

Sustainable insect feed in shrimp aquaculture: Challenges and opportunities in research and practical implementation

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“You cannot get through a single day without having an impact on the world around you. What you do makes a difference, and you have to decide what kind of difference you want to make.”

– Jane Goodall

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1 Synthesis

1.1 Abstract

Aquaculture is a growing global food production sector that aims to meet the increasing demand for human dietary protein. Crustaceans are an important part of aquaculture, particularly the Pacific white shrimp (PWS; *Penaeus vannamei*). Shrimp farming often has a large ecological footprint, partly due to the fishmeal content in commercial aquafeed. An opportunity to improve sustainability and move towards a circular economy would be replacing fishmeal in aquafeeds with insects, such as the black soldier fly larvae (BSF; *Hermetia illucens*), reared on plant by-products. However, limited data is available on the effects of novel plant by-products on the growth performance of BSF larvae and in turn on shrimp. Research into BSF-based feed is in its infancy, and the few existing studies show inconsistent results regarding shrimp growth performance. Although some of these discrepancies can be attributed to the properties of the insect feed used, other unknown factors are probably influencing the results. Therefore, this dissertation aims to assess challenges and opportunities in using insects as a sustainable feed in shrimp aquaculture research and practice. It covers key sustainability concerns, enhances the consistency and reproducibility of feeding trials, and offers a comprehensive view of practical application within the context of a circular economy. This dissertation demonstrates that BSF larvae are a promising, sustainable source of protein for shrimp farming. BSF larvae can efficiently convert plant by-products into high-quality protein. Feeding trials show that replacing up to 50% of fishmeal-based compound feed with BSF larvae achieves excellent feed conversion rates. However, the use of BSF meal in compound feed in industrial aquaculture offers greater potential. Furthermore, compliance with reporting guidelines and transparency will be essential for reproducibility and food safety when using insects as feed in the future. The implementation of circular economy concepts remains largely theoretical and depends on optimised rearing systems, increased inclusion rates, industrial scalability and economic profitability. Even though there are still challenges regarding scalability, regulation and consumer acceptance, BSF-based feed has great potential for commercial shrimp farming.

1.2 Zusammenfassung

Die Aquakultur ist ein weltweit wachsender Bereich der Lebensmittelproduktion. Ihr Ziel ist es, den steigenden Bedarf an Proteinen für die menschliche Ernährung zu decken. Krebstiere sind ein wichtiger Bestandteil der Aquakultur, insbesondere die Pazifische Weißbeingarnele (PWS; *Penaeus vannamei*). Die Garnelenaquakultur hinterlässt jedoch oft einen großen ökologischen Fußabdruck, unter anderem aufgrund des Fischmehlanteils in kommerziellem Futter. Eine Möglichkeit, die Nachhaltigkeit zu verbessern und den Übergang zu einer Kreislaufwirtschaft zu fördern, wäre der Ersatz des Fischmehls im Futter. Dieses könnte durch Insekten, wie die Larven der Schwarzen Soldatenfliege (BSF; *Hermetia illucens*), substituiert werden. Die Larven können mit pflanzlichen Nebenprodukten aufgezogen werden. Allerdings liegen nur begrenzt Daten über die Auswirkungen neuartiger pflanzlicher Nebenprodukte auf das Wachstum von BSF-Larven und somit auf das Wachstum von Garnelen vor. Die Forschung zu Futtermitteln auf BSF-Basis steht noch am Anfang und die wenigen vorhandenen Studien zeigen uneinheitliche Ergebnisse hinsichtlich der Wachstumsleistung der Garnelen. Einige dieser Diskrepanzen können auf die Eigenschaften des verwendeten Insektenfutters zurückgeführt werden, jedoch beeinflussen wahrscheinlich auch andere unbekannte Faktoren die Ergebnisse. Auf Grund dessen zielt diese Dissertation darauf ab, die Herausforderungen und Chancen der Verwendung von Insekten als nachhaltiges Futtermittel in der Forschung und Praxis der Garnelenaquakultur zu beleuchten. Dabei werden wichtige Nachhaltigkeitsaspekte behandelt, die Einheitlichkeit und Reproduzierbarkeit von Fütterungsversuchen verbessert und ein umfassender Überblick über die praktische Anwendung im Kontext einer Kreislaufwirtschaft geboten. Die Arbeit zeigt, dass BSF-Larven eine vielversprechende Proteinquelle für die Garnelenaquakultur darstellen. BSF-Larven können pflanzliche Nebenprodukte effizient in hochwertiges Protein umwandeln. Fütterungsversuche zeigen, dass durch den Ersatz von bis zu 50% des Fischmehl-basierten Futters durch BSF-Larven hervorragende Futtermittelnutzungsraten erzielt werden. Die Verwendung von BSF-Mehl in Mischfuttermitteln in der industriellen Aquakultur bietet jedoch ein größeres Potential. Damit die Verwendung von Insekten als Futtermittel zukünftig sicher ist und die Ergebnisse reproduzierbar sind, ist es entscheidend, dass die Berichterstattungsleitlinien beachtet werden und Transparenz herrscht. Die Umsetzung von Kreislaufwirtschaftskonzepten ist nach wie vor weitgehend theoretisch und hängt von optimierten Aufzuchtssystemen, erhöhten Ersatzraten, industrieller Skalierbarkeit und wirtschaftlicher Rentabilität ab. Trotz der noch bestehenden Herausforderungen in Bezug auf Skalierbarkeit, Regulierung und Verbraucherakzeptanz hat BSF-basiertes Futter großes Potential für die kommerzielle Garnelenaquakultur.

1.3 Introduction

1.3.1 1.3.1 Shrimp aquaculture & sustainability crisis

Aquaculture provides a crucial alternative to traditional fishing, given the alarming rate at which marine life is being depleted. As a consequence, aquaculture has become one of the fastest growing sectors of the food industry, characterised by an increasing intensification in farming practices (Fantatto et al., 2024). The annual production of aquaculture accounts for approximately 94.4 million tons, which is half of the aquatic production worldwide (Sharma et al., 2025). In light of the growing global human population (Kumar et al., 2022), proteins from marine sources that are particularly rich in polyunsaturated fatty acids (PUFA's), minerals and vitamins (Dîrvariu et al., 2025) are essential for global food security. Nevertheless, this intensification of production has both advantages and disadvantages, with significant consequences for the environment. Aquaculture increases greenhouse gas emissions (Yuan et al., 2019), massive habitat destruction and overfishing of feed species (Pauly et al., 2002) and social problems such as increasing inequalities and injustice (Brugere et al., 2023). The reliance on fishmeal and fish oil as feed ingredients for many of the farmed aquaculture species, especially marine species, can lead to economic vulnerability due to the volatility of feed costs and can constrain growth (Naylor et al., 2000). Furthermore, aquaculture might significantly disrupt natural fish stocks by introducing parasites and pathogens, as well as altering the gene pool through escapees, which has clear economic consequences, for example in the case of salmon in Norway (Olaussen, 2018). Nevertheless, aquafeed is responsible for approximately 90% of the ecological impact of fed aquaculture (Little et al., 2018).

Similar challenges have also been observed in crustacean aquaculture, which accounts for 9% of the global aquaculture production (Röthig et al., 2023), in which the Pacific white shrimp (PWS; *Penaeus vannamei*, Boone, 1931) is the predominant species, with an annual production of 6.8 million tons (Barth, Bendag, et al., 2025). The majority of PWS farming takes place in coastal regions, where vast areas of mangrove forests are cleared and destroyed to construct extensive shrimp farming ponds (Noguera-Muñoz et al., 2021). Alternatively, some production occurs in modern closed recirculation aquaculture systems, which, while reducing habitat destruction, require significant energy inputs (Röthig et al., 2023). In relation to the three pillars of sustainability (environmental, economic and social dimensions), several disadvantages associated with shrimp farming become evident. The environmental impacts, which are significant, manifest across a variety of dimensions, including land use, habitat loss, water quality degradation, biodiversity loss, and soil and ecosystem concerns (Ahmad et al., 2022). It

is important to note that the effect varies depending on the production system (intensive vs extensive), type of feed utilised and the application of pesticides and antibiotics (Ahmad et al., 2022; Gan et al., 2024; Li et al., 2024). Economically, challenges arise from long-distance transportation and high energy consumption (Barth, Bendag, et al., 2025). Moreover, escalating feed costs and disease related losses exert pressure on the profitability and economic resilience of farms (Villarreal, 2023). The intensification of shrimp farms, characterised by higher stocking densities and technological innovations, has the potential to enhance productivity. This approach requires substantial capital investment, higher operating costs and entails risks (Villarreal, 2023). Socially, shrimp farming in coastal areas generates significant income and employment opportunities, but it consistently favours landowners and wealthy investors, intensifying local inequality and accelerating the conversion of agricultural land for aquaculture (Husni et al., 2023; Macusi et al., 2022; Roy et al., 2024; Taher et al., 2023; Xuan et al., 2021). In the Sundarbans, 91% of agricultural land has been converted into shrimp ponds (Taher et al., 2023). Even though shrimp farming in Indonesia increased community incomes, investment capital and infrastructure were distributed unequally (Husni et al., 2023). In Vietnam, productivity and profitability vary greatly depending on the production system, highlighting the effect that education, feed management and environmental hazards such as flooding and pollution have on performance (Roy et al., 2024). However, external environmental pressures (salinisation, mangrove destruction and wastewater pollution) compromise food security and increase the vulnerability of landless households (Taher et al., 2023). Furthermore, weak governance, uncertain land tenure, market volatility and limited organisation among smallholders restrict the equal distribution of profits along the value chain (Husni et al., 2023; Taher et al., 2023). Shrimp farming can potentially improve people's lives in rural areas, if it is done sustainably. This requires strong policies, the protection of land rights, environmental protection measures, and support for farmers to ensure the sector grows sustainably and equitably (Husni et al., 2023; Roy et al., 2024; Taher et al., 2023).

1.3.2 Ecological Sustainability of Insect-Based Shrimp Feeds

A further noticeable issue pertains to the utilisation of shrimp feed, it is estimated that 90% of crustacean aquaculture production relies on formulated compound feeds (Malcorps et al., 2019), which represent a significant portion of total production costs, estimated at 50-70% (Ferrer Llagostera et al., 2019; Gong et al., 2019). The composition of these compound feeds typically includes proteins, carbohydrates, lipids, vitamins, minerals, pigments and additives to optimise

growth, increase appetite, reduce stress and boost the immune system (Röthig et al., 2023). One of the most important components in this regard is fishmeal, followed by soybean meal as protein source. Fishmeal is a high-quality protein source with excellent digestibility and palatability. It provides all essential amino acids required for shrimp growth, as well as vitamins (A, D, E, B12), minerals (Ca, P) and polyunsaturated fatty acids (PUFA's) (Hussain et al., 2024). However, the production causes serious environmental, economic and social concerns. One third of global fish stocks are harvested unsustainably and 60% at maximum capacity (Sharma et al., 2025). It also carries the risk of transmitting marine pathogens, creates additional transport routes and increases emissions (Barth, Bendag, et al., 2025; Barth, Stelbrink, et al., 2025). The rising fish consumption of wealthier consumers often shifts small fishes away from vulnerable communities (Röthig et al., 2023). Aquaculture already consumes over 70% of fishmeal and fish oil (Hua et al., 2019), and demand is expected to exceed supply by 2050. Furthermore, the quality of the meal is decreasing as consumption increases (Froehlich et al., 2018; Hua et al., 2019). Rising prices, which have tripled since 2000, are predicted to continue, resulting in a significant increase in the cost of fishmeal and fish oil by 2030 (Röthig et al., 2023). Consequently, the development of sustainable and environmentally friendly feed is essential for the sustainability of shrimp farming.

The urgency to address this challenge has led to the exploration of alternative and sustainable shrimp feeds, particularly those incorporating insect-based diets, such as those derived from the black soldier fly (BSF; *Hermetia illucens*) (Röthig et al., 2023). A cosmopolitan fly that is present in tropical, subtropical and tempered regions worldwide, and is usually farmed extensively (Guilliet et al., 2022). BSF larvae are grown by the majority of insect farmers in Europe (Derrien & Boccuni, 2018). The primary product are late larval stages, and the nutrient-rich larval meal is fed to aquaculture, livestock and poultry (Henry et al., 2015; Makkar et al., 2014; Spranghers et al., 2017). All stages of this insect's life cycle – from egg to adult fly (imago) – are cultivated for the purpose of producing larvae, which in turn produces certain by-products (Fricke et al., 2024). Specialised digestive enzymes and nutrient- absorbing microvilli in the midgut of BSF larvae are essential for enzymatic digestion. Their mandibular maxillary complex is strong enough to handle semiliquid food, while gut microbes such as *Enterococcus* and *Bacillus* facilitate the digestion of complex organic materials, absorb nutrients and provide antimicrobial defence, thereby increasing overall biological resistance (Tariq et al., 2025). The promising attributes of BSF include rapid reproduction, low risk as disease vectors, versatility in substrate utilization, a high protein content of 50-60%, an amino acid profile comparable to fishmeal, suitability for vertical farming, a short production cycle and ability to be reared locally

(Barth, Bendag, et al., 2025), especially in tropical and subtropical regions (Da Silva & Hesselberg, 2020). Depending on the substrate and surrounding conditions, BSF larvae can minimise organic waste by up to 84.5% and reach feed conversion ratios of about 16% (Tariq et al., 2025). These characteristics contribute to its suitability as a protein source (Figure 1). Genomic research indicates a diverse spectrum of detoxifying enzymes, immune-related genes and metabolic pathways for amino acid and lipid production, allowing the BSF to thrive in harsh conditions. Additionally, they contain antimicrobial peptides (AMP's) and chitin which may stimulate the immune response upon insect-derived feeding (Koutsos et al., 2022). However, the integrity and efficacy of these compounds can be compromised by the choice of substrate and the processing methods employed (Vogel et al., 2018). Furthermore, the BSF is a genetically highly diverse taxon with a complex phylogeographical structure and increasing regional domestication across the globe (Barth, Stelbrink, et al., 2025). One major disadvantage of BSF larvae is their high fat content and fatty acid composition, as they contain few omega-3 fatty acids but have a high content of saturated fatty acids (Fantatto et al., 2024). The nutritional composition of BSF larvae is affected by factors such as their developmental stage, feeding medium, rearing conditions and post-processing. The substrate has negligible influence on the crude protein content and the amino acid profile of the larvae, whereas the fat and ash content appear to be related to the rearing substrate (Spranghers et al., 2017; Tegtmeier et al., 2021). In addition, it was shown that the substrate composition affects the growth and survival of the BSF larvae (Broeckx et al., 2021; Klüber et al., 2022; Tegtmeier et al., 2021). This variability offers opportunities for the food and feed industry. By managing the feed composition and collection timing, tailored products with optimized nutritional profiles can be developed for human diets, aquaculture, or animal feeds (Huseynli et al., 2023). To date, only the use of bran and a bran-cereal mixture as BSF substrates has been described in shrimp feeding trials (He, Liu, et al., 2022; Richardson et al., 2021), apart from one study by Wang et al. (2021) in which kitchen waste was used.

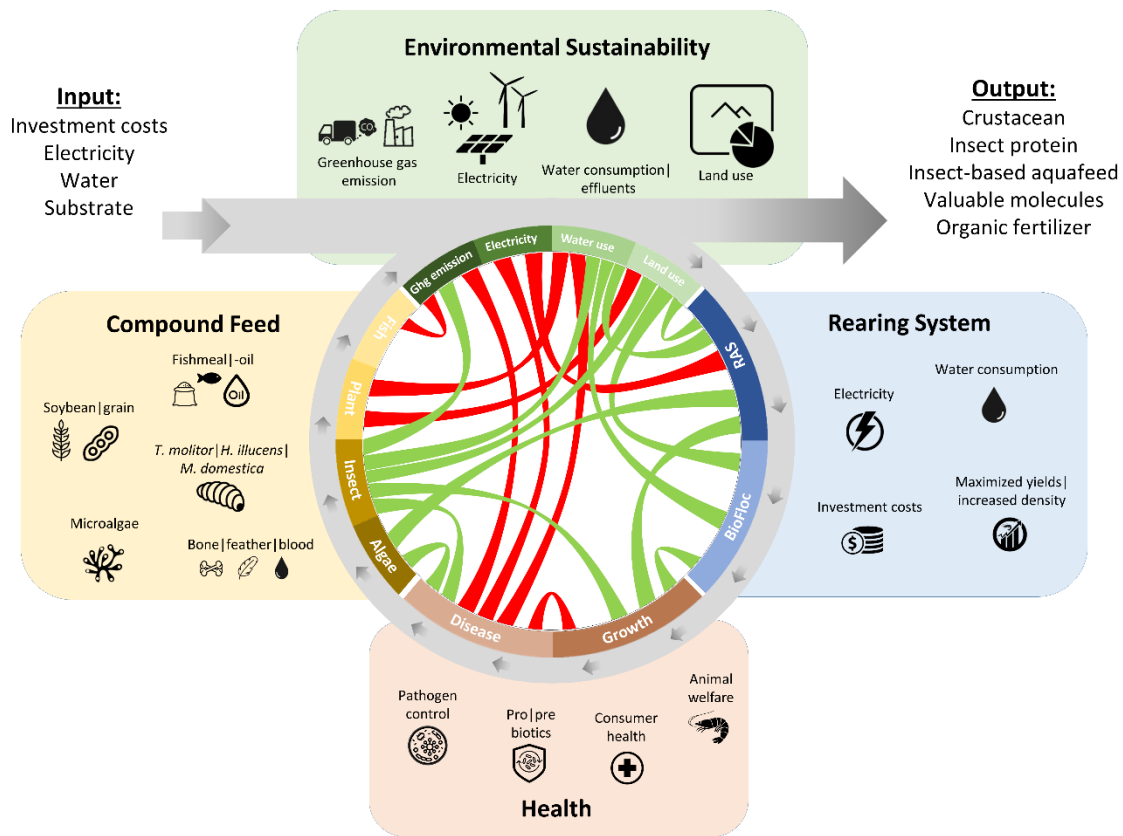


Figure 1: Sustainable, insect-based crustacean aquaculture: A conceptual overview. The environmental sustainability of production and the profitability of the value chain are affected by specific features of the rearing system, health aspects and aquafeed compounds (gray arrows). Green arrows represent interactions that have a positive effect on profitability and/or sustainability, while red arrows represent those with a negative effect. Legal and socioeconomic restriction (not displayed) further govern all these factors. GHG = greenhouse gas. RAS = recirculating aquaculture system. (Röthig et al., 2023)

Particularly the exploration of sustainable substrates for insect rearing becomes crucial in addressing the potential environmental repercussions associated with current practices. Approximately one-third of the global food production is lost or wasted annually, with the highest losses in roots, tubers oil-bearing crops, fruits and vegetables (47%). Food loss occurs during all stages of production and at consumer level (Cattaneo et al., 2024). Using agricultural by-products and food industry side streams to produce insects recovers nutrients that would otherwise be lost (Magee et al., 2021; van Huis, 2020). In addition, it reduces the water footprint (Joly & Nikiema, 2019; Rumpold & Schlüter, 2013) and land loss compared to plant proteins (Salomone et al., 2017), making protein production more sustainable. Consequently, research have started to explore previously underutilized plant by-products as feed for BSF with an attempt to optimize the larvae's nutritional profile. Compared to natural microbial decomposition, this process emits 70% less CO₂, and it also offsets some of the environmental impact of the shrimp production (Röthig et al., 2023). Particularly the by-products from the food production are of interest, since those are available in large quantities and have a low economic value (Nayak et al., 2024). Plant materials like cocoa bean shells, apple pomace and

potato peelings are accessible worldwide and can be used as feed for BSF larvae either directly or after slight processing (Barth, Bendag, et al., 2025). However, there are some potential risks and trade-offs with using plant by-products. On the one hand, there is a nutritional variability when reared on low-value plant by-products which can lead to reduced larval growth. On the other hand, there is a risk for substrate derived contaminants (toxins and heavy metals) (Barth, Bendag, et al., 2025). Recent findings, show that BSF can reduce antibiotic resistance genes (ARGs) and pathogens in biosolids. This highlights how valuable BSF are for eco-friendly treatment of organic residues (Tariq et al., 2025).

1.3.3 Methodological Challenges in Feeding Trials

Current research shows that BSF meal and BSF larvae are the most studied insect-based protein sources for PWS. For BSF larvae, protein-rich substrates improve growth and conversion rates. However, a high fibre content or an imbalance of nutrients can negatively impact performance (Barth, Stelbrink, et al., 2025; Tariq et al., 2025). Both processed and fresh forms of BSF can partially replace fishmeal in shrimp compound feeds without compromising growth or survival up to certain inclusion levels shown in Figure 2 (Barth, Stelbrink, et al., 2025). Processed defatted BSF meal can replace 60% of the total protein source without negative effects (Wang et al., 2021) whereas, non-defatted BSF meal is limited to 25-30% (Chen et al., 2022; Cummins et al., 2017), while BSF larvae can replace up to 50% of the total compound feed (Barth, Bendag, et al., 2025; He, Liu, et al., 2022). Nevertheless, replacement rates below 50% still can negatively impact the health if not balanced in composition (Chen et al., 2022; Cummins et al., 2017). Especially the fatty acid composition, in particular the omega-3 and omega-6 fatty acid balance, significantly affecting the shrimp growth and survival performance (Chen et al., 2021, 2022; He, Liu, et al., 2022).

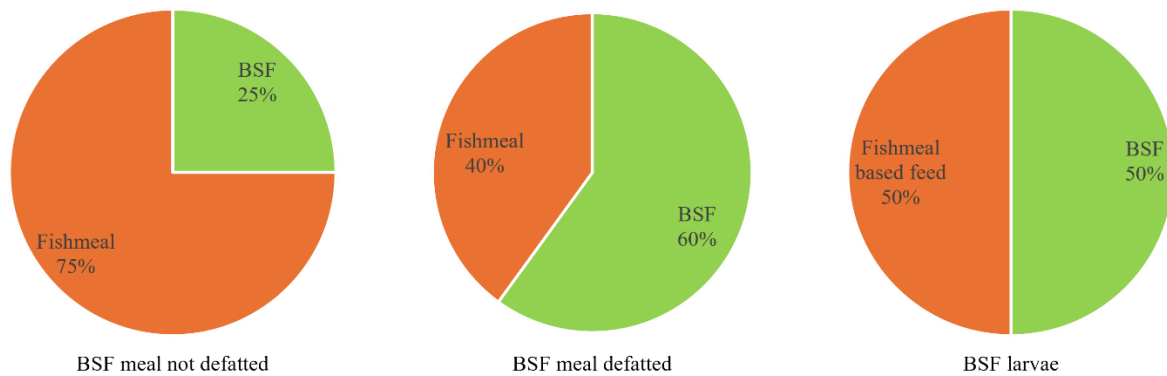


Figure 2: Replacement of fishmeal or fishmeal-based feed without negative effects on shrimp based on literature (November 2025), orange indicates the fishmeal part and green indicates the BSF part. Under each circle is given in which form the BSF were fed.

However, current research faces a major limitation, as it lacks in comparability between the studies since there are a variety of potentially modulation factors which remain unidentified. The various methods used in different studies demonstrate the importance of standardised protocols for ensuring consistent comparisons, as well as the practical application of BSF-based feed for shrimp and their ability to recycle nutrients from plant by-products. Therefore, it is essential to have as much information as possible for comparison and reproducibility of those trials. In particular, information about the BSF larvae or meal as age, genetics, substrate and processing are missing (Barth, Stelbrink, et al., 2025). The age of the larvae at harvest is an essential factor, as the nutritional composition of the larvae changes during development. As they mature, the larvae accumulate more fat, and the protein content reduces (Schneider et al., 2025). Given the genetic heterogeneity of BSF, it is possible that this affects the nutrient composition of the larvae and utilization of substrates (Barth, Stelbrink, et al., 2025). Depending on how the BSF larvae are processed, their nutrition profile may be modified. Furthermore, it is unknown to what extent bioactive compounds such as AMP's are altered (Vogel et al., 2018). In this regard, the implementation of standard reporting guidelines for future studies, as proposed by Barth, Stelbrink, et al. (2025) and Deruytter et al. (2025), would be beneficial. These guidelines could enhance the comparability of research, facilitating the detailed reporting of insect and shrimp genotypes, age, feed, processing, environment and experimental setup. Additionally, it would be adventurous to encourage transparency via open data and alignment with aquafeed reporting norms (Barth, Stelbrink, et al., 2025).

