 100% replacement. Notably, these diets were supplemented with lysine and methionine, emphasizing their strategic formulation. Achieved equivalent protein retention levels with Nile tilapia (Oreochromis niloticus) with a 14% FM substitute using BSF larvae, aligning with the control diet. However, this retention was significantly reduced when the remaining diets had substitutes of 12%, 16%, or 18% (Kurniawan et al., 2018). A similar trend was observed with energy retention. Unfortunately, data concerning diet compositions, growth, FCR, or other pertinent indices are absent from the study's documentation (Kroeckel et al., 2012).

#### Nutritional Aspects of Housefly Larvae and Their Effect on Aquaculture

Housefly larvae (HFL) are poised to yield a bountiful supply of nourishing maggots. These larvae are preserved for their rapid reproductive pace, costeffectiveness in terms of food, ease of processing, and impressive durability (Makkar et al., 2014). Housefly larvae offer copious amounts of protein, energy, and essential micronutrients such as Copper (Cu), Iron (Fe), and Zinc (Zn), as well as vital amino acids (EAAs) and fatty acids (FAs). These larvae are not only economical but also present a superior nutritional profile, offering a hassle-free alternative to conventional resource of animal protein. Broadly speaking, HFL-based meals boast significant levels of Lysine (Lys), Threonine (Thr), and Methionine (Met), making them valuable supplements for low protein cereal and legume based livestock diets (Moreki et al., 2012; Khan, 2018).

Housefly larvae exhibit a substantial CP content (63.99% of DM) and Ether Extract (EE) content (24.31% of dry matter). However, the EE and CP levels may vary due to drying methods and the age of the larvae, with CP declining and EE increasing as the larvae mature (Hwangbo et al., 2009). Research by Makkar et al. (2014) and Khan (2018) emphasized that ash (10.68% of DM), CP (60.38% of DM), Gross Energy (GE) (4,800.80 kcal/kg), and EE (14.08% of DM), are all components of HFL feed. Ash (7.73 percent of DM), CP (76.2 percent of DM), EE (14.3 percent of DM), and GE (4,877.23 kcal/kg) are also included in HFL. Notably, the apparent metabolic energy of HFL pupae and larvae are 3,398.77 kcal/kg and 3,618.51 kcal/kg, correspondingly. In comparison to Soybean Meal (SBM), the larvae showcase elevated levels of Calcium (Ca), metabolic energy (4,140 kcal/kg), and Phosphorus (P), in contrast to SBM's 2,250 kcal/kg. Within feeds have Housefly larvae and pupae; Linoleic acid constitutes 26.3% to 36.3% of the total fat (Jayanegara et al., 2017). Additionally, while Housefly pupae are abundant in critical FAs including linoleic acid and oleic acid, these maggots have significant amounts of palmitoleic acid. In terms of both amino acids (AAs) and essential amino (EAAs), maggots also outperform acids SBM. Particularly, maggots present elevated height of EAAs, including Lysine (Lies), Arginine, Phenylalanine (Fi), Threonine (Trap), and Valine (Val), albeit Methionine (Meth) and Sesame (Ses) are found in lower concentrations. Hence, a supplementary supply of Methionine is advisable when incorporating maggotbased diets. Furthermore, CP, Phenylalanine (Phy), Lysine (Lys), and HF pupae had more methionine (Meth) content than mealworms (MWs) did (Makkar et al. 2014).

Similar to other Diptera species, the common housefly has an Essential Amino Acid (EAA) profile like that of fish. Since the early 2000s, study has focused on its possible role in fish and crustacean diets (Makkar et al., 2014). Nevertheless, it's important to highlight the scarcity of information available regarding the application of this Diptera in the context of Spanish and European fish species. With a CP content ranging from 40% to 60%, coupled with a lipid substance that spans a wide spectrum between 9% and 26%, the housefly's nutritional composition is highly variable and contingent on the substrates comprising its diet. Typically harnessed in the form of larva meal, the housefly's efficacy as a dietary source has also manifested positively in live larva feeding approaches, as documented by Ebenso and Udo (2011) and Makkar et al. (2014). In a study conducted by Wang et al. (2017), the impact of substituting fishmeal (FM) with Musca domestica larva (MM) meal was investigated across four experimental diets, containing substitutions of 25%, 50%, 75%, and 100%, in the context of Nile tilapia (Oreochromis niloticus). The findings revealed that FM replacement of up to 75% could be accomplished without engendering significant effects across the various analyzed parameters (Makkar et al., 2014). Intriguingly, the inclusion of MM exerted a modest enhancement on fillet quality, rendering them firmer in texture. A notable observation emerged as well: certain experimental diets led to an observable enhancement in water quality when contrasted with the control diet. This enhancement was manifested by lower levels of total nitrogen and Total Ammonia Nitrogen (TAN), indicating a positive influence on the aquatic environment (Ebenso and Udo 2011; Wang et al., 2017).