1.3.4 Practical Implementation and Circular Economy Integration

The circular economy model attempts to eliminate waste and pollution by decoupling economic growth from environmental effect (Nobre & Tavares, 2021). A roadmap for a clean, circular economy is outlined in the European Green Deal, which is in line with the Sustainable Development Goals (SDGs) of the UN. As part of this strategy the European Union has developed a Circular economy action plan, which, among other goals, seeks to reduce food loss and waste (Röthig et al., 2023). Insects are directly related with seven of the 17 SDGs and indirectly with another six (Moruzzo et al., 2021), and they are recognized as an important component in closing organic material cycles (Huis et al., 2013). The European Union (EU) has permitted the utilisation of proteins from seven insect species for aquaculture 1) Black soldier fly (*H. illucens*), 2) Housefly (*Musca domestica*), 3) Yellow mealworm (*Tenebrio molitor*), 4) House cricket (*Acheta domesticus*), 5) Tropical house cricket (*Grylloides sigillatus*), 6) Jamaican field cricket (*Gryllus assimilis*) and 7) Lesser mealworm (*Alphitobius diaperinus*) (EC Regulation (EU) 2017/893). The European Commission's Directive No. 2008/98 gives management options for by-products priority, placing more emphasis on recovery, reuse, recycling and prevention than on disposal (Broeckx et al., 2021). It takes close examination to comprehend EU regulations regarding insect-based feeds in aquaculture. The use of proteins from BSF as feed for aquaculture (EC Regulation (EU) 2017/893), poultry, and pig farming has been approved by the EU (EC Regulation (EU) 2021/1372). Producers of insects and their derivatives are subject to regulatory obligations under EU legislation on animal by-products; however, live insects for feed are exempt (Regulation No. 142/2011). Regulatory challenges remain in the United States, while in nations that have historically supported entomophagy, laws are more receptive to new insect products (Röthig et al., 2023).

Overall insect production in Europe was estimated to be 2000 metric tons in 2018 (Fricke et al., 2024) and potential 1,5 million metric tons by 2030 and is expected to exceed 23 million tons worldwide (Elleby et al., 2021). Insects are perfect nutrient recyclers which turning waste into high-value protein while generating co-products such as chitin, AMP's and insect frass as fertilizer. Despite the potential of insect-fed shrimps in terms of health and sustainability branding, the consumer acceptance is dependent on transparency and trust in safety certifications, including those based on established regulations (Röthig et al., 2023). The industrial scalability of BSF systems is limited by a variety of issues, including heavy metal bioaccumulation, regional variations in nutritional output depending on substrate composition and a lack of regulatory clarity (Tariq et al., 2025). Incorporation of the insect-based feed must fit to the different shrimp farming operations so that it does not interfere with established

operations (Röthig et al., 2023). However, most research to date remains lab-scale and upscaling to industrial compound feed might change the preservation of nutrients, digestibility, overall feed quality and requires further assessment (Fantatto et al., 2024). In terms of socioeconomic considerations, shrimp farming in coastal areas has provided an essential income for those communities historically. The inclusion of insect-based feed would add local economic value since it can be managed by small or medium companies, with the use of local plant by-products (Röthig et al., 2023). However, the high initial capital requirements for insect farming infrastructure and processing may prohibit access to larger industrial operations without presence of financial incentives or designated pathways (Barth, Bendag, et al., 2025). Current BSF production is expensive compared to fishmeal and soybean meal production, with labour and the used substrate accounting for ~90% of costs (Tariq et al., 2025). Government incentives, such as waste management fiscal policies, could, nevertheless, aid in lowering these expenses (Matheson, 2022). However, the future of the aquaculture industry is uncertain due to factors like macroeconomic constraints, changing trade laws and climate change, all of which could affect markets and production. Strong governance, prudent aquaculture expansion, and research and innovation spending can help to reduce these risks (Engle & van Senten, 2022).

1.3.5 Scope and aims

Based on the recent state of research and its knowledge gaps, this doctoral thesis aims to assess problems and opportunities in using insects as a sustainable feed in shrimp aquaculture. The first publication (Chapter 2.1) covers the general challenges and chances of using insects as an alternative protein for crustaceans (Specific objective 1). Building on this, comparability and reproducibility of the use of insects as feed are main topics in the second publication (Chapter 2.2). For this purpose, a best practice study was conducted and compared with the current literature in order to develop reporting guidelines to enable effective future research (Specific objective 2). The third publication (Chapter 2.3) provides a deeper understanding of the practical application of BSF larvae within the framework of the circular economy. It provides a comprehensive investigation of the use of BSF larvae reared on plant by-products as sustainable shrimp feed (Specific objective 3).

Specific objectives:

- 1) To address sustainability challenges in using insects as shrimp feed.
- 2) To increase the comparability and reproducibility of shrimp feeding studies.
- 3) To provide a holistic perspective on a practical implementation within the context of a circular economy.

1.4 Publications outlined

This cumulative dissertation is based on two scientific research articles and one review that have been published or accepted for publication in renowned, peer-reviewed scientific journals. The dissertation topic was addressed in the review, which provided a literature review on the sustainable insect-based crustacean aquaculture (Specific objective 1). Building on this, the first article, which is a best practice study on insect-based feed for shrimp, focuses on issues of comparability and reproducibility in the use of insects as feed (Specific objective 2). The second article, which is a holistic study of the use of BSF larvae as sustainable shrimp feed, provides deeper insights into the practical implementation of BSF within the context of circular economy (Specific objective 3).

1st publication

Röthig, T.; Barth, A.; Tschirner, M.; Schubert, P.; Wenning, M.; Billion, A.; Wilke, T.; Vilcinskis, A. Insect feed in sustainable crustacean aquaculture. *J. Insects Food Feed*. 2023, 9, 1115–1138. <https://doi.org/10.3920/JIFF2022.0117>

The full article can be found in Chapter 2.1.

Contributions of the author of the dissertation:

Investigation	Contributed to literature collection
Visualization	Developed figures and tables
Writing – Original Draft	Contributed to writing and editing

2nd publication

Barth, A.; Stelbrink, B.; Klüber, P.; Schubert, P.; Bendag, S.; Wilke, T. Broad acceptance of sustainable insect-based shrimp feeds requires reproducible and comparable research. *Aquac. Int.* 2025, 33, 101. <https://doi.org/10.1007/s10499-024-01769-w>

The full article can be found in Chapter 2.2.

Contributions of the author of the dissertation:

Formal Analysis	Statistical analysis
Investigation	Contributed to data collection
Methodology	Contributed to development of methodology

Project administration	Contributed to research activity planning and execution
Visualization	Developed figures and tables
Writing – Original Draft	Writing initial draft

3rd publication

Barth, A.; Bendag, S.; Klüber, P.; Kreft, D; Schubert, P.; Tegtmeier, D.; Wilke, T. From Plant By-Products to Insects to Shrimp: A Pathway to Sustainable Aquaculture Feed in a Circular Economy. *Aquaculture Nutrition*. 2025, 1. <https://doi.org/10.1155/anu/7288318>

The full article can be found in Chapter 2.3.

Contributions of the author of the dissertation:

Formal Analysis	Statistical analysis
Investigation	Contributed to data collection
Methodology	Contributed to development of methodology
Project administration	Contributed to research activity planning and execution
Visualization	Developed figures and tables
Writing – Original Draft	Writing initial draft

1.5 Discussion

The use of insects, especially BSF larvae, as sustainable feed in shrimp aquaculture presents both opportunities and concerns. BSF larvae can convert plant by-products into high-protein biomass, thereby reducing the ecological footprint associated of standard fishmeal-based feeds (Röthig et al., 2023). However, the nutritional composition of BSF larvae is highly dependent on the substrate utilized, the processing method and the larval age, which can make it difficult to achieve consistent outcomes in terms of shrimp growth performance (Barth, Bendag, et al., 2025). Furthermore, feeding trials are difficult to compare due to a lack of standardised protocols, incomplete reporting of BSF larvae rearing parameters and inconsistencies in experimental design (Barth, Stelbrink, et al., 2025). Despite these limitations, incorporating insect-based feed into circular aquaculture systems has the potential to increase sustainability, close nutrient loops and support decentralised feed production.

1.5.1 Sustainability challenges in using insects as shrimp feed

The nutritional composition of BSF larvae plays a key role when they are used as feed for shrimp. Especially the protein and fat content, as well as the type of fatty acids, are particularly important. Those parameters are influenced by the type of feeding substrate of the BSF larvae (Röthig et al., 2023). Plant by-products with a low-fat content, like apple pomace and potato peeling, lead to a reduced fat content within the BSF larvae compared to a grain-based control substrate (Barth, Bendag, et al., 2025). In a study by Broeckx et al. (2021), BSF larvae were fed twelve different by-products, including apple pulp, and their performance was compared to a grain-based substrate (chicken mash). They observed a positive correlation between substrate protein content and growth (Broeckx et al., 2021). Additionally, it has been observed that whereas protein-rich substrates increase the protein content, carbohydrate-rich substrates cause the larvae to accumulate more lipids (Dîrvariu et al., 2025). However, the BSF larvae showed overall poor growth rates resulting in a low final weight (0.04 g) when fed with apple pomace (Barth, Bendag, et al., 2025), which is consistent with studies by Bonwoo Koo et al. (2023) 0.08 g, Broeckx et al. (2021) 0.04 g and Ribeiro et al. (2022) 0.01-0.03 g. This could be due to the high tannin content or possible anti-nutritional phenolic compounds in apple pomace. Additionally, the high crude fibre and cellulose content could influence digestive processes (Ribeiro et al., 2022). The use of apple pomace in combination with bran or a mixture with other fruits and vegetables showed a higher growth performance (Barbi et al., 2020; Horgan et al., 2023; Lalander et al., 2019). In a mixture of fruits and vegetables (consisting of 50% lettuce,

30% apples and 20% potatoes) (Lalander et al., 2019), the BSF larvae developed similarly to larvae reared on apple pomace. Nevertheless, the prepupae grew larger compared to other protein-rich substrates (Lalander et al., 2019). Protein rich plant by-products like cocoa bean shells, appear to be suitable for BSF larvae rearing, they lead to a high fat content in the larvae. Prior to feeding the larvae to shrimp it would need an additional processing step to defat the larvae which increases the energy consumption of the production (Barth, Bendag, et al., 2025). In contrast, cocoa pods have been shown to result in a decrease in growth rate of BSF (Dzepe et al., 2023). Combining cocoa pods with tithonia leaves (20% replacement) likewise led to improved growth and higher protein content in the larvae (Dzepe et al., 2023). The presence of steroidal alkaloids such as α -solanine and α -chaconine in potatoes (which belong to the nightshade family) necessitates extensive processing before use as livestock feed (Mulder et al., 2024). Despite this, no negative effects on BSF larvae have been observed (Barth, Bendag, et al., 2025). Future studies are required to ensure that there is no accumulation of steroidal alkaloids present and that they do not pass on through the food chain. Furthermore, the nutrient profile of plant by-products varies considerably depending on their place of origin and plant cultivar (Barth, Bendag, et al., 2025; Broeckx et al., 2021). Nevertheless, the growth rates of BSF larvae on plant by-products are lower compared to grain-based substrates, which could be due to toxins or a lack of nutrients (Barth, Bendag, et al., 2025). Consequently, the supplementation of plant by-products with specific essential amino acids that are missing may be instrumental in optimising their use as feed for BSF larvae and enhancing their growth performance (Barth, Bendag, et al., 2025). Therefore, micro- and macroanalysis of the plant by-products are essential. Another possible solution for enhanced larval growth might be the combination of different plant by-products or in combination with grain instead of a mono-substrate.

However, when feeding BSF larvae (50% replacement of the fishmeal-based compound feed) reared on plant by-products to PWS no differences in growth rates were observed. Instead, the use of these larvae had a positive effect on the feed conversion ratio (FCR) (Barth, Bendag, et al., 2025). Comparable to the results of He, Liu, et al. (2022), where replacing 50% of the compound feed with BSF larvae (bran substrate) had no adverse effects on growth performance and survival rate, digestive enzyme activities and antioxidant enzyme activities. There were only two other studies in which the substrate of the BSF larvae was specified with defatted BSF meal. One study, conducted by Wang et al. (2021), found that it was possible to replace up to 60% of fishmeal with defatted BSF larvae meal (kitchen waste substrate) without any adverse effects on the growth performance, antioxidant and immune enzyme activity and digestive

enzyme activity of the shrimp. The other study used a Food Grade GMP+ (wheat bran and cereal mill offal) certified substrate for rearing the BSF larvae. The researchers utilized defatted meal for the feeding trial, which led to improved growth of the shrimp (Richardson et al., 2021). The FCR from the two papers with defatted meal ranged between 1.1 – 1.4 (Richardson et al., 2021; Wang et al., 2021), which is consistent with the FCR from the grain-based group from Barth, Bendag, et al. (2025). In a study by Motte et al. (2019), defatted meal from *T. molitor* (yellow mealworm) reared on vegetal feed material was shown to improve growth and feed conversion (best at 50% replacement), improve disease resistance and reduce immunosuppression in shrimp. In comparison to other studies that have employed BSF as a fishmeal substitute, in which the utilized substrate was not specified, there are notable differences. The utilization of non-defatted BSF meal has been observed to result in adverse effects on shrimp when the replacement exceeds 25-30% (Chen et al., 2021, 2022; Cummins et al., 2017). Similar findings were shown using full fat BSF larvae there were no adverse effects on the shrimp up to a replacement of 22.5% (Usman et al., 2021). However, the water quality was slightly reduced at the end of the trial, and they noticed an increased mortality due to cannibalism after moulting (Usman et al., 2021). Conversely, feeding the shrimp fresh larvae seems to keep them occupied for longer and allows them to satisfy their natural behaviours more effectively. This leads to less observed aggression and cannibalism. The observed low replacement rates with non-defatted BSF meal are probably due to the lack of omega-3 and omega-6 fatty acids, which are essential for shrimp nutrition and the comparatively high proportion of saturated fatty acids in BSF meal (Röthig et al., 2023). In fish aquaculture, replacing fishmeal with BSF has equally varied effects, which depend on species, life stage and replacement level. Substitution rates range widely, with 10-100% of fishmeal being replaced by BSF meal without negative effects. However, the lack of polyunsaturated fatty acids and lower levels of methionine and lysin seems to be a key issue that has a negative impact on fish, especially marine/carnivorous species, as well (Dîrvariu et al., 2025). Nevertheless, other factors may have contributed to this heterogeneous picture of research results in shrimp and fish. A key challenge arising from the lack of comparability between studies due to missing details in the reporting.

1.5.2 Reproducibility and comparability of feeding trials

Worldwide, concerns regarding food safety, hygiene during processing, and potential contamination during production chains have been raised. To promote the use of insects as food

and feed, researchers suggested standardising rearing and processing procedures and implementing effective strategies to enhance food safety (Tang et al., 2019). Reproducibility and comparability are essential for high quality research yet there are no standards for feeding trials with shrimps and insect-based feed (Barth, Stelbrink, et al., 2025). In addition, a wide range of variables must be considered during the preparation of insect larvae for use as feed. For trials with BSF larvae and yellow mealworm larvae, reporting guidelines have been established by Deruytter et al. (2025) to ensure increased comparability. However, some factors have a higher impact on comparability and reproducibility in shrimp feeding trials than others. The genetics of the BSF vary widely depending on location and breeding, raising the question of whether this is still considered one species (Barth, Stelbrink, et al., 2025). In addition, the substrate used and the timing of the BSF larvae harvest plays a key role in their nutrient profile, with protein and fat content being particularly important for PWS. Despite this, the substrate used is only mentioned in six feeding trials (Barth, Bendag, et al., 2025; Barth, Stelbrink, et al., 2025; He, Liu, et al., 2022; He, Zhang, et al., 2022; Richardson et al., 2021; Wang et al., 2021) and the life stage of the larvae in only three (Barth, Bendag, et al., 2025; Barth, Stelbrink, et al., 2025; Richardson et al., 2021). So far, the genetics of the BSF larvae have only been specified in two feeding trials by Barth (Barth, Bendag, et al., 2025; Barth, Stelbrink, et al., 2025). Therefore, it is nearly impossible to compare the results of previous trials on BSF-based feed for shrimp and determine the parameters required to custom tailor this feed for shrimp. In particular, six categories are crucial for comparability and reproducibility: 1) characteristics of the insect, 2) shrimp species, 3) characteristics of the insect-based feed, 4) pre-treatment of shrimp, 5) water parameters and 6) experimental design of the shrimp feeding trial (Barth, Stelbrink, et al., 2025). Based on these categories, a list of reporting guidelines was compiled with information relevant to each category, which should be included in future shrimp feeding trials to improve comparability between trials (Barth, Stelbrink, et al., 2025). This should enable the identification of further modulating factors and development of a custom-tailored BSF based feed for shrimp.

1.5.3 Circular economy and practical implementation

The BSF is a promising source of high-quality protein for use in sustainable shrimp aquaculture. However, to remain economically viable, the maximum price for BSF meal must not exceed USD 3.04/kg (Nunes et al., 2023). The current price in Europe is around EUR 3.50/kg. This price could be significantly reduced through upscaling with automation and the use of plant by-

products and the utilization of co-products such as frass (Dírvariu et al., 2025). For BSF production to be profitable, 110 tons of plant by-products must be processed daily to produce 7.7 tons of insect meal. Underutilized plant by-products will be key to the expansion of the insect feed business (Tariq et al., 2025). The conception of an insect-based shrimp feed, in particular using BSF larvae reared on plant by-products, would enable circular economy. Due to the valorisation of plant by-products and transforming them into high quality protein which reduces the environmental impacts compared to fishmeal (Barth, Bendag, et al., 2025). Approximately 50-60% of the carbon in the substrate is converted into insect biomass by BSF larvae, resulting in far lower CO₂ emissions than composting or vermicomposting (Tariq et al., 2025). The frass (insect excreta), a byproduct of BSF larvae production, can serve as a nutrient-rich biofertilizer that has a higher nitrogen content than conventional organic alternatives. It contains plant-accessible nitrogen, growth promoting biomolecules and microorganisms, thus creating nutrient loops in integrated farming systems to support agroecological cycles and enhance crop tolerance to stress, pathogens and pests (Beesigamukama et al., 2020; Poveda, 2021). Over 80% of plant by-products can be converted into larvae and frass in less than two weeks (Tariq et al., 2025).

Insects as a protein source for shrimp can be used in both low-tech and high-tech approaches. In developing countries in the global south, local communities use low-tech approaches for BSF rearing. In these regions people focus on self-sufficiency and selling small surpluses, therefore many value-chain and business initiatives fail since they overlook local limitations including weak public support, poor infrastructure and social inequalities. For a sustainable and realistic approach, they must first develop local markets and then build on these to expand into more complex markets and higher-value products (Barragán-Fonseca et al., 2023). Possible approaches involve the use of local plant by-products for BSF farming, creating jobs and reducing dependency on large corporations (Barragán-Fonseca et al., 2023; Piercy et al., 2022). The BSF larvae can then be fed directly to the shrimp in semi-extensive pond farming, in addition to compound feed (Barragán-Fonseca et al., 2023). Consequently, no further processing or energy consumption is required. Furthermore, the local climate provides optimal rearing conditions for BSF larvae (Shumo et al., 2019). Wealthier industrialized nations generally have high-tech, closed, recirculating and land-based shrimp farms. To accommodate the large number of shrimp in a compact space, custom-tailored compound feed is necessary (Röthig et al., 2023). BSF meal can be used to reduce or completely replace the fishmeal content. Larval rearing (maintaining optimal rearing temperature, humidity and aeration) and processing are the major drivers for energy consumption therefore it is essential to use

renewable energy for an optimal Life Cycle Assessment (LCA) (Ramzy et al., 2025). For BSF larval production, a substrate such as apple pomace should ideally be used, as fat content of the larvae is quite low at harvest, eliminating the need for defatting (Barth, Bendag, et al., 2025). In addition, the heat-waste from large datacentres or other industrial facilities can be used for BSF larval rearing in urban areas.

There are numerous benefits of using BSF larvae reared on plant by-products as an alternative protein source in shrimp aquaculture, within the framework of the three pillars of sustainability (ecological, economic and social). From an ecological standpoint, promoting insects as a protein source can reduce reliance on wild fish stocks and other natural resources, conserving biodiversity and ecosystem health (Auzins et al., 2024). Protein-rich biomass can be produced from plant by-products, such as apple pomace and potato peels, by BSF larvae, while significantly less land and water are required compared to conventional protein sources like fishmeal or soy meal. LCAs show that BSF larvae reared on plant by-products can result in lower greenhouse gas emissions compared to conventional livestock feed provided that the energy required for processing (e.g., drying, freezing, or grinding) is sourced from renewable energy systems (Tariq et al., 2025). However, challenges remain in maintaining consistent feed quality, as nutritional composition and safety of BSF larvae can be affected by substrate variability and potential contamination with heavy metals or mycotoxins (Barth, Bendag, et al., 2025; Jucker et al., 2025). BSF production becomes economically feasible at an industrial scale, processing up to 110 tons of by-products per day, when co-products like frass, oil and chitin are effectively valorised. Nevertheless, its large-scale implementation remains limited by infrastructural costs ranging from 1-2 million euros (Europe) and substantial energy demands for processing (Cattaneo et al., 2024; Tariq et al., 2025). Political initiatives prompting research into sustainable feed, as is already the case in Norway, can have an impact (Auzins et al., 2024). Additionally, insects can function as powerful tools for disease control hence their economic importance (Röthig et al., 2023). The social benefits of insect farming include job creation, feed autonomy in rural areas, and aligns with circular economy principles. However, from a socio-economic perspective, BSF meal has a negative cost and benefit balance in the Baltic-Nordic region. The primary challenge in enhancing insect farming productivity arises from the variability in production processes, operational scale, and regulatory constraints encountered by farmers (Auzins et al., 2024). Factors such as technical issues, operational and investment costs and consumer acceptance all contribute to the overall complexity of the situation. To achieve a positive balance, further research, up scaling, reduction of operating and investment costs are necessary (Auzins et al., 2024). Regulatory uncertainty regarding acceptable substrates

and the classification of by-products is an important barrier to commercial acceptance. So is limited consumer familiarity with insect-fed shrimp (Abenaim & Conti, 2025; Auzins et al., 2024; Röthig et al., 2023). Scientifically and economically, BSF larvae reared on plant by-products represent a promising approach to sustainable aquafeed, given that regulatory frameworks, processing efficiency and market transparency are effectively addressed.

1.5.4 Limitations and future directions

Currently there are several limiting factors. It's not known to what extent the nutrient profile is altered by processing, particularly the immune-stimulating components such as AMPs. Therefore, the results of feeding fresh BSF larvae may not be directly transferable to compound-based shrimp aquaculture. In addition, the feeding habits of shrimp differ from those of fish, as shrimp do not consume the larvae or pellets whole. Shrimp can be considered sloppy feeders since they manipulate the feed particles with their appendages in front of their mouthparts and may consume only parts of the feed (Stadtlander et al., 2025). This can lead to selective feeding, notably in the case of BSF larvae, where the cuticle remains uneaten. Moreover, this feeding behaviour may result in the loss of nutrients and increased water pollution. Comparability of previous study findings is further limited by missing information and high variability. The trials have been conducted mostly under laboratory conditions and on a laboratory scale, and there is a lack of information on scalability to an industrial scale.

1.6 Conclusions and outlook

This Synthesis shows that BSF can be used as a sustainable alternative protein source in aquafeeds, given that remaining issues are addressed. It was demonstrated that BSF larvae can convert low-value plant by-products into high-quality protein, thereby BSF may buffer suboptimal substrate quality. However, further research must be conducted on the optimization of these plant by-products as substrates, either by mixing them strategically or adding necessary amino acids to ensure proper larval rearing. Likewise, the bioaccumulation of potential toxins by of these substrates in the larvae must be investigated to obtain a safe product for the consumer.

In terms of feed performance, it has been shown that BSF larvae with 50% replacement of the fishmeal-based compound feed in shrimp feeding results in excellent FCRs. Nevertheless, feeding BSF larvae directly to shrimp is only possible in small scale farms or extensive farming systems. For intensive farming, it's not feasible due to the amount of larvae which would be needed, and it probably would harm the water quality or lead to a huge intensification in filtration effort and energy consumption. Therefore, future studies should focus on the use of BSF meal derived from larvae reared on plant by-products in compound feed. Achieving comparable and reliable results will depend on the transparent reporting of all essential parameters, following the proposed reporting guidelines.

The viability of circular economy approaches in this context remains largely theoretical and dependent on multiple conditions. A baseline would be an optimized BSF rearing with the use of plant-by products and scale up in production. Further research is required to increase the inclusion rates in shrimp feeding, for direct feeding of the larvae and the replacement of fishmeal in compound feeds. Additionally, investigate the scalability of these processes on an industrial scale, ensuring the economic viability of the proposed solutions. To achieve these objectives, there is a necessity to promote interdisciplinary research, thereby facilitating the integration of nutrition, genetics, insect biotechnology and aquaculture engineering. Ideally, laboratory research should be linked with industry partners to address all requirements.

BSF based feed holds great potential for commercial shrimp farming if the three pillars of sustainability are considered. The key challenges in the future are scalability, political regulatory and consumer acceptance in areas where insects a not yet part of the human diet.

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2 Publications

2.1 1st publication: Insect feed in sustainable crustacean aquaculture

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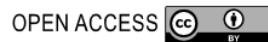
Insect feed in sustainable crustacean aquaculture

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REVIEW ARTICLE

Abstract

Aquaculture is a growing global food production sector that aims to meet the increasing demand for dietary protein. Crustaceans are an important and predominantly high-priced aquaculture segment that could support the transfer of sustainable new technologies to other sectors. Areas of interest include disease management and compound feeds, both of which have the potential to improve both the profitability and sustainability of aquaculture. Modern compound feeds are largely composed of fishmeal and/or terrestrial plant materials, the production of which is unsustainable, leading to the depletion of finite resources. Insects are a promising protein-rich alternative to fishmeal that can reduce the environmental footprint of aquafeeds and crustacean aquaculture. First research data have shown that insect meal has a favourable nutritional composition with positive health effects, and is environmentally sustainable with a strong economic potential, particularly supporting circular economy by valorising otherwise unused side-streams. In this article, we discuss the current state of crustacean aquaculture and highlight the benefits of insect meal compared to today's compound aquafeeds in terms of health and growth-promoting properties as well as environmental benefits. We then consider the molecular mechanisms that confer immunity and disease resistance in crustacean aquaculture and show how insect feeds can support disease management and thus consumer health. Next, we assess the environmental sustainability of crustacean aquaculture and insect farming, the legal framework for insect-based feeds and consumer acceptance issues. Lastly, we identify research gaps, socioeconomic considerations and the potential of insect-based sustainable crustacean aquaculture for the global market.

Keywords: *Litopenaeus vannamei*, *Hermetia illucens*, *Tenebrio molitor*, shrimp

1. Introduction

Marine animal proteins are needed to support globally increasing food demands (FAO, 2018). In 2019, 120 million tons of fish, crustaceans, mollusks, and other aquatic animals contributed to global nutrition (FAO, 2021a). An additional 37.4 million tons are required by 2025, but deteriorating ecosystems will not support an increase in the sustainable foraging of aquatic species (Hua *et al.*, 2019). The catch rate of global fisheries has remained largely stable since 1990, while aquaculture production increased from ~15 million tons in 1999 to more than 82 million tons in 2018, and further growth is anticipated (FAO, 2020). However, the expansion of aquaculture increases

greenhouse gas emissions (Yuan *et al.*, 2019). The growth of aquaculture is projected to slow down between 2019 and 2030 due to stricter environmental regulations, the restricted availability of water and production locations, disease outbreaks exacerbated by intensified production, and lower productivity gains (FAO, 2020). Importantly, feed and energy consumption, and the discharge of effluents, are the main reasons why intensive and semi-intensive crustacean aquaculture is unsustainable, and these factors also govern profits (Cao *et al.*, 2011; Marín-Riffo *et al.*, 2021).

Recent developments in the field provide promising avenues to improve the sustainability of crustacean aquaculture. Modern technologies for water treatment and pathogen

control can improve animal health and reduce water consumption, although such benefits are offset by higher energy demands (Ruiz *et al.*, 2019; Zaibel *et al.*, 2022). The availability of these cutting-edge technologies is mainly limited to premium target species. The demand for high-value species such as sustainably sourced shrimps is predicted to grow, unlocking new production locations in land-based systems, for example in Europe (FAO, 2020). Here, water-efficient recirculating aquaculture systems (RAS) can substantially reduce aquaculture's environmental impact, particularly when powered by renewable energy (Badiola *et al.*, 2018). Furthermore, new insect-based compound feeds offer an alternative and sustainable source of proteins to improve health and enhance growth (Cottrell *et al.*, 2020), but such feeds are not yet commercially available. Facilitated by high product prices, new technologies (including feeds) can be transferred to lower-priced species and regions, facilitating profitable and sustainable production.

Recent work on insect-based feed in aquaculture encompasses primarily fish data (Tran *et al.*, 2022a,b). However, given their broad phylogenetic and physiological differences, this review synthesises current developments in crustacean aquaculture and the potential for innovative and sustainable food production. First, we briefly describe current crustacean aquaculture systems. We then characterise commercial aquafeeds for crustaceans and compare the suitability of insect-based feeds. We next elaborate on recent crustacean health perspectives and assess the benefits conferred by insect-based feeds. To outline the economic and social framework, we then discuss the ecological and economic sustainability of aquafeeds and insect proteins, the current legal framework, and the consumer acceptance of insect-fed crustacean products. We conclude by evaluating insect production for aquafeeds and its potential socioeconomic consequences.

2. Crustacean aquaculture

Aquaculture is the controlled farming of freshwater and saltwater organisms (mariculture), including fish, mollusks, crustaceans and aquatic plants. Such organisms are usually farmed in ponds, tanks or raceways, using land-based (onshore), coastal (nearshore) or offshore facilities. Aquaculture can be traced back more than 4,000 years in China (Beveridge and Little, 2002) and its global importance is increasing (FAO, 2020; Tacon, 2020). Since 1960, annual fish consumption has outpaced world population growth driven by increasing incomes and the adoption of well-balanced diets. For example, the annual per capita consumption of fish increased from 9.0 kg in 1961 to 20.3 kg in 2017 (FAO, 2020, 2021b; Noguera-Muñoz *et al.*, 2021).

Crustaceans account for 9% of production in the global aquaculture sector (Figure 1). Since 2000, crustacean

production has grown continuously at an annual rate of nearly 10% (Tacon, 2020), reaching 10.5 million tons and a market value of US\$ 276.6 billion in 2019 (FAO, 2021b). Marine shrimps dominate the sector, followed by crayfish, crabs and prawns (Figure 1). Pacific whiteleg shrimp (*Litopenaeus vannamei*), Red swamp crawfish (*Procambarus clarkii*), Chinese mitten crab (*Eriocheir sinensis*), Giant tiger prawn (*Penaeus monodon*), Oriental river prawn (*Macrobrachium nipponense*) and Giant river prawn (*M. rosenbergii*) make up ~93% of global crustacean production (FAO, 2021a; Tacon, 2020) (Figure 1). Their market value ranged between US\$ 5.64/kg (shrimps) and US\$ 16.93/kg (lobsters and langoustes) in 2018 according to the FAO (FAO, 2021c). Similar to aquaculture in general, nearly 90% of farmed crustaceans are produced in Asia and most are also consumed there, with China alone producing 50% (Costa-Pierce and Chopin, 2021; El-Sayed, 2021; Naylor *et al.*, 2021). The production of *E. sinensis*, *P. clarkii* and *Portunus* spp. (swimming crabs) is mostly restricted to China (FAO, 2021a).

The increasing demand for aquatic products has driven the development of aquaculture systems. For shrimps, the first extensive coastal pond culture was reported in 1957 (Lebel *et al.*, 2002). Intensified production started in the 1980s and can be quantified by the degree of water exchange, aeration, input of (pelleted) feeds, fertiliser and medication, and the resulting yields (Lebel *et al.*, 2002). Today's shrimp culture can be classified by the type of culture vessel (pond, raceway or tank), the type of water exchange system (open, semi-closed or closed), the relative stocking density, and the energy demand for water maintenance, feed and transport (extensive, semi-intensive, intensive or super intensive) (Figure 2).

Intensified production is associated with several challenges, often related to sustainability and the corresponding ecological, economic and social issues. Traditional coastal shrimp farming in ponds has caused extensive mangrove deforestation (Noguera-Muñoz *et al.*, 2021) and related ecological and economical losses (Walton *et al.*, 2006). Accordingly, social licenses in aquaculture are being considered to improve community-industry relations (Mather and Fanning, 2019). Alternative in-land pond systems compete for space with agriculture and may intensify greenhouse gas emissions (Yuan *et al.*, 2019). Stocking densities are increased to boost the economic output of semi-intensive and intensive pond cultures compared to extensive ponds, which requires enhanced feeding and water quality management (aeration and water exchange) to ensure the better control of pathogens, parasites and pests. Furthermore, intensified production in traditional systems increases the release of effluents that may lead to the eutrophication of adjacent ecosystems (Boyd *et al.*, 2007).

In modern super-intensive crustacean aquaculture, water quality management and specific compound feeds are

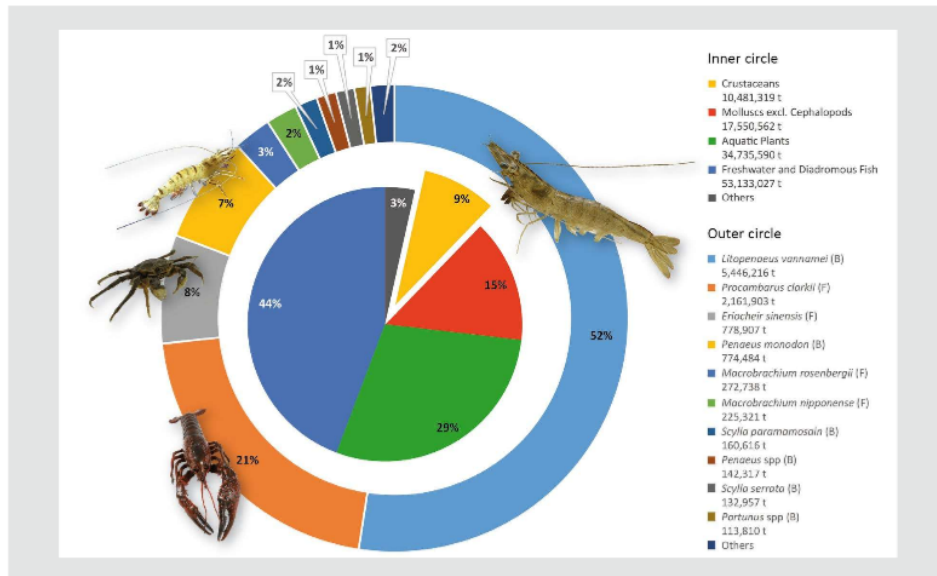


Figure 1. Global aquaculture production and main crustacean species in 2019. Inner circle represents total global aquaculture production in 2019 (120,098,422 tons, including 10,481,319 tons of crustaceans). Outer circle represents the 10 commercially most important crustacean aquaculture species (65 listed species). B = brackish and marine water; F = fresh water (data from FAO, 2021a).

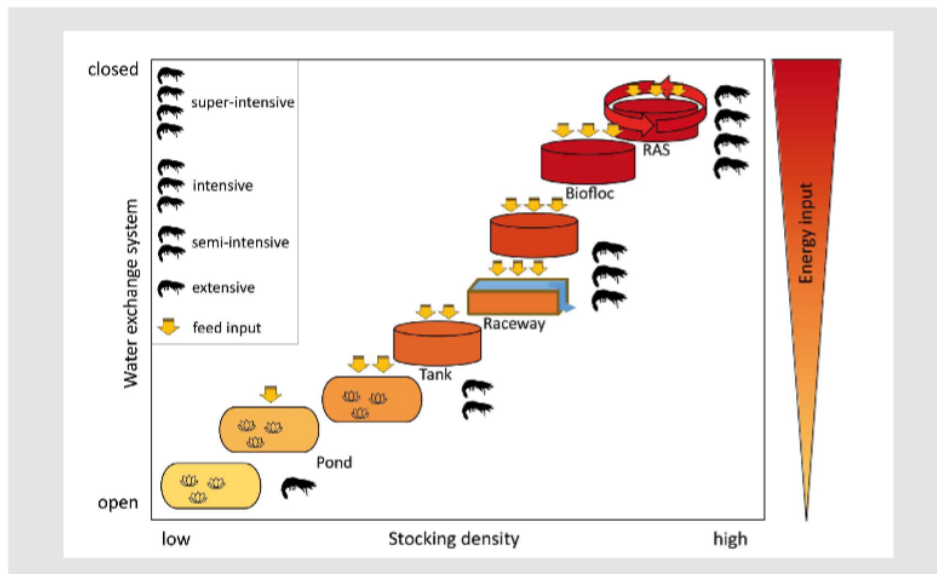


Figure 2. Crustacean culturing systems. Culture vessel (pond, raceway or tank), type of water exchange system (open, semi-closed, or closed/recirculating), and energy demand for water maintenance, feed and transport (extensive, semi-intensive, intensive or super-intensive). Stocking density is generally higher at higher intensities and feed inputs. RAS = recirculating aquaculture system.

necessary to avoid the excessive discharge of effluents and to ensure good culturing conditions. Furthermore, new technologies allow for 'smart aquaculture', a combination of artificial intelligence, Internet of Things technology, and traditional aquaculture (Hu *et al.*, 2020; Mustapha *et al.*, 2021). For example, real-time monitoring of vitality and behaviour, risk prediction, and the control of physical and chemical parameters can optimise the health and growth of farmed species. There are two main forms of super-intensive farming: clear-water RAS and biofloc. In the former, high-performance filters exchange less than 1% of the water per day by combining ammonia-removing biofilters and UV or ozone water sterilisers, although this involves high upfront investment costs and consumes a lot of energy (Badiola *et al.*, 2018; Ray *et al.*, 2017) (Figure 2 and 3). These costs can be offset in part by very high stocking densities, which in turn increase the risk of catastrophic failure due to disease (Naylor *et al.*, 2021). Therefore, disease management is a core element of modern aquaculture, including the development of specific pathogen free (SPF) broodstocks and (feed) additives such as antimicrobial peptides (AMPs), double-stranded RNA (dsRNA) and immunostimulants

(Flegel, 2019). In contrast, biofloc technology encompasses aggregates of bacteria, algae or protozoa embedded in a matrix of particulate organic matter with a high carbon/nitrogen ratio. When added to the culturing system, the mostly heterotrophic microbial communities respire carbon and incorporate bioavailable nitrogen, thereby avoiding harmful ammonia levels in the water and providing edible aggregates that consist of microbes, algae and protozoa for the cultured (crustacean) species (El-Sayed, 2021). While the investment costs for biofloc systems are lower than those for clear-water RAS, the technology is only suitable for a limited number of species and the water quality is generally lower (Dauda, 2020; El-Sayed, 2021).

Modern (crustacean) aquaculture systems can increase production while reducing water consumption, effluents and land use (Figure 3). However, it is important to acknowledge that the high costs of such systems currently limit them to wealthy countries, which contribute a negligible fraction to global crustacean production. Unlike expensive technologies, compound feeds – once developed – are more readily available. Such formulations can reduce

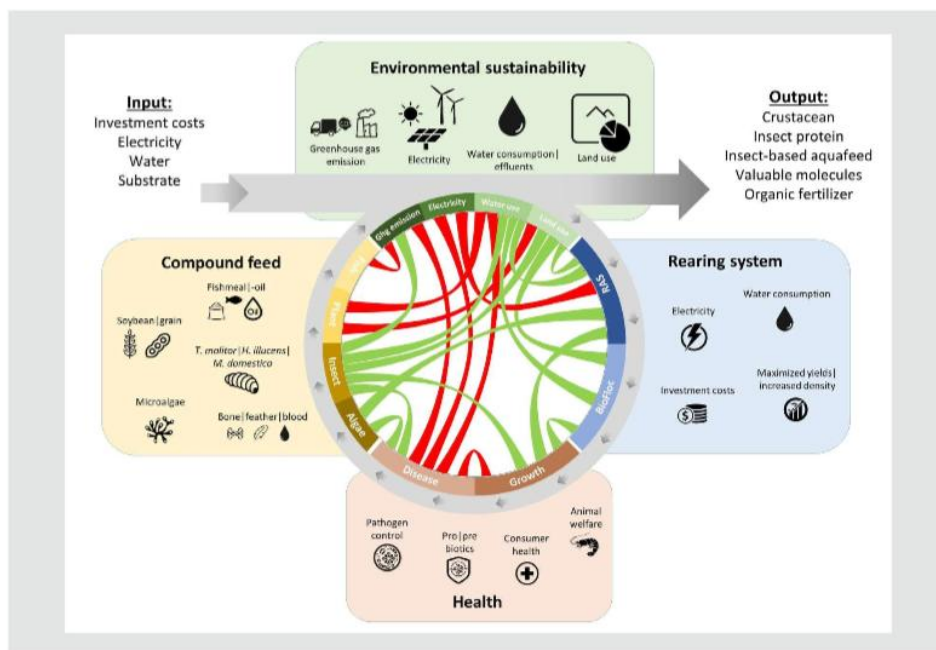


Figure 3. Conceptual overview of sustainable, insect-based crustacean aquaculture. Specific features of the rearing system, health aspects and aquafeed compounds affect each other, the environmental sustainability of production, and the overall profitability of the value chain (gray arrows). All these factors are further governed by legal and socioeconomic restrictions (not displayed). Interactions with positive and negative effects on profitability and/or sustainability are represented by green and red arrows, respectively. Ghg = greenhouse gas; RAS = recirculating aquaculture system.

costs, enhance yields, limit greenhouse gas emissions and thus help to conserve marine resources (Badiola *et al.*, 2018; Cortés *et al.*, 2021; Shah *et al.*, 2018; Verbeke *et al.*, 2015; Yuan *et al.*, 2019; Ziegler *et al.*, 2013).

3. Insects in aquaculture feed

Compound feed

Fed aquaculture accounts for 69.5% of global aquaculture and nearly 90% of crustacean aquaculture (Malcorps *et al.*, 2019). Production relies largely on formulated compound feeds (FAO, 2020), which represent 50-70% of total production costs (Ferrer Llagostera *et al.*, 2019; Gong *et al.*, 2019). State-of-the-art aquaculture feeds contain proteins (including essential amino acids), carbohydrates, fats (including polyunsaturated fatty acids, PUFAs), vitamins, minerals and pigments (Ansari *et al.*, 2021; Hua *et al.*, 2019). Depending on species and life stage, the composition is adjusted to meet macronutrient and micronutrient demands, maximise growth, and promote health to yield high-quality and safe products that meet consumer requirements (Ansari *et al.*, 2021; Hua *et al.*, 2019). Further additives include functional feed components that reduce oxidative stress, boost appetite or confer disease resistance (Hua *et al.*, 2019). Typical functional feed additives include vitamins, PUFAs and highly unsaturated fatty acids (HUFAs), amino acids (e.g. methionine), carotenoids, prebiotics and probiotics.

The key challenge in the development of sustainable aquafeeds is their reliance on fishmeal and fish oil, which are not environmentally friendly (Checkley *et al.*, 2017; FAO, 2016, 2018; Froehlich *et al.*, 2018; Pauly and Zeller, 2016) and particularly crustacean aquaculture has been a major consumer (27.5% of total fishmeal fed in aquaculture in 2003) (Tacon *et al.*, 2006). Alternative compound feeds are often unsustainable, unavailable in sufficient amounts, or too expensive. Proteins contribute most to these costs because feeds must include large amounts of high-quality protein (Ansari *et al.*, 2021) (Table 1). Protein sources must provide an adequate amino acid profile while also ensuring digestibility and palatability (Sánchez-Muros *et al.*, 2020). Depending on species and age, the standard formulation of shrimp feed, for example, consists of 35-50% proteins (National Research Council, 2011) and 6-14% lipids (Ayisi *et al.*, 2017b). Potential alternatives to fishmeal and fish oil include plant-based proteins, animal by-products, algae, yeast and insects. Plant-based feeds may provide a good source of protein although many are characterised by unbalanced amino acid profiles, a low protein content, anti-nutritional factors, or low attraction and palatability. Soybean meal is often included in aquafeeds and offers digestible, high-quality protein with a beneficial amino acid profile at low cost (Jatobá *et al.*, 2017). However, it cannot fully replace fishmeal and is also considered

unsustainable, particularly because large areas of land are needed for soybean cultivation (Gil-Núñez *et al.*, 2020; Sánchez-Muros *et al.*, 2020). Terrestrial plant materials for aquafeeds generally intensify the pressure on agricultural resources by increasing the demand for fresh water, land and fertilisers (Richardson *et al.*, 2021). In aquaculture, the nutritional properties of animal protein sources are considered superior to those of plants, including better digestibility, more suitable amino acid profiles, the absence of anti-nutritional factors, and a higher crude protein content (Sánchez-Muros *et al.*, 2020). However, the essential amino acid and fatty acid profiles of blood meal, hydrolysed feather meal and poultry by-product meal, for example, differ from those of marine sources and are only partly suitable as aquafeeds (Bandara and Tharindu Bandara, 2018; Sánchez-Muros *et al.*, 2020).

In addition to proteins, PUFAs and HUFAs are recognised for their health benefits in crustaceans and humans (Patnaik *et al.*, 2006). Currently, only microalgae provide a comparable omega-3 fatty acid profile to fish oil for crustacean nutrition (Cottrell *et al.*, 2020). Some microalgae synthesise HUFAs such as eicosapentaenoic acid and docosahexaenoic acid, which promote sustainable production (Ansari *et al.*, 2021) and confer digestibility and palatability (Han *et al.*, 2019). Natural pigments from microalgae, such as astaxanthin, chlorophyll and carotenes, promote shrimp growth, and (in the case of astaxanthin) confer the pink flesh colour in cooked shrimp (Han *et al.*, 2019). Microalgae as an additive or substitute for fishmeal have positive effects on crustacean growth and quality, and achieve a favourable feed conversion ratio (FCR) (Cottrell *et al.*, 2020; Ju *et al.*, 2012). The environmental impact of microalgae is lower than that of terrestrial plants, fishmeal and fish oil (Beal *et al.*, 2018). They also provide additional benefits such as oxygen production, CO₂ fixation, water quality improvement and disease control (Charoonnart *et al.*, 2018; Han *et al.*, 2019; Nie *et al.*, 2020) (Figure 3). Although microalgae are essential in crustacean hatcheries, their current low global production volume covers only 0.7% of the demand and therefore critically limits their use as a major component in crustacean feed (Oostlander *et al.*, 2020; Sánchez-Muros *et al.*, 2020).