### Nutritional Aspects of Mealworm Larvae and Their Effect on Aquaculture

Mealworm larvae's nutritional composition can vary greatly depending on their feed, environment, and stage of development, according to scientific research. Notably, recent work by Bovera et al. (2016) and Józefiak, et al. (2016) presents a comparative analysis of the chemical composition (CC) and amino acid (AAs) outline between MWs larvae and Soybean Meal (SBM) diet. The findings indicate that mealworm larvae exhibit a composition ratio of 51.93%, Crude Protein (CP) at 21.57%, Ether Extract (EE), and Acid Detergent Fiber (ADF) at 7.20%. This contrasts with the CP content of 44.51%, EE of 1.84%, and ADF of 4.79% in SBM diet. Moreover, the two protein sources exhibit distinctive EAAs compositions, with notable variations in cystinone and methionine (SBM content being 3.27 times greater than MW), while other AAs like Arginine, (1.70%), Isoleucine, (1.75%), and Lysine, (1.68%) are more abundant in mealworm larvae. Conversely, the SBM diet boasts higher levels of histidine (hist) (1.19%), Leucine, (1.25%), Threonine, (1.26%), and Valine, (1.10%). Interestingly, the methionine concentration is higher in MW larvae than in SBM diet (Ravzanaadii et al., 2012). Notably, the additional AAs in MW larvae prove sufficient to meet the requirements of broiler chickens (Pieterse & Pretorius, 2013). In a current study, Hussein et al. (2017) reported a notable 44.9% CP content, along with a substantial 33.9% content of essential amino acids (EAAs), particularly Lysine (Lys) at 4.51% and Methionine (Meth) at 1.34%, signifying their relatively high levels within MW larvae.

The research by Ravzanaadii et al. (2012) underscores the presence of a minor attentiveness of Calcium, (434.59 mg/kg) and a heightened attentiveness of Phosphorus, (7,060 mg/kg) in mealworm larvae. The Ca: P ratio, however, falls short for poultry production, particularly in chickens. This challenge can be addressed by administering a calcium-enriched diet to mealworms for a day or two (Makkar et al. 2014). Notably, micromineral analysis revealed Copper (Cu) at 13.27 mg/kg, Iron (Fe) at 66.87 mg/kg, and Zinc (Zn) at 104.28 mg/kg within the larvae. Furthermore, significant synthesis of long-chain Fatty Acids (FAs) was observed in larvae, with the highest content being linoleic acid at 30.23%, oleic acid at 43.17%, and palmitic acid at 15.79% (Jayanegara et al., 2017).

The yellow mealworm, a Coleoptera species, boasts convenient breeding and feeding traits. Typically administered live, it's also available in meal form (Veldkamp et al., 2012). Notably, a key advantage is its substantial production scale, notably in countries like China (Piccolo et al., 2017). With protein substance varying among 47% and 60%, and lipid substance ranging from 31% to 43%, its nutritional profile is noteworthy. Yellow mealworm inclusion has yielded impressive outcomes, particularly in trout (Oncorhynchus mykiss). Gasco et al. (2014) achieved a remarkable feat, incorporating 50% T. molitor larva food into the fast while keeping identical ultimate weight compared to the control fast, and even saw improvements in FCR, PER, and EGR. Notably, disparities emerged in the hepatosomatic index (HSI), Polyunsaturated Fatty Acids (PUFAs), and protein Apparent Digestibility Coefficient (ADC), all minor in the investigational diets.

However, the consequences of Gasco et al. (2014) failed to showcase an enhancement across the board, as no significant differences emerged with insect meal inclusion. Iaconisi et al. (2018) discovered no variation in growth with the equal additions. Their research centered on morphometry, skin characteristics, and physical characteristics including color. The only differences seen in fish fed experimental diets were in the fish's skin tone, HSI, and PUFA levels. This led to their recommendation of not surpassing a 25% inclusion of T. molitor meal due to these observed effects. In a 2017 study by Piccolo et al., gilthead sea bream (Sparus aurata) subjected to a 25% addition of T. molitor food showed incremental growth, specific growth rate, and protein efficiency ratio, as well as a decrease in FCR, though not statistically significant. Remarkably, apparent digestibility coefficients (ADCs) were comparable between the control diet and the 25% inclusion diet, yet decreased with the 50% inclusion diet.