Bacteria, yeast and insects are other alternatives that can be used as additives or major protein sources in aquafeeds. Bacteria and yeast have high protein contents and amino acid profiles comparable to fishmeal (Hua *et al.*, 2019). Indeed, bacterial components in aquafeeds constitute the functional base of biofloc technology. Furthermore, microbial bioactive products can reduce the amount of fishmeal required in the diet of the carnivorous Black tiger shrimp (*Penaeus monodon*), but they are expensive (Hua *et al.*, 2019) and dose control is important (Cottrell *et al.*, 2020). Yeast derived from hydrolysed lignocellulosic biomass is also suitable for carnivorous species such as Atlantic

Table 1. Composition of crustacean compound feeds.¹

Compound	Fishmeal-based (%)		Insect-based (%)		References
	min	max	min	max	
Fishmeal	15	42.6	0	38.4	Choi <i>et al.</i> , 2018; Cummins <i>et al.</i> , 2017; Mazlum <i>et al.</i> , 2021; Motte <i>et al.</i> , 2019; Panini <i>et al.</i> , 2017a,b; Peh <i>et al.</i> , 2021; Rahimnejad <i>et al.</i> , 2019; Richardson <i>et al.</i> , 2021
<i>Hermetia illucens</i> meal	NA	NA	0	36	Cummins <i>et al.</i> , 2017
<i>Tenebrio molitor</i> meal	NA	NA	0	30.5	Panini <i>et al.</i> , 2017a,b
<i>Gryllus bimaculatus</i> meal	NA	NA	0	27.6	Peh <i>et al.</i> , 2021
<i>Bombyx mori</i> meal	NA	NA	0	24.2	Rahimnejad <i>et al.</i> , 2019
Insect hydrolysate	NA	NA	0	2	Terrey <i>et al.</i> , 2021
Soybean meal	10	37.6	10	45.1	Choi <i>et al.</i> , 2018; Foyosal <i>et al.</i> , 2019; Peh <i>et al.</i> , 2021; Rahimnejad <i>et al.</i> , 2019; Wang <i>et al.</i> , 2021
Shrimp meal	0	5	0	5	Chen <i>et al.</i> , 2021a; Hu <i>et al.</i> , 2019b; Peh <i>et al.</i> , 2021; Rahimnejad <i>et al.</i> , 2019; Usman <i>et al.</i> , 2021; Wang <i>et al.</i> , 2021
Peanut meal	0	16	0	16	Hu <i>et al.</i> , 2019b
Wheat flour	0	42	0	42	Terrey <i>et al.</i> , 2021
Yeast	NA	NA	0	2	Choi <i>et al.</i> , 2018
Meat bone meal	0	20	0	23	Mazlum <i>et al.</i> , 2021
Poultry by-product meal	0	7	0	7	Richardson <i>et al.</i> , 2021
Corn meal	0	21.5	0	20	Usman <i>et al.</i> , 2021
Tapioca meal	0	5	0	5	Usman <i>et al.</i> , 2021
Squid	0	10	0	10	Choi <i>et al.</i> , 2018
Zeolite powder	0	3.2	0	2.5	Wang <i>et al.</i> , 2021
Rice bran	0	4.1	0	2.5	Wang <i>et al.</i> , 2021
Kaolin	0	14	0	13.5	Panini <i>et al.</i> , 2017a,b
Fish oil	0.25	5	0	4.6	Mazlum <i>et al.</i> , 2021; Panini <i>et al.</i> , 2017a,b; Terrey <i>et al.</i> , 2021; Usman <i>et al.</i> , 2021
Soy oil	0	5.1	0	3.5	Panini <i>et al.</i> , 2017a,b; Shin <i>et al.</i> , 2020
Krill oil	NA	NA	0	1	Terrey <i>et al.</i> , 2021
Lecithin	0	2	0	2	Motte <i>et al.</i> , 2019; Panini <i>et al.</i> , 2017a,b
Vitamin / mineral premix	0	6.9	0	7.1	Choi <i>et al.</i> , 2018
Vitamin C	0	0.1	0	0.1	Chen <i>et al.</i> , 2021a; Hu <i>et al.</i> , 2019b; Panini <i>et al.</i> , 2017a,b; Usman <i>et al.</i> , 2021; Wang <i>et al.</i> , 2021
Choline chloride	0	1	0	1	Shin <i>et al.</i> , 2020
Cholesterol	0	0.5	0	0.5	Foyosal <i>et al.</i> , 2019

¹ The data are based on 19 selected studies using and detailing insect-based crustacean feeds. (For all studies: Supplementary Table S1); NA = not available.

salmon and Rainbow trout, but methionine supplements are necessary. Insects are considered a promising source of animal protein for human consumption and animal feed. They have a suitable nutritional profile and can be produced sustainably by utilising and valorising organic side-streams (Cottrell *et al.*, 2020; Hua *et al.*, 2019).

Insect-based crustacean feed

Aquafeeds must promote growth, maintain health and ensure consumer safety (National Research Council, 2011; Tacon and Metian, 2008). Crustacean compound

feed consists of 20-50% fishmeal that contains high levels of digestible essential amino acids such as methionine, lysine and leucine (Cummins *et al.*, 2017; Henry *et al.*, 2015; Sánchez-Muros *et al.*, 2020) (Table 1). Fishmeal replacement can lead to a higher FCR, where more feed is required to achieve a given weight gain. This is particularly relevant for bacteria, yeast and macroalgae, whereas moderate inclusion of microalgae and insects seem to have little impact on the FCR or even reduce it (but see Cai *et al.*, 2022), thus achieving greater feed efficiency especially at moderate replacement levels (Cottrell *et al.*, 2020; Sánchez-Muros *et al.*, 2020). Incorporating insects into compound aquafeeds is

a novel approach even though insects are part of the natural diet of many fish (Howe *et al.*, 2014; Whitley and Bollens, 2014) and crustaceans (Muangyao *et al.*, 2019). Insect supplements improve growth, gut health and immunity, although a high content of insect meal can be detrimental (Table S1) (Cummins *et al.*, 2017; He *et al.*, 2022; Wang *et al.*, 2021, 2022). Specific effects depend on the processing method, feed composition and insect/crustacean species.

Insects are highly diverse (Stork *et al.*, 2015) thus offering many candidate aquafeeds with different characteristics in terms of nutritional composition. Insect species requirements are important when considering the design of their farms at appropriate scales. A wide range of insects has traditionally been used for food and feed, especially in Asia. In contrast, modern industrialised approaches have focused on a few well-characterised species including the Black soldier fly (*Hermetia illucens*), the Yellow mealworm (*Tenebrio molitor*), the Housefly (*Musca domestica*) and the House cricket (*Acheta domestica*), all of which can be produced at large scales. *H. illucens* in particular is becoming more commercially attractive because it can reproduce rapidly, grows on a broad range of substrates, and has low potential as a disease vector (Müller *et al.*, 2017; Wang and Shelomi, 2017). The nutritional composition of insects varies depending on species, life stage, rearing conditions and diet, and the protein content is generally similar to that of fishmeal (Freccia *et al.*, 2020). On average, the protein content of insect meal is 50–82% dry matter depending on the species and processing method, compared to $\leq 73\%$ in fishmeal and $\sim 50\%$ in soybean meal (Henry *et al.*, 2015). The lipid content of insects is generally high (10–49%) (Makkar *et al.*, 2014) and is influenced mainly by diet and species (Galassi *et al.*, 2021; Taufek *et al.*, 2021; Van Huis *et al.*, 2020).

As a replacement for fishmeal, 20 years of research focusing on insects in fish aquaculture has yielded promising results, whereas insect meal in crustacean aquaculture has received comparatively little attention (Alfiko *et al.*, 2022; Hua *et al.*, 2019). As of July 2022, 31 peer-reviewed studies are listed in the Web of Science (<https://www.webofscience.com>; keywords: 'crustacean', 'insect', 'aquaculture', '*Hermetia*', '*Tenebrio*', '*Litopenaeus*', combinations thereof, and literature citing the appropriate hits/publications), which were published from 2013 to 2022 (Table S1). Most data have been generated for *L. vannamei*. In this species, fishmeal can be fully replaced with *T. molitor* meal without detrimental effects, whereas high proportions of *H. illucens*, *M. domestica* or the Domestic silk moth (*Bombyx mori*) can hamper growth and affect health (Chen *et al.*, 2021a; Choi *et al.*, 2018; Cummins *et al.*, 2017; De León-Ramírez *et al.*, 2018; Huang *et al.*, 2020; Rahimnejad *et al.*, 2019; Wang *et al.*, 2021). For example, insect meal can affect the fatty acid composition of crustaceans (Shin and Lee, 2021), and a high proportion of insect meal can lead to hepatopancreatic damage in shrimps, potentially driven by the high lipid content and particularly

by monounsaturated and saturated fatty acids (Chen *et al.*, 2021a,b; Cummins *et al.*, 2017; He *et al.*, 2022; Wang *et al.*, 2021). Defatted *H. illucens* meal was able to replace 60% of the fishmeal without negative effects in *L. vannamei* compared to $< 30\%$ for full-fat insect meal (Chen *et al.*, 2021a; Hu *et al.*, 2019b; Wang *et al.*, 2021). This demonstrates that post-processing techniques can influence the nutrient composition and quality of the product and its suitability as a feed (Ravi *et al.*, 2020). The amino acid composition of insect meal also influences crustacean growth. The essential amino acid methionine seems to be a limiting factor in *L. vannamei* because fishmeal could only be fully replaced with insect meal if methionine supplements were also provided (Cai *et al.*, 2022; Motte *et al.*, 2019; Panini *et al.*, 2017b). Importantly, 100% replacement of fishmeal with insects in crustacean aquaculture is possible if these species-dependent constraints and feed processing options are considered.

At low supplementation rates, insects seem generally beneficial for crustaceans and can be considered as a form of functional feed (Table S1). For example, compound feed containing $\sim 15\%$ *H. illucens* and 25% *T. molitor* meal improved the growth parameters of *L. vannamei* (Choi *et al.*, 2018; Cummins *et al.*, 2017; Motte *et al.*, 2019; Richardson *et al.*, 2021; Shin *et al.*, 2020). Even 1% *H. illucens* meal significantly improved the palatability of the compound feed (Terrey *et al.*, 2021). Positive effects on crustaceans such as the Longarm river prawn (*Macrobrachium tenellum*), Danube crayfish (*Pontastacus leptodactylus*), Smooth marron (*Cherax cainii*), Australian red claw crayfish (*Cherax quadricarinatus*) and *M. rosenbergii* were also observed when they were fed on insects, including other species such as *Trichocorixa* sp., *M. domestica*, *B. mori*, Two-spotted cricket (*Gryllus bimaculatus*), Japanese rhinoceros beetle (*Allomyrina dichotoma*), Short-horned grasshopper (*Oxya chinensis*) and *Protaetia brevitarsis* (De León-Ramírez *et al.*, 2018; Foyal *et al.*, 2019, 2021; Martínez-Córdova *et al.*, 2013; Mazlum *et al.*, 2021; McCallum *et al.*, 2021; Peh *et al.*, 2021; Shin and Lee, 2021; Wang *et al.*, 2022) (Table S1, Figure 3). Although the precise mechanisms have not been identified in crustaceans, there is ample evidence that insect supplements confer growth and health benefits (Table S1). Insects can therefore be considered as promising alternative protein sources for aquaculture (Hua *et al.*, 2019; Sánchez-Muros *et al.*, 2020).

Two key challenges are associated with the development of insect-based aquafeeds. First, the compound feed must be customised for each target species. Comparable data are still scarce for crustaceans (Table S1), but juvenile Mirror carps (*Cyprinus carpio* var. *specularis*) showed negative effects when more than 50% of the fishmeal was replaced with *H. illucens* meal (Xu *et al.*, 2020), whereas African catfish (*Clarias gariepinus*) can effectively utilise replacements up to 75% (Fawole *et al.*, 2020). The natural food regime of the target species (e.g. omnivorous versus

carnivorous) may be an important determinant of the optimal supplementation rate. Second, a consistent and high quality is required for insect-based feed to achieve efficient growth and maximum yields. In addition to post-processing (see above), the substrate fed to the insects is a major determinant of meal composition, especially the lipid content and fatty acid spectrum (Ewald *et al.*, 2020; Hopkins *et al.*, 2021; Schreven *et al.*, 2020; St-Hilaire *et al.*, 2007), the protein content and amino acid spectrum (Fuso *et al.*, 2021; Meneguz *et al.*, 2018; Tschirner and Simon, 2015), and the mineral content (Chia *et al.*, 2020). Accordingly, insect species, substrates and post-processing must remain consistent to ensure the production of stable, high-quality compound aquafeeds.

Compound feeds must also ensure consumer safety. In this context, insects could affect consumer health in two ways. First, insect-associated substances or pathogens could accumulate in the crustacean and have detrimental effects in humans. Specifically, the contamination of substrates can lead to toxin accumulation in insects (Purschke *et al.*, 2017). Cadmium, arsenic and lead can accumulate in *T. molitor* and *H. illucens* larvae (Diener *et al.*, 2015; Van der Fels-Klerx *et al.*, 2018; Vijver *et al.*, 2003), whereas other heavy metals, mycotoxins and pesticides seem to pose a lower risk (Purschke *et al.*, 2017). Production methods, substrate, stage of harvest, insect species, and processing may all affect the presence and concentration of biological (microbes) and chemical (toxins and heavy metals) contaminants in insect-based feeds (EFSA, 2015). It is therefore necessary to monitor insect production and particularly the substrate, especially for cadmium and lead (Diener *et al.*, 2015). In many jurisdictions, the monitoring of such substances in feed products is already required by law, as in the European Union (EU Regulation (EC) No 178/2002). Second, insects can directly or indirectly affect crustacean health and cause disease, which in turn may affect consumers. For example, microbial pathogens that are either associated with insects or introduced during farming and processing may impair shrimp health and consequently harm consumers. For consumers, the risk of insect-derived pathogens is generally regarded as low, in part because insects are phylogenetically very different to higher animals and humans (EFSA, 2015). However, insect pathogens can nevertheless hamper insect production (Eilenberg *et al.*, 2018), despite the broad resistance demonstrated by *H. illucens* (Joosten *et al.*, 2020). In principal, insects can also act as pathogen vectors for crustaceans, however examples of such terrestrial pathogen transmission to aquatic systems are rare (but see Stentiford *et al.*, 2009), limited by differences in transition modes, a lack of host-parasite coevolution and a generally high pathogen specificity (McCallum *et al.*, 2004). Data on pathogen transmission via insect-based aquafeeds should be carefully considered, but are lacking at large. To be approved in the EU, insects for aquafeed must not be pathogenic or have other adverse effects on plant, animal or human health, for example by acting as

pathogen vectors (EU Regulation No 2017/893). *H. illucens* can even reduce the pathogen load in aquafeeds (Swinscoe *et al.*, 2019) and increases disease resistance in crustaceans (Table S1). Furthermore, given that most economically relevant crustacean diseases appear to be caused by marine pathogens (Shinn *et al.*, 2018), replacing marine compounds such as fishmeal with insect meal should enhance disease management and reduce disease risk particularly in land-based aquaculture (Figure 3).

Thus far, only a few insect species have been considered as crustacean feed, with most of the available data concerning *H. illucens*, *T. molitor* and *L. vannamei*. Based on the relevant literature (Table S1), it is reasonable to assume that the proportion of insect meal can be increased further and can eventually replace fishmeal completely. To this end, it is important to consider growth and health benefits – even at low supplementation rates – beyond the fulfilment of nutritional requirements.

4. Disease and immunity

Global aquaculture has been affected by a wide range of diseases (Lafferty *et al.*, 2015; Stentiford *et al.*, 2017) that reduce the production volume and influence prices on the crustacean market (Thitamadee *et al.*, 2016). For example, ~\$4 billion in losses were recorded during the period 2009-2018 in the Asian shrimp industry (Asche *et al.*, 2021; Shinn *et al.*, 2018). To control this so-called disease crisis (Doyle, 2016), the industry has introduced strategies to identify, monitor and control viruses, bacteria and parasites (Flegel and Alday-Sanz, 1998; Seethalakshmi *et al.*, 2021), and to enhance the crustacean immune system (Aweya *et al.*, 2021; Kulkarni *et al.*, 2021; Nguyen *et al.*, 2018). Like most multicellular organisms, crustaceans are metaorganisms (or holobionts) that consist of a host and its associated microbial community (microbiome) (McFall-Ngai *et al.*, 2013). The complex microbiome of aquatic organisms often includes bacteria, fungi, viruses, archaea and others (Rosenberg *et al.*, 2007). The crustacean microbiome literature is largely focused on bacteria (Holt *et al.*, 2021) and offers additional insights on microeukaryotes (Dai *et al.*, 2017; Li *et al.*, 2019; Xiong *et al.*, 2018, 2020). The bacterial microbiome contributes to host nutrition and health, for example by expanding the enzymatic repertoire to enhance nutrient availability, growth and immunity (Rajeev *et al.*, 2021). The bacterial gut microbiome in particular is involved in digestion, reproduction and immunity, and the balance between these bacteria and the host is essential for overall health (Rajeev *et al.*, 2021). The bacterial microbiome is selected by the host, and can be acquired from the environment (horizontal) and/or via parental mucus feeding (vertical) (Rajeev *et al.*, 2021; Wang and Wang, 2015). The composition of the associated bacterial communities is dynamic – for example, opportunistic, pathogenic and beneficial bacteria are present in the healthy *L. vannamei* intestine but vary

according to the developmental stage, health (including stress), environmental factors (salinity, temperature, nutrients and toxins) and diet (Infante-Villamil *et al.*, 2021; Rajeev *et al.*, 2021). The disruption of any of these factors can cause dysbiosis (Li *et al.*, 2018), an imbalance in the microbiome often associated with disease (Infante-Villamil *et al.*, 2021; Rajeev *et al.*, 2021). In aquaculture, the early detection of dysbiosis therefore facilitates prompt intervention against unsuitable water conditions and diseases.

Over the last decades, the control of bacterial disease, including vibriosis, has largely been achieved through the widespread application of chemicals, antibiotics and other antimicrobials (Vaiyapuri *et al.*, 2021). Antimicrobial resistance has therefore increased, leading to the emergence of superbugs that threaten production, the environment, and ultimately human health, leading to more restrictive regulations governing the application of these substances (Murray *et al.*, 2022; Vaiyapuri *et al.*, 2021). Modern molecular biology methods (especially affordable culturing and sequencing technologies) and a more in-depth knowledge of aquaculture microbiology has allowed researchers to focus on the control and manipulation of bacterial communities in the water and those associated with the host as an effective and sustainable alternative to chemicals and drugs (Dawood *et al.*, 2019; Knipe *et al.*, 2021). Crustaceans rely on an innate immune system, where pathogen-like molecular structures are recognised by specific host proteins (e.g. antimicrobial, clotting and pattern recognition proteins), which activate cellular or humoral immunity response mechanisms to eliminate the threat (Aweya *et al.*, 2021; Vazquez *et al.*, 2009). RNA interference (RNAi), which is a form of sequence-dependent post-transcriptional gene silencing, and antimicrobial peptides (AMPs), which can bolster innate immunity, are particularly promising molecular approaches for immunoprophylaxis (Kulkarni *et al.*, 2021; Matos and Rosa, 2021; Nguyen *et al.*, 2018). These can be combined with SPF broodstocks, biosecure management, genetic modifications, sensitive monitoring/detection tools, immunity training and nanotechnology to mitigate or control diseases (Flegel, 2019; Luis *et al.*, 2019; Roy *et al.*, 2020). However, such measures remain expensive and are difficult to implement particularly in low-and-middle-income (LMIC) regions. Probiotics, prebiotics and synbiotics offer a more affordable approach for disease prevention, the maintenance of a healthy gut microbiome, growth stimulation, and water quality management. They aim to establish (or re-establish) symbiosis between the host and its microbiome, ensuring strong innate immunity and promoting growth while suppressing pathogens (Dawood *et al.*, 2019; Li *et al.*, 2018).

Probiotics are (parts of) living or dead microbial cells that alter host-associated microbial communities, and their health benefits in crustacean aquaculture have been described in detail (Akhter *et al.*, 2015; Dawood *et al.*, 2019; Farzanfar,

2006; Hoseinifar *et al.*, 2018; Jamal *et al.*, 2019; Knipe *et al.*, 2021; Kumar *et al.*, 2016; Lakshmi *et al.*, 2013; Ninawe and Selvin, 2009; Shefat, 2018; Toledo *et al.*, 2019; Van Hai and Fotedar, 2010; Zorriehzahra *et al.*, 2016). Briefly, probiotics aim to improve the crustacean immune response while promoting growth and/or improving water quality (Lakshmi *et al.*, 2013; Van Hai and Fotedar, 2010). Probiotics can modulate host immune responses, induce antimicrobial compounds, and compete with pathogens for colonisation sites or nutrients (Kumar *et al.*, 2016). To this end, the genera *Bacillus*, *Lactobacillus*, *Vibrio*, *Pseudomonas* and others are used as feed supplements or added directly into the water (Amoah *et al.*, 2020; Hoseinifar *et al.*, 2018; Shefat, 2018; Van Hai and Fotedar, 2010). Alternatively, probiotic effects can be achieved by co-culturing species such as shrimps with the macroalga *Ulva lactuca* (Mangott *et al.*, 2020). Probiotics offer an effective and sustainable tool to control disease and stimulate growth in shrimp (Dawood *et al.*, 2019; Hoseinifar *et al.*, 2018) and crab (Ayisi *et al.*, 2017a) cultures, but screening for probiotics is challenging because the precise mode of action is dependent on the host species and microbial strain (Knipe *et al.*, 2021; Rajeev *et al.*, 2021). In contrast to probiotics, prebiotics are substances that are not digested by the host but that influence the microbial community, thus promoting the growth and health of the host indirectly (Akhter *et al.*, 2015; Anadón *et al.*, 2019; Ayisi *et al.*, 2017a; Rajeev *et al.*, 2021; Valcheva and Dieleman, 2016). For example, fructo-oligosaccharides, inulin, β -glucan and polyhydroxybutyrate enhance immunity and promote growth in shrimps (Li *et al.*, 2018; Rajeev *et al.*, 2021) and crabs (Ayisi *et al.*, 2017a). However, compared to probiotics, there is much less information available concerning the effects of prebiotics in crustacean aquaculture. Prebiotics and probiotics can also be combined as synbiotics to achieve synergistic effects (Rajeev *et al.*, 2021). For example, prebiotics can enhance the survival of probiotic bacteria (Anadón *et al.*, 2019). In shrimps, specific probiotic bacterial strains can be combined with prebiotic compounds, such as oligosaccharides, to modulate the microbiome, thus increasing productivity by improving digestion, immunity and growth (Rajeev *et al.*, 2021).

Given their profound effect, feed additives including probiotics have a long history in aquaculture and include functional feed components such as vitamins, enzymes and pigments (Conklin *et al.*, 1978). For example carotenoids are antioxidative pigments that act as immunostimulants and promote growth and reproduction (Babin *et al.*, 2010; Boonyaratpalin *et al.*, 2001; Linan-Cabello *et al.*, 2002; Wade *et al.*, 2017). The more recent term phytobiotics refers to plant-derived bioactive compounds that stimulate the immune system and enhance productivity (Cristea *et al.*, 2012; Jana *et al.*, 2018). Such compounds have been tested in fish (Cristea *et al.*, 2012), and the benefits of algae in crustacean feed are also well-known as discussed above. Natural feed additives that promote growth often contain

phytobiotics and prebiotics, but some compounds from animals – particularly insects – may have similar properties.

Insects are directly consumed by humans (Van Huis, 2020), but they are also used as a source of probiotics and health-promoting substances (Milanović *et al.*, 2021), and as feed for pigs, poultry and aquaculture (De Souza Vilela *et al.*, 2021; Kar *et al.*, 2021; Richardson *et al.*, 2021). Insect meal confers a wide range of health benefits by modulating immunity and/or the gut microbiome to enhance disease resistance. Much of our current knowledge stems from fish aquafeeds containing *H. illucens*, *T. molitor* or *M. domestica*, which exert their immunomodulatory effects by inducing genes encoding hepcidin, major histocompatibility complex II, HSP70 and several pro-inflammatory cytokines, modulating phagocytic and T-cell activity, and improving the hepatic and/or intestinal antioxidant response (Gasco *et al.*, 2021).

Insect meal in crustacean feed has been shown to enhance growth, improve immunity (including disease resistance), and maintain gut health by supporting the gut microbiome and the bacterial communities in the surrounding water (Table S1, Figure 3) (Choi *et al.*, 2018; Cummins *et al.*, 2017; Feng *et al.*, 2019; Foysal *et al.*, 2021, 2022; Hu *et al.*, 2019b; Mastoraki *et al.*, 2020; McCallum *et al.*, 2021; Motte *et al.*, 2019; Rios *et al.*, 2021; Wang *et al.*, 2021). The antioxidant activity of insect chitin may also improve immunity (Wang *et al.*, 2021), because chitin and its derivative chitosan support the innate immune response, increase gut microbiome diversity and gut health, and reduce the abundance of pathogens in fish (Gasco *et al.*, 2021). Chitin and its derivatives mimic pathogen-associated molecular patterns, inducing the production of cytokines and other mediators of immunity that counteract fungal infections and parasites (Stenberg *et al.*, 2019). However, chitin digestibility appears to be limited in *L. vannamei* and high levels of this compound may be detrimental (Shin and Lee, 2021). Chitin is also an anti-nutritional factor that can inhibit feed consumption and nutrient availability, although no direct link has been identified (Freccia *et al.*, 2020; Wang *et al.*, 2021). Insects contain further antibacterial and anti-inflammatory substances that may contribute to positive gut health in aquaculture. These include lauric acid, propionate and butyrate (Gasco *et al.*, 2021), and a large repertoire of AMPs that directly inhibit pathogenic bacteria, fungi, parasites and viruses (Chen *et al.*, 2021a; Gasco *et al.*, 2021; Vogel *et al.*, 2018). Notably, the *H. illucens* AMP profile can be modulated by the presence of specific dietary components to target particular bacterial pathogens, a strategy that could be replicated to address common pathogens in crustacean aquaculture (Vogel *et al.*, 2018). Furthermore, *M. domestica* larvae have been used to transport recombinant protein antigens into *L. vannamei*, demonstrating an effective oral delivery system for aquatic vaccines (Huang *et al.*, 2021).

These recent findings provide powerful insect-based tools for disease management and are therefore of considerable economic interest.

In summary, the supplementation of aquafeeds with insect meal that not only meets the nutritional needs of crustaceans but also provides functional components to minimise losses caused by dysbiosis and disease can thus increase health and productivity in aquaculture.

5. Sustainability

Sustainability of aquafeeds

Aquafeeds account for ~90% of the environmental impact of fed aquaculture (Little *et al.*, 2018). The most common proteins in aquafeeds – fishmeal and soybean meal – raise several environmental, economic and social challenges. Globally, 34% of fish stocks are harvested unsustainably and almost 60% at maximum capacity (FAO, 2020), leaving no room for sustainable increases in fish supplies for fishmeal. From a social perspective, the increasing consumption of fish by the middle classes is often addressed by rerouting resources, particularly small fish, from the poor and vulnerable (FAO, 2015; Isaacs, 2016; Tacon and Metian, 2009). Wild fish production declined by 26.5% between 2000 and 2018 (Jannathulla *et al.*, 2019) and is expected to reach its ecological limits by 2037 (Froehlich *et al.*, 2018). To provide sufficient fishmeal, additional sources, including fish residues (offal, trimmings and cuttings) now contribute ~30% of the global supply (FAO, 2020). However, the processing of bones, heads and tails results in lower-quality meal and higher consumption rates (Hua *et al.*, 2019). Aquaculture consumes more than 70% of all the fishmeal and fish oil currently produced (Hua *et al.*, 2019). Globally, fishmeal production uses ~15 million tons of whole fish (Terrey *et al.*, 2021), and the demand for feed is projected to surpass fishmeal availability by 2050 (Froehlich *et al.*, 2018). The historically high proportion of fishmeal and fish oil in aquafeeds has decreased as prices have tripled from below \$500 per ton in 2000 to \$1,500 per ton in 2020 (FAO, 2020; McLean *et al.*, 2020). Price increases of 20-90% for fishmeal and 16-70% for fish oil are expected by 2030 (FAO, 2020; Kobayashi *et al.*, 2015).

Fishmeal alternatives are already widely used, including soybean, which offers comparable nutritional properties. Although the nutritional value of soybean meal is much higher than many other plants (National Research Council, 2011), its environmental footprint is unfavourable. Soybean production is very land-intensive (Lathuilière *et al.*, 2017), adding to the already large area required for conventional shrimp aquaculture (Davis *et al.*, 2021). Furthermore, soybean is usually cultivated in intensive monoculture systems associated with severe biodiversity loss (Altieri, 2009; Lathuilière *et al.*, 2017), deforestation and land degradation

(Barona *et al.*, 2010; Fehlenberg *et al.*, 2017; Richards *et al.*, 2012). The cultivation of soybean consumes large amounts of water, and the replacement of fishmeal therefore increases the water footprint of the resulting compound feed (Pahlow *et al.*, 2015). The current main ingredients of (crustacean) aquafeeds – fishmeal, fish oil and soybean meal (Table 1, Table S1) – are therefore highly unsustainable and will become more expensive in the near future (Figure 3).

Sustainability of insect protein

Compared to fishmeal and soybean meal, insect proteins have the potential to improve the overall sustainability of aquafeeds (Hua *et al.*, 2019; Liland *et al.*, 2021) (Figure 3). The aspect of insect production that contributes most to global warming is energy consumption, so renewable energy and industrial waste heat can improve sustainability where available (Salomone *et al.*, 2017). Although insect production systems are a source of greenhouse gas emissions (Parodi *et al.*, 2020), the carbon footprint is low compared to fishmeal and soybean meal (Perednia *et al.*, 2017) because local insect production removes the 30% of emissions caused by global shipping (Mertenat *et al.*, 2019). The use of agricultural by-products and food industry side-streams as substrates for insect mass production is also advantageous (Tschirner and Kloas, 2017), and the ability to recover otherwise lost nutrients from these substrates is major contribution to sustainable protein production (Magee *et al.*, 2021; Van Huis *et al.*, 2020). Many proposed input substrates are decomposed naturally by microbes, emitting 70% more CO₂ equivalents than nutrient valorising by insects (Perednia *et al.*, 2017; Smetana *et al.*, 2019). The valorising of by-products thus further compensates for the environmental impact of the main product (in this case crustaceans) (Kim and Kim, 2010). Saprophagous insects such as *H. illucens* and *M. domestica* satisfy their water demand directly via the input substrate, so proteins produced by insects feeding on side-streams have a much lower water footprint even compared to plant proteins (Joly and Nikiema, 2019; Rumpold and Schlüter, 2013). Furthermore, insect farming does not need a large area of land (Doi and Mulia, 2021). Life-cycle assessments for *H. illucens* (Salomone *et al.*, 2017) and *M. domestica* (Van Zanten *et al.*, 2015) revealed land requirements of 0.05 and 0.032 m²/yr/kg protein, respectively, compared to almost 9 m²/yr/kg protein for soybean (Salomone *et al.*, 2017). Importantly, insect production does not compete with human food production in terms of land, water and fertiliser use (Hua *et al.*, 2019).

By-product utilisation can improve the sustainability (and profitability) of insect-based aquafeeds and may be synergistically used alongside crustacean by-products. Several compounds are particularly valuable, including: (1) insect (and crustacean) pigments (astaxanthin and its esters, β-carotene, lutein, astacene, canthaxanthin and zeaxanthin),

which are commercially valuable as antioxidants and vitamin A precursors for biomedical and nutritional applications (Hu *et al.*, 2019a; Irna *et al.*, 2018); (2) chitin as a structural biopolymer and a potential source of antimicrobials (Trung and Phuong, 2012); and (3) chitosan, which has a range of applications in wastewater treatment, cosmetics, toiletries, foods, beverages, agrochemicals and pharmaceuticals (Islam *et al.*, 2016; Muñoz *et al.*, 2018; Pakizeh *et al.*, 2021). Chitosan is a particularly promising derivative because it is a water-soluble, non-toxic, biocompatible substance with antibacterial, antifungal, antitumor and immunostimulatory properties (Muñoz *et al.*, 2018). Furthermore, insect frass – a mixture of moulting skins (exuviae) and excrement – is a valuable organic fertiliser (Klammsteiner *et al.*, 2020; Xiao *et al.*, 2018) with a higher nitrogen content than commercial organic fertilisers (Beesigamukama *et al.*, 2020). Insect frass is also very rich in plant-accessible nitrogen, contains various plant growth-promoting biomolecules and microorganisms, and increases the tolerance of crops to abiotic stress, pathogens and pests (Poveda, 2021). Overall, industrial insect-based feed production can improve the environmental impact of aquafeeds, significantly reduce food waste and organic waste, provide further commercially interesting substances, and thereby facilitate the path to a closed circular economy (Figure 3).

A circular economy can be defined as an economic system that aspires to zero waste and pollution by decoupling environmental pressure from economic growth (Nobre and Tavares, 2021) and is considered essential to reach the United Nations' Sustainability Development Goals (SDGs) (Schroeder *et al.*, 2019). For example, the EU published a circular economy action plan aiming, among others, to reduce food loss and waste, which accounts for one third of all agricultural products worldwide (EEA, 2015; Gustavsson *et al.*, 2011). Insects can be linked directly to seven and indirectly to further six of the 17 SDGs (Moruzzo *et al.*, 2021) and are regarded as the missing link in organic material cycles (Huis *et al.*, 2013). For example, *H. illucens* is able to convert a wide range of otherwise unused side-streams into high-quality protein, fat and fertiliser (Magee *et al.*, 2021). Given the small land footprint of insect production, side-streams can be upcycled at their location of origin to support short transport ways. In less developed regions, insects can facilitate effective waste management (Shumo *et al.*, 2019). Based on their favourable environmental and economic characteristics, insects may improve the sustainability of (crustacean) aquafeeds. However, consumer safety, acceptance and the corresponding legislation pose considerable challenges (Cammack *et al.*, 2021).

6. The legal framework and consumer acceptance

Insect production is growing and becoming increasingly industrialised (Wade and Hoelle, 2019). Different market reports project a global volume of 730,000 tons by 2030 (Alliedmarketresearch, 2020) and nearly 1,400,000 tons by 2027 (PRNEWswire, 2021) for edible insects. The success of insect-based feeds will predominantly depend on consumer acceptance and regulatory approval, the latter differing widely across global jurisdictions. For example, the EU has permitted the utilisation of proteins from seven insect species (*H. illucens*, *M. domestica*, *T. molitor*, *A. domesticus*, Tropical house cricket (*Grylodes sigillatus*), Jamaican field cricket (*G. assimilis*) and *Alphitobius diaperinus*) as feed ingredients for aquaculture (EC Regulation (EU) 2017/893) and poultry and pig production (EC Regulation (EU) 2021/1372). In the USA, only one species (*H. illucens*) is an approved animal feed ingredient and the application of *H. illucens* larvae or meal is currently limited to feed formulations for salmonid aquaculture (Lähteenmäki-Uutela *et al.*, 2021). In countries that traditionally endorse entomophagy, legislation is more open toward novel insect products. In China, a catalogue of approved raw feed materials compiled in 2012 lists insects and degreased insect powder (U.S. Department of Agriculture (USDA), 2021). Each product must be labelled by species, and meticulous hygiene and labeling standards must be met (Lähteenmäki-Uutela *et al.*, 2017). The global regulatory disharmony is challenging for international producers, which must comply with different regulations depending on the location of their production facilities and customers. With nearly 90% of crustacean aquaculture located in Asia and over 50% in China (FAO, 2020; Naylor *et al.*, 2021), the favourable local legislation facilitates the development of insect-based aquafeeds.

The consumer acceptance of insect-based products may also pose a barrier (Onwezen *et al.*, 2019). Consumer acceptance varies across the world. In the Global South (Latin America, Africa and South Asia), insects are part of the food culture and are consumed regularly by more than two billion people (Feng *et al.*, 2018; Huis *et al.*, 2013; Raheem *et al.*, 2019). Research data from the Asian markets are scarce, but the drivers of consumer acceptance appear similar to those in Western cultures (Liu *et al.*, 2019; Park and Choi, 2020; Sogari *et al.*, 2019a) and seem to depend mostly on availability, traditions, food processing/preparation, quality, price, and background knowledge, whereas the health risks of insects are rated low (Arena *et al.*, 2020; Hamerman, 2016; Sogari *et al.*, 2019c; Szendrő *et al.*, 2020). Beyond the Global South, insects are only just emerging as a novel food and are more regarded as pests and indicators of low hygienic standards, reducing consumer acceptance (Hartmann and Bearth, 2019; Sogari *et al.*, 2019c). However, despite this attitude toward food, Western consumers are more amenable to insect feed

in agriculture and aquaculture (Ferrer Llagostera *et al.*, 2019; Rumbos *et al.*, 2021). Importantly, aroma and texture of aquaculture products are not affected by insect feeds (Cunha and Ribeiro, 2019; Gasco *et al.*, 2019; Panini *et al.*, 2017b).

Appropriate strategies to facilitate the acceptance of edible insects are considered important (Cunha and Ribeiro, 2019; Ferrer Llagostera *et al.*, 2019; Gasco *et al.*, 2019; Hartmann and Bearth, 2019). For example, advertising the numerous benefits of insect protein (Table S1) and their positive role in sustainability and a circular economy (Figure 3) can enhance consumer acceptance (Borrello *et al.*, 2017; Cunha and Ribeiro, 2019; Gasco *et al.*, 2019). Information campaigns could be integrated into or supported by certificates or labels – for example, ‘fishmeal free’ – that extend existing ones such as the ASC (Aquaculture Stewardship Council), BAP (Best Aquaculture Practices), and ATBF (Antibiotic Free) to promote sustainable, insect-based aquaculture. Consumer acceptance could be further improved by implementing higher animal welfare standards for insects and crustaceans, because current widespread production techniques such as eye ablation to enhance crustacean reproductive success remain controversial (Bardera *et al.*, 2019; Gjerris *et al.*, 2016; Rodrigues *et al.*, 2021; Zacarias *et al.*, 2019). For example, EU regulations on animal welfare currently exclude most invertebrates (cephalopods are an exception), which contradicts the stated aim of protecting sentient beings (Gjerris *et al.*, 2016). Therefore, some researchers propose extending the regulations to cover at least decapod crustaceans (Birch *et al.*, 2021). Overall, consumer acceptance is not considered a major barrier, but further rigorous studies specifically assessing the acceptance of sustainable insect-fed aquaculture are required (Ferrer Llagostera *et al.*, 2019; Ngo and Moritaka, 2021; Sogari *et al.*, 2019b).

7. Outlook

Aquaculture is a growing food production sector that must achieve greater sustainability to ensure its future productivity. Crustaceans are an important market segment that – based on their price – have the potential to drive the development of new sustainable technologies, such as disease management and alternative feeds. Compound feeds are essential for modern aquaculture but their ingredients, including fishmeal, fish oil and terrestrial plant materials, are often unsustainable, for example due to high water and land use, large carbon footprints, and the depletion of other resources. Therefore, sustainable aquafeeds offer a promising toehold to significantly improve the environmental impact and yields of crustacean aquaculture. Existing data indicate that insect meal has a favourable nutritional composition as a fishmeal replacement for aquaculture, and offers health benefits, environmental sustainability and economic advantages, but there is an urgent need for research on the specific mechanisms of action.

Similar to the industrialised production of crustaceans, the insect industry is not yet established globally and may struggle to become a short-term solution to replace fishmeal and fish oil for the global aquafeed industry, despite evidence of economic efficiency (Arru *et al.*, 2019; Rawski *et al.*, 2021). Small production volumes, extensive manual labour and substrate costs result in prices of \$470-5,615 per ton larvae meal (Hua *et al.*, 2019; Niyonsaba *et al.*, 2021; Roffeis *et al.*, 2018) (compared to ~\$1,430 and ~\$520 per ton fishmeal and soybean meal, respectively, in January 2022 (www.indexmundi.com)). More widespread regulatory approval (IPIFF, 2021), the utilisation of formerly unused side-streams, automation, upscaling, and increasing investments in production capacity may result in the more competitive pricing of future insect-derived products. The growth and health benefits of insect-based crustacean aquafeeds reflecting their probiotic and/or prebiotic effects may also drive production, particularly by helping to prevent catastrophic disease outbreaks in aquaculture.

The aquaculture sector is globally heterogeneous, making it difficult to predict future social developments. Case studies in nine developing countries in Asia, Africa and South America, representing 16% of global aquaculture production, showed that small-scale farms account for the highest employment share (65%) but less than 30% of the production volume (Phillips *et al.*, 2016). Large-scale aquaculture is therefore important to meet global protein demands, which generally involve long transportation routes causing additional greenhouse gas emissions (Liu *et al.*, 2016). Local livelihoods, by comparison, often depend on small and medium-sized enterprises (SMEs) (Phillips *et al.*, 2016). Here, insect farming can also support and benefit from waste management (Diener *et al.*, 2011). Food waste that accumulates on farms and potentially attracts pests can be transformed by SME insect farms, thus reducing farm inputs and transportation costs and enhancing household resilience through income diversification (Huis *et al.*, 2013). By combining sustainable crustacean and insect production, SMEs have an opportunity to increase regional production, thereby strengthening supply chain resilience to economic, political and environmental challenges. A bright future may await SMEs that deploy insect-based aquaculture, particularly because insects appear suitable as a supplementary feed for many crustaceans and fish. However, active support from policymakers is required to integrate such SMEs into a modernising market, compensating for the lack of access to information, services, technology and credit and the capacity to offer larger volumes of quality products (Phillips *et al.*, 2016), particularly in the case of export-oriented shrimp production (Phillips *et al.*, 2016). Given the financial scope of the aquafeed sector and the investment costs required to produce high-end compound feeds, it seems likely that large companies will continue to drive aquafeed development and crustacean production for the global market.

On a global scale, all stakeholders strongly depend on more favourable legislation, communication, management, and controls along the value chain to intensify aquaculture yields and enhance sustainability (Joffre *et al.*, 2018). Given the current market size, insect-based feed will struggle to provide a global short-term solution. However, it offers the potential to improve production yields, disease management, environmental and social sustainability, arguing for the global intensification of sustainable crustacean aquaculture based on insect feed.

Supplementary material

Supplementary material can be found online at <https://doi.org/10.3920/JIFF2022.0117>

Table S1. Insect species assessed in crustacean feeds.

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Author contributions

TR conceived the article and TW, PS, AV, MT and AB contributed to its design and development. TR led and PS contributed to the coordination of the study. TR, AIB, MT, PS and MW led and all authors contributed to drafting of subsections. PS, AIB, AB and TR developed figures and tables. AV and TW provided tools. All authors contributed to the writing and editing of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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2.2 2nd publication: Broad acceptance of sustainable insect-based shrimp feeds requires reproducible and comparable research

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RESEARCH



Broad acceptance of sustainable insect-based shrimp feeds requires reproducible and comparable research

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Abstract

The black soldier fly (BSF) has great potential as a sustainable aquaculture feed. However, for shrimp aquaculture, research on BSF-based feeds has only recently started and the few available studies show a heterogeneous picture in terms of growth parameters of shrimp. While some of these varying results may be explained by the properties of the insect feed used, it is likely that yet unknown parameters also play a role. Moreover, pre-treatment, experimental design, and statistical analyses performed may influence the outcome of feeding experiments. Therefore, the general goal of this study is to establish basic reporting guidelines for BSF-based feeding trials in Pacific white shrimp (PWS), building on literature data and a best-practice study. Here, we (i) identify several contributing parameters that may influence life-history parameters of shrimp fed with BSF, including the genetic composition or strain of BSF used; (ii) show that the partial substitution of fishmeal-based compounds with BSF larvae could be a sustainable alternative to fishmeal-based feeds for PWS as the production index did not differ significantly between treatment and control groups in our best-practice study; and (iii) propose reporting guidelines with 39 parameters to be considered in future insect-based feeding studies in PWS to facilitate comparability and reproducibility. Overall, our study may contribute to improving the ecological, economic, and social sustainability of shrimp aquaculture through insect-based feeds.

Keywords Shrimp aquaculture · Pacific white shrimp · Black soldier fly · Aquaculture feed · Reporting guidelines

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Introduction

Aquaculture is one of the fastest-growing food production sectors (Ahmad et al. 2021) and a potential sustainable alternative to fisheries (Naylor et al. 2009). However, aquaculture intensification may also have negative impacts on sustainability, such as increased greenhouse gas emissions (Cao et al. 2019; Yuan et al. 2019; Marín-Riffo et al. 2021), massive habitat destruction, overfishing of feed species (Pauly et al. 2002), and social problems such as increasing inequalities and injustice (Brugere et al. 2023).

This conflict between the three pillars of sustainability—economy, ecology, and society—is particularly evident in shrimp aquaculture, which is dominated by the Pacific white shrimp (PWS; *Penaeus vannamei* Boone, 1931). While this species accounts for 50% of all crustacean aquaculture with an annual production of 6.3 million tons in 2021 (FAO 2023), the conversion of valuable coastal ecosystems to farmland, the high carbon footprint, the spread of foodborne marine pathogens, and increasing overfishing associated with fishmeal-based shrimp feeds remain important issues (Boyd et al. 2020). The key to improving the sustainability of shrimp aquaculture could be a locally produced insect-based feed (Röthig et al. 2023). This might help to reduce overfishing of feed species, transportation-related carbon footprints, and the use of antibiotics to control marine pathogens.

Currently, the black soldier fly (BSF; *Hermetia illucens* Linnaeus, 1758) has a particularly high potential as a shrimp feed, mainly due to its rapid reproduction rate, low risk as a disease vector, and its ability to be reared on a variety of substrates, including by-products from agriculture (Müller et al. 2017; Wang and Shelomi 2017; Klüber et al. 2022; Röthig et al. 2023). Furthermore, its protein content is relatively high (i.e., 50–70%; Richardson et al. 2021), and the amino acid profile is largely comparable to that of fishmeal (Barroso et al. 2014). Therefore, the BSF is increasingly becoming a sustainable source of protein in pond culture in tropical and subtropical regions.

Although insect-based shrimp feeds are of great ecological, economic, and social interest, systematic application-oriented research has only recently started and the few available studies show a heterogeneous picture (see review by Röthig et al. 2023). Despite the paucity of data, several trends seem to emerge from these studies: (i) substantial portions of commercial PWS feed may be replaced by BSF without adverse effects on PWS growth and survival rates (Cummins et al. 2017; Chen et al. 2021; Wang et al. 2021; He et al. 2022a, b); (ii) both fresh BSF larvae and processed BSF meal may be used as a replacement (Röthig et al. 2023); (iii) the fatty acid composition of BSF feeds, particularly the content of omega-3 and omega-6 fatty acids, may have significant effects on PWS growth and survival rates (Cummins et al. 2017; Chen et al. 2021, 2022; Wang et al. 2021; He et al. 2022a, b); (iv) defatted BSF meal appears to allow higher replacement rates (up to 60%; Wang et al. 2021) than non-defatted BSF meal (25–30%; Cummins et al. 2017; Chen et al. 2022); and (v) even replacement rates of <50% may have negative effects on PWS health (Cummins et al. 2017; Chen et al. 2021).

Despite these general findings, there is no consensus on optimal BSF replacement rates. In particular, the inferred health effects in PWS associated with BSF feeding vary widely between studies (Röthig et al. 2023). For example, some authors (He et al. 2022a, b) found that as little as 25% BSF feed content led to a significant increase in antioxidant enzymes in PWS, indicating oxidative stress. In contrast, other studies (Wang et al. 2021) inferred an increase in the total antioxidant capacity of PWS only at a BSF replacement rate of 80%.

While some of these widely varying results in growth and survival rates as well as health effects may be explained by the proportion of insect feed used, its fat content, the fatty acid composition, or the manner in which the BSF are processed (see also Röthig

et al. 2023), it is very likely that other unknown parameters, such as the (genetic) strain of the BSF used, also play a role. Ignoring such factors may also limit the applicability of the results and explain the lack of reproducibility in some cases (Wang 2018).

For example, the BSF is a genetically highly diverse taxon with a complex phylogeographical structure and increasing regional domestication across the globe (Kaya et al. 2021). At the same time, introgression of genetic clusters into domesticated strains is accelerating due to international trade (Kaya et al. 2021; Guilliet et al. 2022). This high genetic diversity of commercial BSF strains used globally may therefore also influence their life-history, physiological, and behavioral traits (Kaya et al. 2021; Klüber et al. 2024). Such effects of the genetic structure on growth-related traits could also play a role in domesticated PWS, although baseline genetic data are still scarce (but see Casado et al. 2022).

Studies of farmed aquatic animals have also shown that the pre-experimental treatment of organisms, experimental design, statistical analyses performed, and reporting of results influence our understanding of how animals respond to experimental treatments (Wang 2018; McDonald et al. 2021).

In addition, experimental methods in aquatic research are often poorly described, and researchers are increasingly raising concerns about a potential lack of comparability and reproducibility (Wang 2018; Cowger et al. 2020; see also Bosch et al. 2020). At the same time, complex experimental designs and statistical models may increase the risk of data misinterpretation (McDonald et al. 2021).