According to Sánchez-Muros et al. (2015) used two experimental diets for tilapia (Oreochromis niloticus) and untreated diet consisting of fish meal and soybean meal; the first involved a 75% FM substitution (50% SM and 25% TM), and the second replaced 50% of FM with T. motorli meal. Contrary to expectations, outcomes from the experimental diets were suboptimal, as both final weight and PER dwindled compared to the control. Interestingly, a decrease in FCR was also experiential. The study revealed a distinct alteration towards specialized gut bacterial communities for rainbow trout after the substitution, contrasting the outcomes observed in the other two species (Piccolo et al., 2017). Interesting dietary role emerged in the gut communities of gilthead sea bream and European sea bass as a result of the insect meal replacement, distinguishing them from trout. These investigations highlight intriguing insights and emphasize the necessity for further research to validate the potential of insect meal in enhancing disease resistance and its distinct implications across diverse farmed species (Sánchez-Muros et al., 2015).

#### Comparing the Nutrient Profiles of Insects to Traditional Fish Feed Sources

Insects stand out as the most diverse category within the animal kingdom, serving as a vital normal food supply for fish, particularly omnivorous and carnivorous species. These fish require substantial protein intake in their diets, as documented by Makkar et al. (2014) and van Huis (2020). Analyzing the nutritional composition of three distinct insect species, including measurements of CP, AA, fat content, FA profiles, and mineral levels, has been conducted by Sanchez-Muros et al. (2014), with a summarized overview displayed in Table 3. Detailed insights into the nutritional constituents of each insect species are accessible in published works such as de-Souza-Vilela et al. (2019), and Sanchez-Muros et al. (2014). Our focus here is to concisely present the primary nutritional composition of these three species while highlighting potential nutritional concerns. Notably, these insects exhibit elevated levels of crude protein (CP), ranging from 42.1% to 63.3% (Table 3). Although this CP content is lower than that found in fishmeal (FM), it aligns more closely with soybean meal (SM) (Allegretti et al., 2017). Among these insects, the adult pupae of silkworms, locusts, and crickets have comparatively lower CP levels than the larvae of BSFs and houseflies. The amino acid concentration among these insect species exhibit variations across the board. Orthoptera insects (crickets and locusts) and mealworms show decreased lysine content compared to FM, while Diptera insects (black soldier flies and houseflies) and silkworms boast abundant lysine levels. Sulphur amino acid content in insects, excluding silkworms, are below those found in FM. The seven insect species' threonine levels are still rather consistent, with silkworms having greater levels (Sanchez-Muros et al., 2014; Henry et al., 2015). With the exception of silkworms and HF maggot meal, the tryptophan concentrations in the remaining six bug species are typically lower. To promote optimal growth, addition with synthetic amino acids could be advised according on the particular nutritional requirements of the fish species. Comparing their amino acid profiles to soybean meal (SM), silkworms, black soldier flies, and houseflies exhibit more favorable compositions (Table 3), making them promising alternatives to fishmeal (FM) for aquafeed substitution (Henry et al., 2015).

The fat content of these insect species is notably lower than that of FM, with insects often accumulating fat during developing phase (Sanchez-Muros et al., 2014). The fat substance varies among the eight species, ranging from approximately 8% in mature locusts to around 36% in mealworm larvae (van Huis, 2020). It's crucial to acknowledge that lipid concentration varies significantly even within a single species, influenced by factors such as developmental stage and diet (Barroso et al., 2019). Differences in fatty acid composition between insect meals and fish oil are noteworthy. Fish oil has higher rank of omega-3 fatty acids compared to insect meals (Barros-Cordeiro et al., 2014; Makkar et al., 2014). Saturated fatty acids are more common in insect meals than unsaturated fatty acids, which make up about 60-70% of the fat in three insect species. Contrarily, the unsaturated fatty acid content of BSF larvae is lower, only ranging from about 19% to 37% (Gasco et al., 2020). When compared to fish oil, these of insects have lower concentrations EPA (eicosapentaenoic acid, 20:5n-3), and DHA (docosahexaenoic acid, 22:6n-3), Hawkey et al. (2021) but higher quantities of polyunsaturated fatty acids (PUFA), particularly n-6 PUFA (Gasco et al., 2020; van Huis, 2020). These eight bug species can only be used as a secondary oil source in aquafeeds since they lack EPA and DHA. Alpha-Linolenic Acid (ALA, 18:3n-3), which salmonids can synthesis, is a dietary source of EPA and DHA that accumulates more efficiently in fish meat and oil (Tocher et al., 2019). Since the composition of the substrate can be changed, it is now commonly accepted that the lipid content and fatty acid profiles of insect meals are significantly altered by their food (Makkar et al., 2014).