Applying these general findings to sustainable insect-based shrimp aquaculture, further advancement of this emerging field and stakeholder acceptance could be facilitated by conducting comparable and reproducible experimental studies, identifying unknown contributing factors that influence shrimp life-history traits, and establishing reporting guidelines that support the dissemination, interpretation, and synthesis of research findings.

Our general goal is therefore to combine literature data and data from a randomized controlled trial to establish baseline reporting guidelines. We also aim to raise awareness of the need for reproducible and comparable feeding research to increase stakeholder acceptance of sustainable insect-based shrimp feeds.

The specific goals are:

- 1) To perform a literature review to identify contributing parameters that may affect life-history parameters of shrimp fed with BSF meal or larvae
- 2) To evaluate the genetic barcodes of captive BSF and PWS populations as a hitherto neglected contributing parameter
- 3) To conduct a comparable and reproducible best-practice study to assess the effects of BSF-based feed on life-history and physiological traits of PWS, incorporating previously unstudied contributing parameters
- 4) To establish reporting guidelines for reproducible and comparable studies of insect-based feeds in shrimp aquaculture

Materials and methods

Literature review

The literature search systematically evaluated all publications listed in Web of Science (Clarivate, Chandler, USA) and Google Scholar (Dublin, Ireland) as of August 6, 2024,

that dealt with the use of insects as shrimp feed or the comparability of aquaculture studies. Combinations of terms from the following keyword groups were used: (“black soldier fly,” “*H. illucens*,” and “*Hermetia illucens*”) and (“*Litopenaeus vannamei*,” “*Penaeus vannamei*,” and “shrimp”) and (“aquaculture,” “comparability,” “feed,” and “reproducibility”). The publication date was set between 2017 and 2024. The articles retrieved were then screened to determine whether they met at least one of the following criteria: (i) contained original studies on the use of insects as shrimp feed; (ii) addressed contributing parameters that affect the life-history parameters of shrimp fed BSF meal or larvae; (iii) reported comparability or reproducibility of aquaculture or aquatic studies; and (iv) provided guidelines for aquatic studies. In particular, the following information was extracted: species involved, pre-experimental treatment, and experimental conditions.

Species studied and DNA barcoding

The main characteristics of the BSF and PWS studied are shown in Table 1. For the genetic barcoding, DNA was isolated from two randomly selected BSF and two randomly selected PWS individuals stored in 80% ethanol, using a DNeasy Blood and Tissue kit (QIAGEN, Hilden, Germany) according to the manufacturer’s instructions. A fragment of the mitochondrial barcoding gene, cytochrome *c* oxidase subunit I (COI), was amplified using the primers from Folmer et al. (1994). Purification of the PCR fragments and bidirectional Sanger sequencing were performed by LGC Ltd. (Berlin, Germany). The sequences were deposited in the NCBI GenBank under the following accession numbers: BSF (PQ187607–PQ187608) and PWS (PQ187640–PQ187641).

For comparison, all available COI sequences for BSF (463 sequences; e.g., Khamis et al. 2020; Stähls et al. 2020; Nguyen et al. 2023; Pazmiño et al. 2023) and PWS (189 sequences; e.g., Shen et al. 2016; Günther et al. 2017; Korzik et al. 2020) as of February 7, 2023, were retrieved from GenBank and aligned with the newly generated sequences. The alignment was performed using the MAFFT web server (Katoh and Standley 2013). Sequences with more than 200 ambiguous positions (N) were removed from the datasets, resulting in a final alignment of 275 and 122 sequences for the BSF and the PWS, respectively. Haplotype networks (TCS algorithm; Clement et al. 2000) were then generated in PopART v1.7 (Leigh and Bryant 2015).

Pre-treatments of BSF and PWS

Information on the pre-treatment of the investigated BSF and PWS is given in Table 2. The water parameters for the PWS during the acclimation period were the same as in the main experiment (see following section).

Experimental design of the best-practice feeding study

The feeding trial was conducted at the animal facility of the Natural Sciences Campus of the Justus Liebig University Giessen, Germany. The main information for the experimental setup and water parameters are provided in Table 3. The parameters for salinity, temperature, and pH were monitored daily using an aquarium computer (ProfiLux 4, GHL Advanced Technology

Table 1 Characteristics of the black soldier fly (BSF) and Pacific white shrimp (PWS) studied

Characteristic	BSF	PWS
Species name	Black soldier fly, <i>Hermetia illucens</i> Linnaeus, 1758	Pacific white shrimp, <i>Penaeus vannamei</i> Boone, 1931
Origin	Illucens GmbH, Ahaus, Germany	White Panther Produktion GmbH, Rottenmann, Austria
Genetic strain	For DNA barcoding data, see "Results" section	For DNA barcoding data, see "Results" section
Life stage	5th larval instar	Juvenile shrimp (ca. 8 weeks)
Weight	0.06 ± 0.02 g	0.4 ± 0.2 g
Length	1.4 ± 0.1 cm	2.0 ± 0.3 cm

Table 2 Pre-treatment of the black soldier fly (BSF) and the Pacific white shrimp (PWS)

Characteristic	BSF	PWS
Acclimation time to experimental system	N/A	20 days
Rearing system	Vertical farming using a high-rack warehouse with fixed shafts	Indoor clear water recirculation system
Rearing container	4.3 × 0.5 m	680 L fibreglass tank (green)
Water filtration system	N/A	Filter system TMC 5000 M (filter bags, protein skimmer, sand filter, trickle filter, ozone and UV system)
Photoperiod	0 h light/24 h dark	Defined by daylight (ca. 14 h light/10 h dark)
Stocking density	6.0 larvae cm ⁻²	5000 post-larvae, 7.4 specimens L ⁻¹
Feed	Until day 3 before experiment: mixture of rye (90%) and wheat bran (10%) (Illucens GmbH) Last 2 days: apple pomace (to reduced ammonia odor and increased feed acceptance by the PWS); conditions as in (Klüber et al. 2023)	1 × per hour: compound feed (Grower 1, Le Gouessant Aquaculture) 1 × per day: a portion of compound feed enriched with omega-3 fatty acids 1 × per day for days 1–3: supplement of <i>Artemia</i> nauplii
Method of processing	Cleaned and stored in a freezer (−20 °C) until use	N/A

GmbH & Co. KG, Kaiserslautern, Germany). The values for NO_2^- , NO_3^- (test strips QUANTOFIX Peroxid 25, Macherey–Nagel GmbH & Co. KG, Düren, Germany), and PO_4^{3-} (Spectroquant test kit, Merck KGaA, Darmstadt, Germany) were measured weekly (Table 3). The water parameters corresponded to ideal standards proposed by Pedrazzani et al. (2023).

Our randomized controlled trial follows the CONSORT (Consolidated Standards of Reporting Trials) guidelines (Moher et al. 2010). First, 162 juvenile specimens from the shrimp stock were assessed for trial eligibility based on phenotypic criteria (no malformations or injuries and natural body coloration). All 162 specimens met these criteria and were randomly allocated into 8 parallel groups—4 treatment (21, 21, 20, and 20 specimens) and 4 control groups (20, 20, 20, and 20 specimens)—by placing them in the respective tanks one at a time to rule out group effects. With a coefficient of determination of $R^2=0.13$ (medium effect), a statistical power of 0.9, and a significance level of $\alpha=0.05$, a sample size of $n=73$ would be required for a significant overall model with 1 predictor (sensu Cohen 1988). Thus, our 80 and 82 specimens should have a sufficient power to assess differences between the two groups.

In the experiment, the fishmeal-based shrimp compound feed “Grower 1” (Le Gouessant Aquaculture, Lamballe-Armor, France) was used for the control group. For the treatment group, 24% of this compound feed was replaced with BSF larvae (on a dry matter basis). The total amount of feed supplied daily (dry matter basis) was approximately 4% of the PWS biomass.

For the feeding regime, we followed the approach of He et al. (2022a, b). Accordingly, feed was offered four times a day: 8.00 a.m. compound feed for the control groups and BSF larvae for the treatment groups, 11.00 a.m. compound feed for both control and

Table 3 Experimental conditions and water parameters (mean values \pm standard deviations) for the Pacific white shrimp (PWS) feeding study

Characteristic	Information/value
Duration of experiment	42 days
Rearing system	Indoor clear water recirculation system
Rearing tanks	Glass aquaria, 80 L, 50×45×37 cm (length×width×height), covered with transparent light-grid panels, sides and the bottom covered with gray foil from outside, except for front window
Replications	4 replications for control (fishmeal-based diet), 4 replications for treatment (BSF-based diet)
Water filtration	Mechanical and biological filtration (filter system TMC 5000 M: filter bags, protein skimmer, sand filter, trickle filter, ozone and UV system)
Aeration	Each tank equipped with an air supply
Photoperiod	Defined by daylight (ca. 14 h light/10 h dark)
Water parameters	
Salinity	17.0±0.9
Temperature (°C)	28.0±0.9
pH	8.10±0.04
Dissolved oxygen content (mg L ⁻¹)	6.72±0.09
Nitrite content (mg L ⁻¹)	0±0
Nitrate content (mg L ⁻¹)	120±10
Phosphate content (mg L ⁻¹)	2.5±0.5
Stocking density	20/21 shrimp per tank

treatment groups, 2.00 p.m. compound feed for both control and treatment groups, and 6.00 p.m. compound feed for both control and treatment groups.

BSF larvae were fed to the treatment group after thawing and cutting the larvae into 3–4 pieces of approximately 2–3 mm each. This size is comparable to the compound feed with a diameter of approximately 1.7 mm. Both the compound feed and the BSF larvae met the ideal standards of Pedrazzani et al. (2023) for nutrition indicators.

Residual feed, shells, and feces were siphoned off daily. Stocking densities in both pre-experimental and experimental setups met the ideal standards proposed by Pedrazzani et al. (2023).

After the trial, the PWS specimens were collected, chill-killed in an ice water bath to drastically reduce the body temperature, and then placed in plastic storage bags and frozen at -20°C . All PWS specimens were weighed, measured, and photographed at the beginning and end of the experiment.

For the intention-to-treat survival analysis, information from all 80 and 82 specimens in the control and treatment groups, respectively, was used. For the physiological and growth analyses, we used the 62 and 68 surviving individuals from the control and treatment groups, respectively.

Nutritional analyses of fishmeal- and BSF-based diets used in best-practice feeding study

For the nutritional analyses, 200 g of the fishmeal-based feed substrate and 200 g of randomly selected BSF larvae were lyophilized for 72 h and then ground in a mortar. Prior to lyophilization, the moisture content of the diets was determined thermogravimetrically using an M35 Moisture Analyzer (Sartorius, Göttingen, Germany). For crude ash determination, the diets were pre-ashed in a quartz crucible over a Bunsen burner, incinerated twice at 550°C for 6 h in a muffle furnace (L 9/11, Nabertherm, Lilienthal, Germany), and the content calculated by differential weighing. The total nitrogen content was determined by the Kjeldahl method (Matissek et al. 2010). Samples were subjected to sulfuric acid digestion (Kjeldatherm, C. Gerhardt GmbH & Co. KG, Königswinter, Germany), followed by automated steam distillation and titration using the Vapodest 500 (Vapodest 500, Gerhardt, Königswinter, Germany) and TitroLine 5000 (SI Analytics, Mainz, Germany) systems. A conversion factor of 6.25 was used to calculate the crude protein content (Yi et al. 2013). For the determination of crude fat content according to Weibull-Stoldt, samples were first disintegrated manually in 150 mL of boiling $4\text{ mol}\cdot\text{L}^{-1}$ HCl for 30 min. They were then filtered, washed neutrally with hot demineralized water, and dried at 105°C for 2 h. Crude fat extraction was performed automatically with n-hexane in a Soxtherm system (Gerhardt, Königswinter, Germany). The content was determined gravimetrically.

Amino acid analyses of PWS and PWS feeds

For the amino acid analyses, 2 samples were taken for each fishmeal-based and BSF-based diets. Additionally, 2 PWS individuals were randomly selected from each tank

(i.e., 8 individuals fed the fishmeal-based diet and 8 individuals fed the BSF-based diet). The samples were hydrolyzed and the amino acid analyses performed according to a standard protocol (Bickel Haase et al. 2024). Briefly, total hydrolysis included all amino acids except tryptophan, cysteine, and methionine. For tryptophan and the sulfur-containing amino acids (cysteine and methionine), alkaline or oxidative digestion was performed. The amino acids were then separated on a cation exchange column, derivatized with ninhydrin, and detected at 570 nm or 440 nm (proline) in the SYKAM S433 amino acid analyzer (SYKAM Chromatographie Vertriebs GmbH, Fürstenfeldbruck, Germany). Note that we also analyzed the amino acid composition of the fishmeal-based diet and the BSF larvae used for the feeding study. The data are available at <https://doi.org/https://doi.org/10.22029/jlupub-18753>.

Statistical analyses

All statistical analyses and data visualizations were performed in the R statistical environment version 4.3.1. Data were tested for normality using the Shapiro–Wilk test. In addition, homogeneity of variance was confirmed using the Levene test to ensure that the assumptions of the model tests were met. Data on the growth parameters, including (a) final weight in g, (b) length gain in cm, (c) weight gain ((WG, %) = $100 \times (\text{final body weight} - \text{initial body weight}) / \text{initial body weight}$), and (d) specific growth rate ((SGR, % per day) = $100 \times (\text{Ln final body weight} - \text{Ln initial body weight}) / t$ (where “t” is time in days)), were analyzed using linear mixed effects models (lmer). In these models, treatment was dealt with as a fixed effect, and tank were included as random effect.

A post hoc contrast analysis (cftest) was then performed to test for significant differences ($p < 0.05$) between treatment groups using the R package multcomp v1.4–16 (Hothorn et al. 2008). Differences in (e) survival ((%) = $100 \times (\text{final number of shrimp}) / (\text{initial number of shrimp})$), (f) production index in g (total weight of surviving shrimp / number of shrimp stocked), (g) crude protein, and (h) crude fat between treatment groups were analyzed by a two-sample *t*-test. Boxplots were generated for each parameter to visually compare the distributions between the treatments. These plots were supplemented with jittered scatter points to display individual data points and significance annotations derived from the statistical tests. All plots were created with the R package ggplot2 v3.5.1. (Wickham 2016) with adjustments for aesthetic presentation.

Reporting guidelines

Our reporting guidelines follow the suggestions of Pedrazzani et al. (2023) and Wang (2018) and the implications of our best-practice study (see “Results” section). Specifically, we have considered the following six categories: information on insect species, shrimp species, shrimp feed, shrimp water parameters, pre-treatment of shrimp, and experimental design of shrimp feeding trial.

Results

Modulating parameters affecting life-history traits of shrimp

The literature search yielded 16 studies investigating the use of BSF as a protein substitute for fishmeal in PWS (summary available at <https://doi.org/https://doi.org/10.22029/jlupub-18753>). Despite significant variation in methodology, materials, and other details across the studies, several potential modulating factors were identified:

- Insect species (Cummins et al. 2017; Hu et al. 2019; Chen et al. 2021, 2022; Richardson et al. 2021; Shin and Lee 2021; Terrey et al. 2021; Usman et al. 2021; Wang et al. 2021; He et al. 2022a, b; He et al. 2022a, b; Li et al. 2022; Chen et al. 2023; Novriadi et al. 2023; Nunes et al. 2023; Keetanon et al. 2024)
- Substrate used for rearing insects (Richardson et al. 2021; Wang et al. 2021; He et al. 2022a, b; He et al. 2022a, b)
- Life stage of insects (Richardson et al. 2021)
- Shrimp species (Cummins et al. 2017; Hu et al. 2019; Chen et al. 2021, 2022, 2023; Richardson et al. 2021; Shin and Lee 2021; Terrey et al. 2021; Usman et al. 2021; Wang et al. 2021; He et al. 2022a, b; He et al. 2022a, b; Li et al. 2022; Novriadi et al. 2023; Nunes et al. 2023; Keetanon et al. 2024)
- Life stage of shrimp (Cummins et al. 2017; Chen et al. 2021, 2022; Richardson et al. 2021; Terrey et al. 2021; Usman et al. 2021; Wang et al. 2021; He et al. 2022a, b; He et al. 2022a, b; Li et al. 2022; Novriadi et al. 2023; Nunes et al. 2023; Keetanon et al. 2024)
- Proportion of insect in feed (Cummins et al. 2017; Hu et al. 2019; Chen et al. 2021, 2022, 2023; Richardson et al. 2021; Terrey et al. 2021; Usman et al. 2021; Wang et al. 2021; He et al. 2022a, b; He et al. 2022a, b; Keetanon et al. 2024)
- Fishmeal replacement rate (Cummins et al. 2017; Hu et al. 2019; Chen et al. 2021, 2022, 2023; Richardson et al. 2021; Usman et al. 2021; Wang et al. 2021; He et al. 2022a, b; He et al. 2022a, b; Li et al. 2022; Novriadi et al. 2023; Nunes et al. 2023; Keetanon et al. 2024)
- Additional feed compounds (Cummins et al. 2017; Hu et al. 2019; Chen et al. 2021, 2022, 2023; Richardson et al. 2021; Usman et al. 2021; Wang et al. 2021)
- Processing of insects (Cummins et al. 2017; Chen et al. 2021; Richardson et al. 2021; Terrey et al. 2021)
- Experimental design of feeding trials (Cummins et al. 2017; Chen et al. 2021, 2022, 2023; Richardson et al. 2021; Usman et al. 2021; Wang et al. 2021; He et al. 2022a, b; He et al. 2022a, b; Li et al. 2022; Novriadi et al. 2023; Nunes et al. 2023; Keetanon et al. 2024)
- Frequencies of shrimp feeding (Cummins et al. 2017; Chen et al. 2021, 2022, 2023; Richardson et al. 2021; Usman et al. 2021; Wang et al. 2021; He et al. 2022a, b; He et al. 2022a, b; Li et al. 2022; Novriadi et al. 2023; Nunes et al. 2023; Keetanon et al. 2024)
- Duration of trials (Cummins et al. 2017; Chen et al. 2021, 2022, 2023; Richardson et al. 2021; Usman et al. 2021; Wang et al. 2021; He et al. 2022a, b; He et al. 2022a, b; Li et al. 2022; Novriadi et al. 2023; Nunes et al. 2023; Keetanon et al. 2024)
- Water parameters during feeding trials (Cummins et al. 2017; Chen et al. 2021, 2022, 2023; Li et al. 2022; Richardson et al. 2021; Terrey et al. 2021; Usman et al. 2021;

- Wang et al. 2021; He et al. 2022a, b; He et al. 2022a, b; Novriadi et al. 2023; Nunes et al. 2023; Keetanon et al. 2024)
- Photoperiod during feeding trials (Cummins et al. 2017; Richardson et al. 2021; Terrey et al. 2021; Wang et al. 2021; Nunes et al. 2023)

None of the studies indicated the strain or the genotype of the insects used as protein source nor the strain or the genotype of the shrimp species as modulating parameter in feeding studies.

DNA barcoding of BSF and PWS used in best-practice feeding study

Because different genetic strains of commercial BSF and PWS may have different life histories and physiological or behavioral traits (Kaya et al. 2021; Casado et al. 2022; Klüber et al. 2024), we performed COI-barcoding and haplotype network analyses for the strains used in our experiment.

The haplotype networks for the BSF and the PWS are provided in Fig. 1A and B, respectively. They include both wild and cultured strains found worldwide (see also Table S1). The network structure shows that the global genetic diversity of BSF is much higher than that of PWS, reflected in 44 and 14 unique haplotypes, respectively (Fig. 1).

The BSF and PWS individuals used in our trials had each genetically identical COI sequences. The BSF shared a common haplotype with 17 other sequences deposited in GenBank. The PWS were genetically identical with 74 other GenBank sequences.

Proximate composition of fishmeal- and BSF-based feeds

The proximate composition of the two diets is shown in Table 4. Both the moisture and crude fat of the BSF larvae were higher and the ash content was lower compared to the fishmeal-based diet. Protein contents were similar.

Amino acid composition of PWS and PWS feeds

A comparison of the amino acid composition of the fishmeal-based feed and the BSF (see <https://doi.org/https://doi.org/10.22029/jlupub-18753>) shows several differences in the content of individual amino acids, particularly for arginine, glutamic acid, histidine, leucine, methionine, phenylalanine, and tryptophan.

Regarding the amino acid composition of the PWS raised on the fishmeal- and BSF-based diets, there were small but significant differences in the content of 4 of the 16 amino acids analyzed (i.e., alanine, histidine, serine, and tyrosine; Table S2).

Effects of fishmeal- and BSF-based feeds on life-history and physiological traits of PWS

A comparative analysis of the fishmeal- and BSF-based diets revealed significant differences in several growth parameters of the PWS (Fig. 2, Table S3). PWS fed the fishmeal-based diet had a significantly higher weight ($p < 0.001$), weight gain ($p < 0.001$), and specific growth rate ($p < 0.001$) than PWS fed the BSF-based diet. Moreover, PWS fed the BSF-based diet had a significantly higher crude fat content ($p = 0.029$) and a significantly

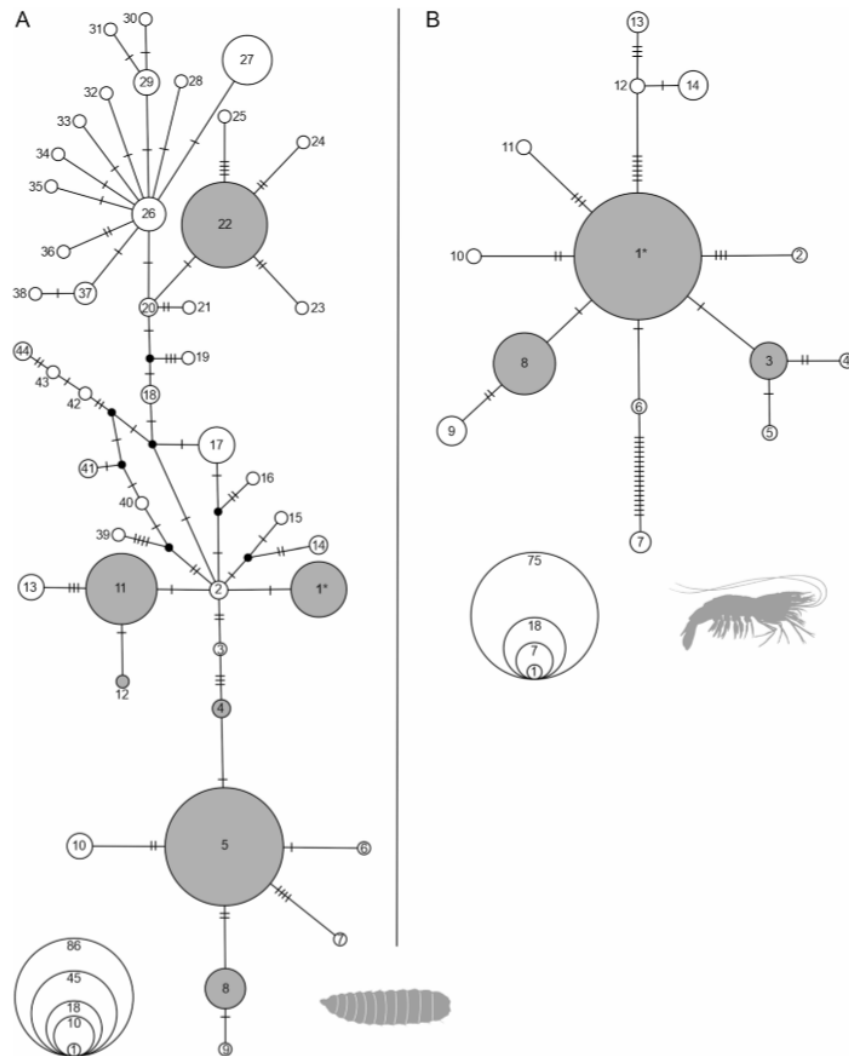


Fig. 1 Cytochrome *c* oxidase subunit I (COI) haplotype networks for **A** the black soldier fly (BSF) and **B** the Pacific white shrimp (PWS). Each circle represents a unique haplotype separated by mutational steps (hatch marks). The size of the circles is proportional to the number of sequences with that haplotype (see also the legend on the bottom left of the respective haplotype network). Numbers in circles indicate the haplotype number according to Table S1. Haplotypes of the individuals used in the present study are marked with an asterisk (*). Gray-shaded circles indicate haplotypes found in cultured species. Note that many literature studies or GenBank data entries do not provide information on the origin of the specimens (wild caught vs. cultured). Therefore, the number of haplotypes found in cultured PWS may be higher

lower crude protein content ($p=0.003$). However, PWS fed the BSF-based diet had a slightly higher survival rate than their counterparts fed the fishmeal-based diet, although the difference was not statistically significant ($p=0.513$). Consequently, the differences

Table 4 Proximate compositions (mean values \pm standard deviations) of fishmeal-based compound feed and black soldier fly (BSF) larvae (DM, dry matter; FM, fresh matter; *according to manufacturer)

Proximate	Composition of PWS feed	
	Fishmeal-based feed	BSF larvae
Moisture (% FM)	6.69 \pm 0.40 (<i>n</i> = 2)	64.28 \pm 0.03 (<i>n</i> = 3)
Crude protein (% DM)	39.61 \pm 0.53 (<i>n</i> = 2)	39.28 \pm 0.03 (<i>n</i> = 2)
Crude fat (% DM)	7.70 \pm 0.01 (<i>n</i> = 2)	25.23 \pm 0.75 (<i>n</i> = 2)
Omega-3 fatty acids (% total fat)	18.5*	0.8*
Omega-6 fatty acids (% total fat)	22.7*	7.6*
Crude ash (% DM)	7.96 \pm 0.03 (<i>n</i> = 3)	5.66 \pm 0.01 (<i>n</i> = 3)

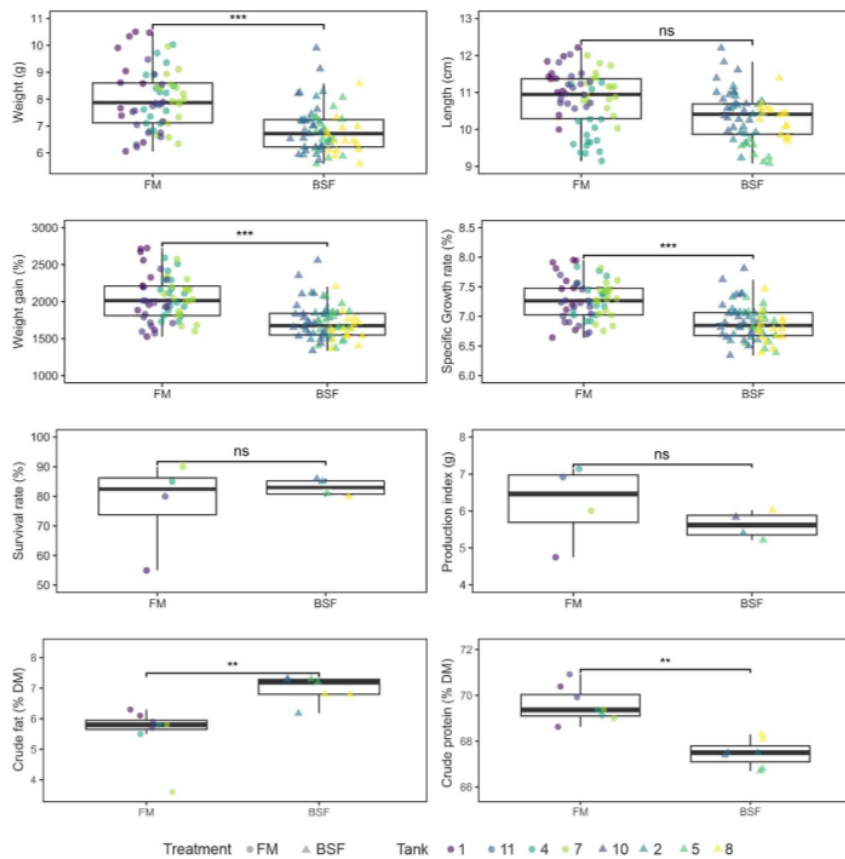


Fig. 2 Differential effects of fishmeal (FM) and black soldier fly (BSF) based diets on life-history and physiological traits of Pacific white shrimp (PWS). Data are presented as box-and-whisker plots, with raw data points for replicates of each treatment colored (tanks 1, 4, 7, and 10: FM-based diet; tanks 2, 5, 8, and 11: BSF-based diet). Lines indicate medians and boxes indicate the first and third quartiles. Asterisks indicate significant differences between treatments (*** = $p < 0.001$, ** = $p < 0.01$, ns, not significant; $p \geq 0.05$)

between the control and treatment groups are not significant when considering the production index of PWS ($p=0.375$).

Reporting guidelines

Based on the literature review of potential modulating factors in studies using insects as shrimp feed and on the results of our best-practice feeding study, a list of basic and supplementary information on these parameters was compiled (Table 5) to improve the comparability and reproducibility of future studies. The list consists of six categories: insect species, shrimp species, shrimp feed, shrimp water parameters, shrimp pre-treatment, and experimental design of the shrimp feeding trial.

Discussion

The overarching goal of this study was to enhance awareness of the necessity for reproducible and comparable feeding research and to facilitate the acceptance of sustainable insect-based shrimp feeds among stakeholders. Specifically, we have identified potential factors in the literature that have been demonstrated to influence the life history of the black soldier fly (BSF) and the Pacific white shrimp (PWS), which are pertinent to feeding studies. We then investigated the effects of a BSF-based diet, in which 24% (on a dry matter basis) of the fishmeal-based compound feed was replaced with fresh BSF larvae, on life-history and physiological traits of the PWS. Finally, we devised a set of parameters that facilitate reproducibility and comparability of research on insect-based diets in shrimp farming.

Previous studies suggest that several modulating parameters influence shrimp traits

Our literature review has identified 15 general factors that potentially affect the outcomes of insect-based feeding trials (see “Results” section). These factors include the characteristics of the insect (i.e., species, rearing substrate, and life stage) and shrimp species (i.e., species and life stage) and characteristics of the insect-based shrimp feed (i.e., proportion of insects in feed, fishmeal replacement rate, feed supplements, and processing of insects). In addition, water parameters during the trials, the pre-treatment of shrimp (i.e., acclimation of shrimp to the experimental system), and properties of the experimental design (i.e., experimental setup, photoperiod during trial, frequencies of shrimp feeding, and duration of trial) are considered.

While some of these parameters, such as the photoperiod, appear to have only a minor influence on shrimp feeding studies, other parameters are of greater importance and may partly explain the lack of consensus among researchers on the optimal BSF replacement rate in shrimp feed (see “Introduction”). These include, for example, the life stage and processing of the BSF, the life stage of the PWS under investigation, the processing and composition of feed (e.g., fat and protein content), the acclimation period of the shrimp, the experimental design and duration of the experiment, and the stocking density. All of these parameters were taken into account and/or reported in our best-practice feeding study (see “Materials and methods”).

Notably, neither the (genetic) strain of the BSF nor of the PWS was proposed as a potential modulating factor. This is surprising because the strain and genetic diversity of

Table 5 Recommended reporting items for future trials using insects as alternative feed for shrimp aquaculture. The table includes basic (minimum) and supplementary (optimal) reporting parameters in six categories. It should be noted that the information presented below represents a preliminary baseline. As research continues, the importance of certain parameters may change and the need to include additional parameters may arise

Categories and parameters	Basic	Supplementary
Insect species		
Species name	●	
Origin of individuals		●
Genetic strain of individuals		●
Life stage of individuals	●	
Rearing substrate	●	
Insect processing	●	
Shrimp species		
Species name	●	
Origin of individuals	●	
Genetic strain of individuals		●
Life stage of individuals	●	
Weight of individuals		●
Length of individuals		●
Shrimp feed		
Feed composition	●	
Feed processing	●	
Feed supplements	●	
Feeding frequency	●	
Feeding method		●
Shrimp water parameters		
Temperature	●	
Salinity	●	
pH	●	
Dissolved oxygen content	●	
Phosphate content		●
Nitrite content	●	
Nitrate content	●	
Pre-treatment of shrimp		
Acclimation time	●	
Rearing system		●
Filtration system		●
Photoperiod		●
Stocking density		●
Experimental design of shrimp feeding trial		
Type of experimental system	●	
Tank dimension		●
Tank material		●
Filtration system	●	
Aeration system	●	
Light source		●
Photoperiod		●
Stocking density	●	
Duration of experiment	●	
Sampling of individuals	●	

cultured species can significantly affect the productivity and sustainability of aquaculture (Zenger et al. 2019), and impact disease resistance (Dunham and Su 2020).

Consequently, we conducted DNA barcoding of the BSF and PWS individuals used in our experiments.

DNA barcoding of BSF and PWS indicate highly different genetic structures

Our haplotype network for the BSF (Fig. 1A), based on the barcoding gene COI, corroborates the high diversity and complex genetic structure of this species. A total of 44 haplotypes was identified in the dataset obtained from GenBank. This is consistent with the results of a previous microsatellite study (Kaya et al. 2021), which reported 16 genetic clusters in 150 wild and captive populations from 57 countries. Moreover, our network also illustrates that haplotypes from captive populations cluster throughout the network, thereby demonstrating their high genetic diversity. This finding is also consistent with previous studies, suggesting regional domestication across the globe (Kaya et al. 2021) and increasing introgression of genetic clusters into domesticated strains due to international trade (Ståhls et al. 2020; Guilliet et al. 2022).

Although the data are not conclusive as to whether the BSF represents a highly polymorphic species or even a (cryptic) species complex, it is probable that the various genetic clusters of the BSF (both wild and cultured) also exhibit disparate physiological traits that may influence the nutrition of PWS (Sukmak et al. 2024). We therefore recommend that the strain of the BSF used in feeding studies be recorded, ideally complemented by DNA barcoding information.

In contrast, our COI haplotype network for wild and captive populations of the PWS (Fig. 1B) is less diverse (only 11 haplotypes) and exhibits a simple star-shaped structure. Thus, the presence of a cryptic species complex in the PWS is less likely (but see the highly distinct haplotype #7 in Fig. 1B). However, previous genetic studies on farmed PWS (Casado et al. 2022) showed that some stocks were genetically moderately distinct and that these stocks differed in certain growth parameters. We therefore recommend that the respective strain be recorded in feeding studies on PWS. A DNA barcoding seems not necessary at present.

Nutritional analyses revealed important differences between fishmeal- and BSF-based diets

Comparing the proximate composition of the fishmeal-based diet and BSF larvae (Table 4), the protein contents were very similar. Thus, BSF larvae are as good as commercial fishmeal-based diets in terms of crude protein content.

In contrast, the moisture content of the BSF larvae was considerably higher and the ash content lower than that of the fishmeal-based feed. More importantly, the fat content of our BSF larvae was also much higher by a factor of > 3. High fat contents in BSF-based diets have been reported previously (Cummins et al. 2017; Chen et al. 2022) and may have adverse effects on PWS growth parameters (Chen et al. 2022), as also seen in our study (see following section). Therefore, some authors suggest the use of defatted BSF meal in shrimp diets to enhance growth performance (Wang et al. 2021). Our preliminary studies (Barth et al. unpublished data) also show that a plant-based diet for the BSF may significantly reduce their fat content, thus improving their ability to be used (directly) as PWS feed (see also He et al. 2022a, b).

We also found slightly more differences in the amino acid composition of the fishmeal-based compound feed and the BSF larvae than previously suggested for insect meal compared to fishmeal (Barroso et al. 2014). In addition, the content of the amino acid methionine in both fishmeal-based diet and BSF larvae (0.24% and 0.09% of the diet, respectively) is much lower than the methionine requirement of PWS feeds (i.e., 0.7–0.9% of the diet) proposed by the National Research Council (2011). However, the amino acid composition of the two PWS groups fed these diets differed less, especially with respect to methionine (i.e., 0.16% in the PWS fed the fishmeal-based diet and 0.15% in the PWS fed with the BSF-based diet). Therefore, at this stage, it is difficult to say whether these aspects contributed to the low growth rates of the PWS fed the BSF-based diet (see following section).

Best-practice study indicates BSF-based diet as sustainable alternative to fishmeal-based diet in shrimp aquaculture

Our best-practice feeding study was prompted by the fact that previous studies with insect-based diets showed very different results in terms of growth and survival rates of the PWS. This could be largely due to the complexity of the parameters to be considered, highlighting the need to provide standardized information to promote comparability and reproducibility.

The results of our study emphasize the multiple effects of feed choice on shrimp growth characteristics. Partial substitution of fishmeal-based compounds with BSF larvae in PWS diets, as done in the current study, had both positive and negative effects on shrimp growth characteristics. PWS fed with the BSF-based diet had a slightly (but not significantly) higher survival rate than those fed a traditional fishmeal-based diet. However, the individuals in the former group had a significantly lower growth rate. Nonetheless, when the production index was considered, the groups were not significantly different. The slightly lower survival rate of PWS in the control group, however, is mainly due to increased mortality in one of the four tanks (see <https://doi.org/https://doi.org/10.22029/jlupub-18753>). This, of course, also affects the production index and could contribute to it being similar in the control and treatment groups.

Despite this limitation, our study shows that supplementing fishmeal-based diets in shrimp culture with fresh BSF larvae could be an ecologically and economically sustainable approach to improve PWS aquaculture, especially in low-tech pond cultures.

Our comparison of the amino acid composition of PWS raised on fishmeal- and BSF-based diets revealed only minor differences in the content of the 16 amino acids analyzed (see Table S2). This finding suggests that a BSF-based diet is unlikely to result in deficiency of specific essential amino acids and therefore does not require amino acid supplementation, at least when the proportion of BSF remains relatively low, as in our study (i.e., 24%).

Although our results are not directly comparable with the few previous PWS feeding studies with insect-based diets (e.g., Cummins et al. 2017; Usman et al. 2021) due to the different experimental conditions and feed compositions, they add to the body of evidence that the fat content of the BSF could be the key to further improve insect-based feeds, especially for high-end recirculating aquaculture systems (see also Cummins et al. 2017; Chen et al. 2021, 2022; Wang et al. 2021; He et al. 2022a, b).

Standardized reporting information facilitate reproducible research

The fact that the results of our best-practice study are not directly comparable to other studies is partly due to the lack of standardized reporting guidelines in shrimp feeding research and highlights the need to establish a set of basic criteria to be reported in PWS feeding studies. Our list (see Table 4) comprises 39 recommended reporting items from 6 categories (i.e., information on insect species, shrimp species, shrimp feed, shrimp water parameters, pre-treatment of shrimp, and experimental design of shrimp feeding trial). The table includes the minimum parameters that should be reported in feeding trials as well as a number of additional parameters that may further improve reproducibility and comparability. In particular, our study emphasizes the need to specify the strains of the BSF and PWS used in the trials, the substrate and processing of the insects, and the fat content of the PWS feed (see also Kaya et al. 2021; Röthig et al. 2023). However, the items listed in Table 4 should be considered as a preliminary framework based on only a small number of experimental studies. As research progresses, the importance of certain parameters may change and there may be a need to include additional parameters or eliminate existing ones. Moreover, in the young field of insect-based shrimp feeds, the effects of the identified reporting parameters on PWS life-history and physiological traits are often not well understood. The list therefore differs from reporting guidelines for fish (Wang 2018), where the potential effects are relatively well understood.

Nevertheless, our reporting guidelines aim not only to increase reproducibility and comparability (see also Wang 2018) but also to reduce the risk of misinterpretation of data in complex experimental studies with aquatic animals (McDonald et al. 2021). This may ultimately lead to greater acceptance of insect-based shrimp feeds among stakeholders.

Limitations and outlook

In our study, we performed DNA barcoding of BSF and PWS specimens using the COI gene. This helped us to better characterize the BSF and PWS strains used in our study and to understand the degree of diversity of domestic strains, especially in the BSF. However, COI is a mitochondrial gene with a relatively low substitution rate (Wilke et al. 2009). It is therefore well suited to characterize past demographic processes, including regional domestication in the population under study. However, for a better understanding of the genetic basis of life-history and physiological traits, genome-wide genetic or even epigenetic analyses in feeding studies are necessary.

Another limitation of our study may be that our experimental design did not allow us to acclimate the PWS to a BSF-based diet. This probably did not have a strong effect on the main implications of our study, as the production index of the control and treatment groups did not differ significantly. However, there is increasing evidence for “nutritional programming” in aquaculture, i.e., programming effects related to growth, survival, and nutrient metabolism caused by endogenous (e.g., parental feeding) or exogenous (e.g., larval feeding) stimuli (Hou and Fuiman 2020). In other words, feeding an insect-based diet to parental individuals could in principle increase the acceptance of insect-based feeds in the offspring via epigenetic mechanisms (Piferrer 2023). Therefore, we recommend future feeding studies with “nutritionally programmed” offspring of parents reared on insect-based diets.

While defatting BSF meal is already a proven approach to reduce the typically high fat content of BSF larvae, which limits high replacement rates in fishmeal-based feeds for shrimp (Wang et al. 2021), we also encourage future nutritional studies with plant-based feeds in BSF to reduce their body fat content through diet selection. In particular, underutilized agricultural by-products, such as apple pomace or potato peelings, have great potential to increase the sustainability of shrimp aquaculture in the context of a circular bioeconomy (Röthig et al. 2023).

We therefore recommend future studies to further improve the sustainability of insect-based shrimp farming by focusing on (i) the selection of local feeds for BSF; (ii) animal welfare in BSF and PWS; (iii) the use of sustainable materials in shrimp farming such as wood or bamboo; (iv) the reduction of energy consumption in shrimp farming, for example, by using excess heat from insect rearing to heat shrimp tanks; and (v) local business models and campaigns to further increase consumer acceptance of insect-based shrimp farming. This would also help to minimize conflicts between the three pillars of sustainability—economy, ecology, and society.

Conclusions

Our barcoding analyses show that the BSF is a diverse taxon with a complex genetic structure. Haplotypes from captive populations cluster throughout the network. As the genetic clusters of the BSF may also exhibit different life-history and physiological traits, they may represent an important modulating factor. We therefore recommend DNA barcoding for future feeding studies with the BSF. In contrast, the PWS haplotype network is less diverse and star-shaped. Due to this relatively limited genetic diversity, DNA barcoding of PWS may not be necessary in feeding studies.

A comparison of the proximate composition of the fishmeal-based diet and BSF larvae shows that they have a similar protein content, but the fat content of the BSF larvae is considerably higher. This suggests that BSF larvae are well suited as a shrimp feed in terms of protein content, but the high fat content may be a major problem.

In line with this observation, the results of our best-practice feeding study demonstrate that the partial substitution of fishmeal-based compounds with BSF larvae in PWS diets resulted in a significantly lower growth rate but a non-significantly higher survival rate. When these two parameters were combined, the production index between treatments was not significantly different. This shows that supplementing fishmeal-based diets in shrimp farming with fresh BSF larvae could be an ecologically and economically sustainable approach to improve PWS aquaculture.

Based on literature data and the results of our best-practice study, we recommend that 39 items from 6 categories be considered in future BSF-based feeding studies to improve comparability and reproducibility. Of particular importance are the strains of the BSF used in the trials, the substrate and processing of the insects, and the fat content of the PWS feed.

Given the high genetic diversity of the BSF, we recommend genome-wide genetic or even epigenetic analyses in feeding studies to better understand the genetic basis of life-history and physiological traits. With regard to the emerging field of “nutritional programming,” we recommend future feeding studies with nutritionally programmed PWS individuals. We also encourage nutritional studies with plant-based feeds in BSF to

reduce the body fat content of the insects through diet selection. Finally, we recommend future studies to further improve the sustainability of insect-based shrimp farming.

Overall, this study supports the notion that widespread acceptance of sustainable insect-based shrimp feeds requires reproducible and comparable research.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10499-024-01769-w>.

Author contribution AB and TW designed the study. AB performed the experiments with assistance from PK, SB, and PS. BS conducted the genetic analyses. AB collected and analyzed the data and prepared drafts of the figures and tables. TW and AB drafted the manuscript. All authors contributed to writing, reviewing, and editing of the final manuscript.

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Data availability The Supplementary information of this paper lists GenBank accession numbers and haplotype information for the BSF and PWS individuals studied (Table S1), the amino acid contents of the PWS (Table S2), and the effects of the feeds on life-history and physiological traits of the PWS (Table S3). The raw data from this study are publicly available on JLUpub at <https://doi.org/https://doi.org/10.22029/jlupub-18753>.

Declarations

Ethics approval In Hesse, Germany, experiments with shrimps (order Decapoda) and flies (order Diptera) are not subject to authorization. All applicable legal requirements have been complied with.

Consent for publication The six authors agree for the submission to the “Aquaculture International” and to nominate Thomas Wilke as corresponding author.

Competing interests The authors declare no competing interests.

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2.3 3rd publication: From Plant By-Products to Insects to Shrimp: A Pathway to Sustainable Aquaculture Feed in a Circular Economy



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Research Article

From Plant By-Products to Insects to Shrimp: A Pathway to Sustainable Aquaculture Feed in a Circular Economy

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Shrimp aquaculture often has a large environmental footprint, partly due to the fish meal content of commercial shrimp feeds. One potential way to improve ecological sustainability would be to replace fish meal-based compounds in shrimp feeds with insect-based compounds, such as black soldier fly (BSF; *Hermetia illucens* s.l.) larvae reared on plant by-products. However, little is known about the effects of novel plant by-products on the growth characteristics of BSF larvae and how these in turn affect aquaculture species, including Pacific white shrimp (PWS; *Penaeus vannamei*). Therefore, the general goal of this study was to evaluate the suitability of BSF larvae reared on three common plant by-products (cocoa bean shells, depectinized apple pomace, and potato peelings) as a feed component for PWS aquaculture based on randomized controlled feeding trials. The study showed that both the nutritional profiles of the tested feeds and the profiles of the BSF larvae reared on these feeds differed, particularly in crude protein and crude fat content. All BSF larvae reared on the plant by-products showed significantly lower growth performance than those reared on a control feed, possibly due to the presence of toxins and insecticides, and a low content of some essential amino acids. However, no significant differences in growth parameters were found between PWS fed BSF larvae reared on plant by-products and the control feed. Possibly, BSF do not bioaccumulate the toxins/insecticides present in the plant by-products and/or partially compensate for amino acid deficiencies. PWS fed BSF larvae reared on plant by-products had feed conversion ratios (FCRs) ranging from 0.8 to 1.0. These excellent values may fully or partially compensate for the lower growth performance of the BSF larvae reared on these by-products. This study may be of economic importance for future shrimp aquaculture, confirming that BSF can be a central link in the transformation of low-cost plant by-products into high-quality protein sources for sustainable shrimp farming in a circular economy.

1. Introduction

As oceans become increasingly overfished, marine aquaculture offers an alternative to fishing. However, many aquaculture practices, such as shrimp farming, are ecologically unsustainable [1]. In particular, the production of shrimp feed has a large environmental footprint. This is partly due to the fish meal content of commercial shrimp diets. Fish meal production is often associated with overfishing of fish species, the prevalence of foodborne marine pathogens, and high CO₂ emissions

associated with transportation [2]. Therefore, the development of responsible and environmentally friendly feed practices is key to sustainable shrimp aquaculture.

Scientists are, for example, working to replace fish meal-based compounds in shrimp diets with insect-based compounds such as fresh larvae or larval meal from the black soldier fly (BSF; *Hermetia illucens* s.l.) [3]. Many characteristics of the BSF, such as its rapid reproductive rate, low risk of disease transmission, and versatility in feed utilization [3, 4], high protein content [5], amino acid profile largely comparable

to fish meal [6], potential for vertical farming [7], short production cycle, and possibility of local rearing [8], make it an attractive candidate for fish meal replacement. In addition, BSF contain antimicrobial peptides and chitin, which may induce beneficial immune responses in the target species [9].

However, the palatability of BSF compounds by shrimp may depend on the nutritional composition of the BSF larvae, including fat and protein contents, which in turn are influenced by factors such as developmental stage, rearing conditions, and feeding medium [10, 11].

Therefore, attempts are being made to use plant by-products as feed for BSF to reduce the fat content of the larvae while increasing the sustainability of insect rearing by exploring previously neglected by-products. In fact, large quantities of unused plant materials are generated as side streams in agriculture and the food industry [12]. These could potentially be used as sustainable feed for BSF rearing [13].