## The Potential Benefits for Fish Health and Growth

#### **Enhancing Growth and Optimizing Feed Utilization**

Numerous researches have examined the effects of different insects on aquaculture growth performance and feed use. The Black Soldier Fly (BSF) has drawn a lot of interest among these insects (Fawole et al., 2020; Peng et al., 2021). In addition, research has been conducted in this area on the T. molitor (Sankian et al., 2018), M. domestica (Hashizume et al., 2019), I. belina, and G. bismasculatus (Taufek et al., 2016). Notably, BSF stands out as the most extensively studied insect in aquaculture. Insects can be integrated into aquaculture through various forms, such as dry meals (Jeong et al., 2021). For instance, in a thorough 60-day experiment, Fawole et al. (2020) examined the effects of replacing fish meal with BSF larvae feed at 25%, 50%, and 75% on the growth performance, nutrient consumption, and health parameters of African catfish (C. gariepinus). Their research showed that compared to other feeding regimens, a 50% inclusion of black soldier fly larval meal resulted in the highest final body weight, weight gain, and specific growth rate.

As reported by Kamarudin et al. (2021), achieving an optimal development performance in lemon fin barb hybrid fingerlings required a substantial inclusion level of 75% black soldier pre-pupae meal. Notably, a study by Belghit et al. (2019) showed the viability of completely substituting BSF meal for fish meal in Atlantic salmon (S. salar), retaining appropriate levels of growth and nutrient digestibility. Moreover, the efficacy of BSF pulp as a dietary component was underscored by its positive impact on the growth performance of *M. salmoides*, as evidenced in studies by Peng et al. (2021). As opposed to this, laconisi et al. (2017) found that when mealworm was utilized to partly alternate fish meal at levels of 25% and 50% for a period of 131 days in blackspot seabream , there was no appreciable contact on growth and feed consumption metrics. Nonetheless, certain fish species, such as those highlighted by Coutinho et al. (2021), exhibited adverse effects on growth performance and feed utilization in response to mealworm supplementation. These observations underscore the importance of refining ingredient processing techniques and underscore the necessity for further investigations to optimize the integration of this ingredient within the realm of aquaculture (Jeong et al., 2021).

The evaluation of immune function response in aquatic animals due to dietary supplementation has emerged as a pivotal standard for assessing the suitability of feed ingredient within aquaculture. The use of insects in aqua-feed has been extensively scrutinized across various immune-related parameters, including examinations of the biochemical makeup of the blood, the histology of pertinent organs, intestinal health, immune-related gene appearance, and illness resistance in various aquaculture species. A compilation of outcomes for these considerations can be found in Table 4. In the context of Atlantic salmon fast, the complete replacement of fishmeal with BSF meal has been accomplished without inducing adverse effects on liver histology (Stenberg et al., 2019) or the dictation of pro-inflammatory genes in the fish's skull kidney (Belghit et al., 2019). The addition of dietary BSF meal supplementation to young Japanese seabass has shown to have no effect on the fish's intestinal histomorphology (Wang et al., 2019). Furthermore, investigations have unveiled that replacing fishmeal with BSF at 25% and 50% in the diets of zebrafish does not yield substantial changes in gut histology, immune response and stress levels (Zarantoniello et al., 2019). Notably, partial replacement of up to 64% of fishmeal with defatted BSF larvae meal has not shown any appreciable effects on the histomorphology of the intestine and liver, nor on the intestinal antioxidant position or immune reaction of the L. japonicus, according to Wang et al. (2019). In terms of the bug species and meal compositions used in aguafeed, the research that has already been done shows subtle variances. The histology of the liver, spleen, and intestine in rainbow trout did not significantly change when fishmeal was substituted with a 50% partly defatted BSF meal (Elia et al., 2018). Conversely, employing full-fat TM meal at levels ranging from 28% to 67% as a fishmeal alternative showcased the potential to enhance the fish's immune response (Henry et al., 2018).

In the context of Nile tilapia, complete replacement of fishmeal was accomplished through the utilization of BSF meal, resulting in observable enhancements in both hematology and immunity within the coat mucus (Tippayadara et al., 2021). In contrast, a mere 15% replacement with superworm larvae contributed to elevated innate immunity in the fish (Alves et al., 2021). Although a few studies have statement on the collective impact of incorporating numerous species of insect meals in aquaculture (Jozefiak et al., 2019), additional investigations are necessary to comprehensively understand this phenomenon. Defatted silkworm pupae meal replacement at greater levels (above 75%) in the case of Pacific white shrimp has been connected to potential negative impacts on the hepatopancreas integrity in the shrimp (Rahimnejad et al., 2019). Bruni et al. (2018) examined the effects of switching from fishmeal to partially defatted BSF meal on the microbial population in the fish's intestines when feeding rainbow trout. The authors of the study came to the conclusion that feeding rainbow trout diets with 50% BSF meal resulted in increased biodiversity and altered microbial community structure. When red seabream (*Pargus major*) were given TM-containing meals after being exposed to the microorganism pathogen Edwardsiella tarda, the fish's continued existence rates were significantly increased (Ido et al., 2019).

The incorporation of insect foods into aquaculture holds promise for facilitating the utilization of plantbased proteins, mainly soybean meal, which has seen diminished application in high-value species culture due to its association with intestinal enteritis. Notably, in the case of rainbow trout, the addition of BSF meal to diets based on soybean meal successfully delayed the onset of intestinal enteritis caused by soybean meal (Kumar et al., 2021). In accordance with the conclusion of Xiang et al. (2020), it's proposed that bioactive peptides present in insect meal could contribute to the avoidance of this illness. Consequently, insect food demonstrates a promising avenue for mitigating intestinal tenderness within aquaculture. Nonetheless, as highlighted by Kumar et al. (2021), a deeper examination is necessary to thoroughly illustrate the bio-active peptides inherent in insect foods.

#### **Exploring Antioxidant Capacity**

The impact of integrating insects into aquafeed on the antioxidant capability of fish has been the subject of yielding talented findings. studies, several Α comprehensive overview of outcomes from various studies is presented in Table 4. On the other hand, the observed results exhibit variations contingent upon the specific insect species and components employed within the agua feed. For instance, the incorporation of dietary insect meal, like BSF, as a substitute for fishmeal, demonstrated adverse effects on antioxidant enzyme dictation and stress-related gene expression in the leukocytes of the head kidney (Stenberg et al., 2019). On the other hand, using 75% BSF in place of fishmeal did not affect the antioxidant position of African catfish (Fawole et al., 2020). Elia et al. (2018) found that consuming at least 20% BSF negatively damaged oxidative equilibrium in rainbow trout, mainly in the liver and kidney. Glutathione peroxidase (GPx) activity was reduced as a result, although ethoxyresorufin Odeethylase (EROD), glutathione S-transferase (GST), and total glutathione (GSH) activity were increased. As a result, the authors advised keeping BSF incorporation levels in fish food to under 20%. In the case of Atlantic salmon, rising the amount of BSF paste in both the fishmeal and plant-based diets from 6.25% to 25% increased the fish's blood's antioxidant capacity (Weththasinghe et al., 2021). The serum antioxidant capacity of Pacific white shrimp was also increased

Species Proximate analysis (% dry matter)				tter)	Amino acid (% total)			Fatty acid (%)								
								Saturated fatty acid (%)				Polyunsaturated fatty acid (%)				
	ASH	EE	СР	NFE	LYS	MET	THR	Lauric,	Myristic,	, Palmitic,	Stearic,	Linoleic,	Linolenic,	Eicosapentaenoic,	, Docosahexaenoic,	
								12:0	14:0	16:0	18:0	18:2n-6	18:3n-3	20:5n-3	22:6n-3	
Fishmeal	17.0	12.1	70.6		7.5	2.7	4.1		3.4	16.1	4.6	1.4	0.6	11.1	29.1	
Soymeal	6.6	4.4	51.8	36.1	6.8	1.32	3.78			10.6	3.8	53.7	5.8			
Hermetia illucens	9.3	18.0	36.2	36.5	7.6	15	5.39	21.4	2.9	16.1	5.7	4.5	0.19	0.03	0.00	
Tenebrio molitor	3.5	30.1	58.4	8.0	6.03	0.64	4.49	0.5	4.0	21.1	2.7	27.4	1.2			
Musca domestica	6.5	31.3	46.9	15.3	8.36	3	4.87		5.5	31.1	3.4	19.8	2.0			
References	Tocher e	Tocher et al., 2019; Gasco et al., 2020; van Huis, 2020; Barroso et al., 2019; Sanchez-Muros et al., 2014; Henry et al., 2015; Allegretti et al., 2017														

#### **Table 3.** Comparing the insects nutrient profiles with traditional fish feed sources

#### Table 4. The potential benefits for fish health and growth

	Aquaculture	Inclusion	Effect	Time (Months)	References
Black soldier fly ( <i>Hermetia illucens</i> )	African catfish	22.5	<ol> <li>Elevated growth performance and restored gut microbiota equilibrium.</li> <li>Enhanced development performance and optimized feed utilization, accompanied by increased antioxidant enzyme activity, contribute to reduced lipid oxidation in the fillet.</li> </ol>	2	Fawole et al. (2020)
	Atlantic salmon	66.5	1. Suppression of stress-related and antioxidant-associated gene expression within the leucocytes.	1.8	Stenberg et al. (2019)
Housefly (Musca domestica)	Baltic prawn	18.2	1. Enhanced growth performance accompanied by a decrease in survival rate.	2	Mastoraki et al. (2020)
Mealworm	Giant river prawn	12.3	1. Greater resistance to infections caused by Aeromonas hydrophila and Lactococcus garvieae as well as improved growth performance and immunological response.	1.8	Feng et al. (2019)
	Rainbow trout	25.4	<ol> <li>Diminished apparent digestibility of crude protein.</li> <li>Creation of innovative nutritional niches within the gut ecosystem.</li> <li>Achieved superior final weight, specific growth rate, weight gain, and protein efficiency ratio, coupled with a reduced feed conversion ratio."</li> </ol>	5.4	Piccolo et al. (2017)

when defatted silkworm pupae meal was substituted for fishmeal (Rahimnejad et al., 2019).

The consequence of insect oils on the antioxidant rank of young mirror carp (C. carpio var. specularis) was examined in recent research by Xu et al. (2020). Their research showed that the liver's antioxidant capacity was increased by the simultaneous addition of BSF oil, silkworm pupae oil, and TM oil at similar quantities. Notably, when comparing individual insect oils, BSF oil exhibited superior outcomes compared to the other two oils. Additionally, mirror carp fed dietary BSF pulp at lower levels had significantly increased serum antioxidant capacity, according to Xu et al. (2020). Additional insect-derived protein sources demonstrating analogous outcomes contain cricket (Gryllus bimaculatus) food within the African catfish diet (Taufek et al., 2016) and maggot meal incorporated into the common carp diet (Ogunji et al., 2011). Furthermore, the nutritional addition of TM in rainbow trout diets showcased enhancements in intestinal antioxidant enzyme activity, accompanied by a reduction in lipid per-oxidation (Henry et al., 2018). Notably, when hybrid tilapia were fed a diet that included maggot meal as a full substitution for fishmeal, their antioxidant capacity remained unchanged (Qiao et al., 2019; Mastoraki et al., 2020).

#### Conclusion

In conclusion, insects hold immense potential as a sustainable feed source for aquaculture, addressing nutritional demands while minimizing environmental impacts. Addressing challenges through collaborative research and industry engagement can pave the way for a more resilient and eco-friendly aquaculture sector. Additionally, the exploration of insects as sustainable feed in aquaculture offers a promising pathway to address the challenges of feed sourcing in the industry. The nutritional richness of insects, coupled with their minimal environmental footprint and waste-recycling capabilities, presents a compelling case for their integration into aquafeed. While challenges like standardization, regulation, and understanding their effects on aquatic organisms persist, collaborative efforts among researchers, industry stakeholders, and policymakers can foster their successful adoption. Embracing insects as a sustainable feed source has the potential to revolutionize aquaculture practices, contributing to both ecological resilience and the ability to meet the rising global demand for seafood products.

#### **Ethical Statement**

It is review paper there not used human and animal.

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#### **Author Contribution**

Ning Bingqian and Assar Ali Shah was involved in conceptualization. Ali Zaman and Shakeeb Ullah designed this experiment. Rifat Ullah Khan, Khalid Muhammad and Muhammad Shuaib Khan was involved in data analysis. Assar Ali Shah wrote the original draft, reviewed and edited the manuscript. All authors approved the final version of the manuscript for submission.

#### **Conflict of Interest**

The authors declare that they have no competing interests.

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