However, there are two critical research needs. First, researchers are interested in finding out how these novel plant-based feeds affect the developmental and growth characteristics of BSF, and second, how feeding BSF reared on plant by-products affects the developmental and growth characteristics of the aquaculture species such as shrimp.

Regarding the first point, studies have shown that the composition of the feed affects the growth and survival of BSF larvae [7, 13, 14]. For example, the use of apple pomace as insect feed resulted in reduced growth rates and an increased larval development time, probably due to the low protein, and high fiber content of apple pomace [7, 11, 15]. Similarly, feeding cocoa pods resulted in reduced growth and delayed development of BSF larvae [16]. In contrast, larvae reared on beer dross reached higher weights comparable to chicken starter mash [7].

Concerning the second point, there are only very few studies that have used BSF reared on plant by-products in shrimp feeding trials. For example, replacing 50% of the fish meal-based feed with bran-fed BSF had no negative effect on the growth performance or survival rate of shrimp [17]. Moreover, a mixture of bran and cereal meal as feed for BSF and the subsequent use of defatted BSF meal (30%–70% replacement of fish meal) led to improved growth of the shrimp compared to a fish meal-based diet [18]. Similarly, the use of food by-products as BSF feed and the replacement of up to 60% of the fish meal with the corresponding defatted BSF meal had no adverse effects on shrimp [19]. However, it remains unclear for most plant by-products to what extent they affect shrimp life-history parameters; and thus, shrimp aquaculture as a whole.

Worldwide, a large number of companies have emerged that produce the economically, most important shrimp species—the Pacific white shrimp (PWS; *Penaeus vannamei*)—locally in closed aquaculture facilities. They are increasingly interested in improving the sustainability of their operations by using shrimp feed with a lower environmental footprint. Therefore, the use of BSF reared on regional plant by-products could be an important step in this direction as part of a circular economy.

There are several plant by-products of low economic value that are available in large quantities worldwide and can be digested by BSF larvae directly or after moderate processing

[10]. These include cocoa bean shells, depectinized apple pomace, and potato peelings.

Cocoa bean shells are a by-product of cocoa production. Cocoa pods are produced in vast quantities, especially in Africa, but also in the Americas and Asia. The beans are extracted, fermented, and dried. The dried beans are shipped around the world for, among others, chocolate production. During the process, the beans are deshelled, and the shells, which make up 10%–17% of the bean's weight, are mostly discarded. As a result, more than 700,000 tons of cocoa bean shells are generated each year, with Europe accounting for more than 250,000 tons [20].

Depectinized apple pomace is a by-product that remains after processing apples to remove pectin, a natural thickening agent used in various foods. The pomace typically contains the peels, seeds, cores, stalks, and remaining soft tissues of the apples. Worldwide, pectin production from apple pomace is an important industry, although exact production figures may vary. It is estimated that apple pomace accounts for a significant portion of the global pectin supply, with millions of tons of apple pomace processed annually [21].

Finally, potato peelings are a plant by-product of potato production and processing. Potatoes are one of the world's most important crops. During processing, 15%–40% of peel waste is generated. This results in at least 70,000–140,000 tons of potato peel by-products per year, most of which are disposed of in landfills [22].

Considering the great potential of neglected plant by-products in a circular economy, the general goal of this study is to evaluate the usability of BSF larvae reared on these by-products as sustainable feed for PWS aquaculture. Based on randomized controlled feeding trials, we specifically:

1. determined the nutritional profiles of three candidate-plant by-products—cocoa bean shells, depectinized apple pomace, and potato peelings—and compared them with a grain-based control feed,
2. evaluated the nutritional profiles of BSF larvae reared on these plant by-products compared to a control feed,
3. analyzed the growth performance of BSF larvae reared on these plant by-products compared to a control feed,
4. determined the growth performance characteristics of the PWS fed with BSF larvae reared on these plant by-products compared to a control feed, and
5. calculated the respective feed conversion parameters.

2. Materials and Methods

2.1. Ethics Statement. In Hesse, Germany, experiments with shrimps (order Decapoda) and flies (order Diptera) do not require a permit. All applicable legal requirements were met.

2.2. Species Studied and Pretreatment. Black soldier flies (BSFs) were obtained from Bio.S Biogas (Grimma, Germany) in July 2018 (Table 1). Since then, the population has been kept genetically isolated from other populations (>37 generations). Adult BSF flies were maintained in 60 cm × 60 cm × 90 cm

TABLE 1: Characteristics of the black soldier flies (BSFs) and Pacific white shrimp (PWS) studied.

Characteristic	BSF	PWS
Species name	Black soldier fly, <i>Hermetia illucens</i> Linnaeus, 1758	Pacific white shrimp, <i>Penaeus vannamei</i> Boone, 1931
Origin	Bio.S Biogas (Grimma, Germany)	Suburban Seafood Germany UG (Nebelschütz, Germany)
Genetic strain	For DNA barcoding information on the strain used see GenBank accession #PQ187607 and PQ187608	For DNA barcoding information on the strain used see GenBank accession #PQ465998 and PQ465999
Life stage	Fifth larval instar	Juvenile specimens (7 weeks old)
Weight	36–253 mg	0.10 ± 0.01 g
Length	0.5–2.5 cm	2.4 ± 0.6 cm

(length × width × height) mesh cages (Bioform, Nürnberg, Germany), located in a greenhouse (Fraunhofer IME, Giessen) at 26 ± 1°C, 60% ± 5% relative humidity, and a 12 h photoperiod (L-PL-ECO623330, Lence Technology, Langen, Germany). Water-soaked paper towels provided drinking water ad libitum, and the mesh cages were sprayed with water daily. Fresh egg clutches were collected from artificial oviposition sites consisting of three wooden boards spaced by washers and held in place with rubber bands [23]. After weighing (ALJ 160-4A, Kern & Sohn, Balingen, Germany), 150 mg of eggs (approximately 6000 eggs) were transferred into 19.5 × 16.5 × 9.5 cm plastic boxes and moistened. Once ≥50% of the eggs had hatched, the neonates were initially fed 10 g of the corresponding feed. The boxes were monitored daily for remaining water and feed, and additional feed was provided ad libitum (typically at 48-h intervals). The moisture content of the diets was measured using a TMT-MC-7828S soil moisture meter (OCS.tec, Neuching, Germany) and adjusted to approximately 70% by spraying. Larvae were reared in a climate chamber at 27 ± 1°C and 65% ± 5% relative humidity in darkness [13].

Juvenile PWS (Table 1) were obtained from Suburban Seafood Germany UG (Nebelschütz, Germany). They were acclimated for 1 week in a wooden tank (Douglas fir, 1200 mm height, 600 mm radius, Wilhelm Eder GmbH, Bad Dürkheim, Germany), located at the marine aquaculture experimental facility of the Justus Liebig University Giessen, at a stocking density of 0.8 larvae L⁻¹. The indoor recirculation system was equipped with a filtration system consisting of a drum filter (35 µm drum mesh, FAIVRE, Baume-les-Dames, France) and a protein skimmer (Bubble King DeLuxe 650 intern, Royal Exclusiv, Wesseling, Germany). The shrimp were fed six times per day ad libitum with the PWS compound feed Grower 1 (diameter 1.7 mm, Le Gouessant Aquaculture, Lamballe, France) through automatic feeders (easyFuttermat, Aqualight GmbH, Bramsche, Germany).

DNA barcoding of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene in two individuals each of BSF and PWS was performed according to Barth et al. [6].

2.3. Nutritional Analyses of BSF Feeds and BSF Larvae. For the nutritional analyses, BSF larvae and the BSF feeds were lyophilized for 72 h, and then ground in a mortar. Prior to lyophilization, the moisture content was determined thermogravimetrically using an M35 moisture analyzer (Sartorius, Göttingen, Germany). For crude ash determination, the diets were pre-ashed in a quartz crucible over a Bunsen burner,

incinerated twice at 550°C for 6 h in a muffle furnace (L 9/11, Nabertherm, Lilienthal, Germany), and the content calculated by differential weighing. The total nitrogen content was determined by the Kjeldahl method [24]. Samples were subjected to sulfuric acid digestion (Kjeldatherm, C. Gerhardt GmbH & Co. KG, Königswinter, Germany), followed by automated steam distillation and titration using the Vapodest 500 (Vapodest 500, Gerhardt, Königswinter, Germany) and Titro-Line 5000 (SI Analytics, Mainz, Germany) systems. A conversion factor of 6.25 was used to calculate the crude protein content [25]. For the determination of crude fat content according to Weibull-Stoldt, samples were first manually disintegrated in 150 mL of boiling 4 mol·L⁻¹ HCl for 30 min. They were then filtered, washed neutrally with hot demineralized water, and dried at 105°C for 2 h. Crude fat extraction was performed automatically with n-hexane in a Soxtherm system (Gerhardt, Königswinter, Germany). The content was determined gravimetrically.

2.4. Plant By-Products and Control Diet Used as BSF Feeds. Four diet groups were established for the feeding trials: cocoa bean shells, depectinized apple pomace, potato peelings, and a grain-based control feed. Cocoa bean shells were provided by August Storck KG (Berlin, Germany). The depectinized apple pomace was obtained from agro Food Solution GmbH (Herbavital F12, Werder, Germany). Both cocoa bean shells and depectinized apple pomace were homogenized in a Thermomix TM6-1 for 1 min at setting 10 (Vorwerk, Wuppertal, Germany). The control feed was ground in a Mockmill 200 grain mill (Wolfgang Mock, Oetzberg, Germany) and served as reference feed (GoldDott Eierglück, Derby Spezialfutter, Muenster, Germany). After processing, control feed, cocoa bean shells, and depectinized apple pomace had a particle size of 0.1–1.5 mm, which was determined with analytical sieves (Retsch, Haan, Germany). Due to their low moisture content (6.5%–9.5%), the feed was stored at room temperature in separate airtight plastic barrels until further use.

Potato peelings were produced by peeling potatoes (variety “Kuras”) on a Flott 16K potato-peeling machine (Flottwerk, Rotenburg a.d. Fulda, Germany) and were supplied frozen by the Fraunhofer Institute for Process Engineering and Packaging in Freising (Germany). Due to the high moisture content (82%), the potato peelings were stored at –20°C and processed directly on the day of feeding. The potato peelings were mixed into a slurry using the Thermomix TM61 for 10 min at setting 6–8. Excess water was drained before feeding. In addition, part

TABLE 2: Experimental conditions of the black soldier flies (BSFs) and the Pacific white shrimp (PWS).

Characteristic	BSF	PWS
Duration of trials	17–82 days ($\geq 50\%$ prepupae)	35 days
Rearing system	Small-scale indoor rearing	Indoor clear water recirculation system
Rearing container	19.5 cm \times 16.5 cm \times 9.5 cm polypropylene boxes, maximum filling capacity of 2 L, 12 boxes in total	35 cm \times 21 cm \times 20 cm plastic tanks, filling capacity of 9.2 L, each divided in two chambers by a plastic wall, 56 tanks in total
Replications	$n = 3$	$n = 28$
Water filtration system	N/A	Vlies Dreambox 3.0 with fleece filter and protein skimmer (Royal Exclusiv), and a flow-through biofilter (Aqualight) with a sponge media
Photoperiod	24 h dark	Blue LED light (ca. 12 h light/12 h dark)
Stocking density	150 mg eggs per box (~ 6000 eggs)	1 postlarva per chamber
Feed	Fed <i>ad libitum</i> (typically at 48-h intervals) with CBS, DAP, PP, and CF	Four times daily at 6:00 am (compound feed), 9:30 am (BSF larvae), 4:00 pm (BSF larvae) and 10:00 pm (compound feed) to a visual satiation each time
Method of processing	Cleaned and stored in a freezer (-20°C) until use	PWS were weighed, measured, and photographed at the start and end of the trials. After 35 days, the shrimp were euthanized, placed in bags, and stored in a freezer (-20°C)

Abbreviations: CBS, cocoa bean shells; CF, control feed; DAP, depectinized apple pomace; PP, potato peelings.

of the potato peelings was dried for 12 h at 50°C (BDA-15 dehydrator, Beeketal Lebensmitteltechnik, Rastdorf, Germany) and ground to adjust the feed moisture during feeding.

2.5. BSF Feeding Trials. For each BSF diet group, developmental and growth parameters were recorded using three replicate boxes (Table 2). The boxes were maintained under the conditions described above. Growth parameter documentation was started when the larvae reached a manageable size of 3–4 mm, which was highly dependent on the feed. To obtain growth curves, the mean weight of 25 randomly selected larvae was determined using an AT261 DeltaRange analytical balance (Mettler, Giessen, Germany). Measurements were stopped and boxes were harvested when $\geq 50\%$ of the population had reached the prepupal stage, indicated by brown coloration. The larvae were separated from the substrate by sieving (AS 200, Retsch, Haan, Germany), cleaned of impurities, and stored at -20°C for subsequent analysis and feeding to shrimp.

2.6. PWS Feeding Trials. For the PWS feeding trials (Table 2), a combined diet was used. It consisted, on a dry matter weight basis, of approximately 50% of the conventional fish meal-based compound feed Grower 1 and approximately 50% BSF larvae. This ratio has been suggested to have no adverse effects on the growth performance and survival rate of PWS [17]. The BSF larvae were reared on cocoa bean shells, depectinized apple pomace, potato peelings, or a control feed. For the latter, we used a grain-based diet, as this is a common feed for BSF larvae [7]. The relative amount of feed supplied daily was approximately 1.5% compound feed and approximately 1.5% BSF larvae of the PWS biomass on a dry matter basis (for total amounts of feed supplied, see <https://doi.org/10.22029/jlupub-19060>). An automatic feeder was used to deliver the compound feed to the PWS. The BSF larvae were fed manually after thawing and cutting into pieces of 2–3 mm. For the calculation of

feed conversion ratios (Section 2.7, Equation (2)), it was assumed that all the feed was consumed by the shrimp.

The PWS were preselected for the trials based on weight and length criteria (Table 1), and randomly distributed to the treatment and control tanks. Shrimp were kept individually to prevent competition and cannibalism. Each plastic tank had a clear front screen, blue-opaque side walls, and an acrylic-glass lid to prevent shrimp escape and to reduce evaporation. Residual feed, shells, and feces were siphoned off once a week. For a detailed description of the “single-shrimp system” used, see [26].

Tank water parameters (Table 3), including nitrogen, nitrate (test strips QUANTOFIX Peroxid 25, Macherey-Nagel, Düren, Germany), salinity, and temperature (Hanna Instruments Deutschland, Vöhringen, Germany), were monitored daily. Dissolved oxygen and pH were measured twice a week with a multiparameter probe (Multi 3620 IDS SET G, Xylem Analytics Germany, Weilheim, Germany). All information provided for PWS (Tables 1–3) complies with the guidelines for reporting experimental studies in shrimp [6].

2.7. Data Processing and Statistical Analyses. Our randomized controlled trials followed the Consolidated Standards of Reporting Trials (CONSORT) guidelines [27]. Statistical analyses of data were performed in Excel 2016 (Microsoft, Redmond, USA) and OriginPro 2022b (OriginLab, Northampton, USA). With a coefficient of determination of $R^2 = 0.26$, a statistical power of 0.85, and a significance level of $\alpha = 0.05$, a sample size of $n = 28$ would be required for a significant overall model with one predictor [28]. These requirements were met for both the BSF and PWS feeding trials. Shrimp survival was analyzed using the nonparametric Kaplan–Meier estimator. The $S(t)$ survival functions were then compared pairwise using log-rank tests ($\alpha = 0.05$). The homogeneity of variance was assessed by Levene’s test. For

TABLE 3: Water parameters for the PWS preexperimental and experimental periods.

Water parameter	Value
Salinity (ppt)	24.3 ± 0.5
Temperature (°C)	27.7 ± 0.2
pH	7.8 ± 0.1
Dissolved oxygen (%)	97.5 ± 2.1
Nitrite (mg L ⁻¹)	0.1 ± 0.2*
Nitrate (mg L ⁻¹)	24.3 ± 9.5

Note: Data represent mean ± SD.

*Only found in the first week, afterward 0.0 mg L⁻¹.

all other parameters, a one-way analysis of variance (ANOVA)

was performed, and mean values were separated using Tukey's test (homogeneous variance). When the assumption of homogeneity of variance was not met, we used Welch's one-way ANOVA, followed by a Games–Howell post hoc test [29]. Linear relationships were calculated using Pearson product–moment correlation [30]. Visualization of the data was conducted in the R statistical environment, version 4.3.1, together with the graphical user interface 'RStudio' (version 2023.06.1 + 524, Posit PBC, Boston, USA).

The following formulas were used to obtain the efficiency of conversion of ingested feed (ECI; Equation (1)), the feed conversion ratio (FCR; Equation (2)), the specific growth rate (Equation (3)), the survival rate (Equation (4)), and the weight gain (Equation (5)); (DM, dry matter; FM, fresh matter):

$$\text{Efficiency of conversion of ingested feed} = \frac{\text{total biomass harvested (g, FM)} - \text{initial biomass (g, FM)}}{\text{total feed provided (g, DM)}}, \quad (1)$$

$$\text{Feed conversion ratio} = \frac{\text{total feed intake (g, DM)}}{\text{final body weight (g, FM)} - \text{initial body weight (g, FM)}}, \quad (2)$$

$$\text{Specific growth rate (\% per day)} = \frac{100 \times (\ln \text{ final body weight} - \ln \text{ initial body weight})}{t(\text{days})}, \quad (3)$$

$$\text{Survival rate (\%)} = \frac{100 \times \text{final number of shrimp}}{\text{initial number of shrimp}}, \quad (4)$$

$$\text{Weight gain (\%)} = \frac{100 \times (\text{final body weight} - \text{initial body weight})}{\text{initial body weight}}. \quad (5)$$

3. Results

3.1. Nutritional Profiles of Plant By-Products and Control Feed. Information on the nutritional profiles of the three plant by-products tested (i.e., cocoa bean shells, depectinized apple pomace, and potato peelings) compared to a grain-based control feed is provided in Table 4. The three plant by-products differed from the control feed in some or all of the nutritional parameters analyzed. In particular, potato peelings contained up to 13 times more water than the control feed and the other diets (Welch's $F_{(3, 3.35)} = 250.77; p < 0.001$). The crude ash content of the control feed was significantly higher than that of any of the plant by-products tested (Welch's $F_{(3, 4.07)} = 1121.34; p < 0.05$). Cocoa bean shells had the highest crude fat content, exceeding the control feed and the other diets by at least 940% (Welch's $F_{(3, 4.19)} = 1792.14; p < 0.001$). The crude protein content of apple pomace and potato peelings was significantly lower than in the control feed and in cocoa bean shells, as was the total nitrogen content (Welch's $F_{(3, 3.88)} = 9550.25; p < 0.001$).

3.2. Nutritional Profiles of BSF Larvae Fed With Plant By-Products or the Control Feed. Information on the nutritional profiles of BSF larvae fed the three tested plant by-products (i.e., cocoa bean shells, depectinized apple pomace, and potato peelings) compared to a grain-based control feed is presented in Table 5. BSF larvae fed apple pomace and potato peelings had a significantly higher moisture content than those fed cocoa bean shells and the control diet (Welch's $F_{(3, 3.39)} = 4197.30; p < 0.05$). The crude ash content of BSF larvae fed potato peelings or apple pomace was also significantly higher than that of those fed cocoa bean shells or the control feed ($F_{(3, 11)} = 36.40; p < 0.001$). The fat content of the larvae varied greatly depending on the diet. Crude fat content was particularly low in BSF larvae fed apple pomace and potato peelings, compared to cocoa bean shells and the control feed ($F_{(3, 11)} = 755.02; p < 0.001$). Both the total nitrogen and crude protein content of BSF larvae fed the three plant by-products were higher than of the larvae fed the control feed ($F_{(3, 11)} = 93.52; p < 0.001$).

TABLE 4: Nutritional profiles of three plant by-products (cocoa bean shells, depectinized apple pomace, potato peelings) and a grain-based control feed.

Parameter	BSF feed substrates			
	Cocoa bean shells (n = 3)	Depectinized apple pomace (n = 3)	Potato peelings (n = 3)	Control feed (n = 3)
Moisture (% FM)	6.5 ± 1.0 ^b	9.5 ± 0.5 ^b	81.9 ± 3.2 ^a	6.7 ± 0.3 ^c
Crude ash (% DM)	6.4 ± 0.0 ^c	3.6 ± 0.1 ^d	2.8 ± 0.1 ^b	12.9 ± 0.9 ^a
Crude fat (% DM)	30.1 ± 0.7 ^c	3.2 ± 0.0 ^d	0.4 ± 0.0 ^b	2.5 ± 0.0 ^a
Crude protein (% DM)	20.3 ± 0.0 ^c	8.0 ± 0.1 ^b	7.5 ± 0.8 ^b	18.2 ± 0.4 ^{a*}
Total nitrogen (% DM)	3.3 ± 0.0 ^c	1.3 ± 0.0 ^b	1.2 ± 0.1 ^b	2.9 ± 0.1 ^{a**}

Note: Different letters (a–d) indicate statistically significant differences ($p < 0.05$). Data represent mean ± SD.

Abbreviations: DM, dry matter; FM, fresh matter.

*n = 4 with data taken from [14].

TABLE 5: Nutritional profiles of black soldier fly (BSF) larvae reared on three plant by-products (cocoa bean shells, depectinized apple pomace, potato peelings) and a grain-based control feed.

Parameters	BSF larvae reared on			
	Cocoa bean shells (n = 3)	Depectinized apple pomace (n = 3)	Potato peelings (n = 3)	Control feed (n = 3)
Moisture (% FM)	70.1 ± 1.1 ^c	81.7 ± 0.5 ^d	79.2 ± 0.2 ^b	64.3 ± 0.0 ^a
Crude ash (% DM)	5.4 ± 0.2 ^a	9.0 ± 0.3 ^c	12.3 ± 0.7 ^b	4.9 ± 0.2 ^a
Crude fat (% DM)	28.9 ± 0.8 ^c	5.3 ± 0.4 ^b	3.1 ± 0.4 ^b	33.9 ± 1.1 ^a
Crude protein (% DM)	46.9 ± 0.2 ^c	52.1 ± 0.3 ^b	50.2 ± 0.9 ^b	40.7 ± 1.3 ^{a,*}
Total nitrogen (% DM)	7.5 ± 0.0 ^c	8.3 ± 0.0 ^b	8.0 ± 0.1 ^b	6.5 ± 0.2 ^{a*}

Note: Different letters (a–d) indicate statistically significant differences ($p < 0.05$). Data represent mean ± SD.

Abbreviations: DM, dry matter; FM, fresh matter.

*n = 5 with data taken from [14].

3.3. Growth Performance of BSF Larvae Fed With Plant By-Products or the Control Feed. Information on the growth characteristics of BSF larvae fed the three plant by-products (i.e., cocoa bean shells, depectinized apple pomace, and potato peelings) compared to a grain-based control feed is summarized in Figure 1.

All BSF larvae fed plant by-products had a significantly longer development time compared to those fed the control feed ($F_{(3, 10)} = 9.58$; $p < 0.001$) (Figure 1B). The development time was longest in larvae fed depectinized apple pomace, followed by those fed cocoa bean shells and potato peelings. Neither crude protein ($r = -0.47$; $p = 0.08$) nor crude fat ($r = 0.00$; $p = 0.93$) content of the feeds was correlated with larval development time.

BSF larvae fed the control feed exhibited the highest and fastest weight gain, followed by those fed potato peelings, cocoa bean shells, and depectinized apple pomace (Figure 1A). Accordingly, final larval weight differed significantly between groups ($Welch's F_{(3, 4.16)} = 504.61$; $p < 0.001$), with the heaviest larvae reared on the control feed, followed by those reared on potato peelings, cocoa bean shells, and depectinized apple pomace (Figure 1C). Final larval weight was positively correlated with crude protein content of the diets ($r = 0.54$; $p < 0.05$), but not with crude fat content ($r = -0.09$; $p = 0.76$). The specific growth rate of BSF larvae differed significantly among diets, with all plant by-products showing lower rates than the control feed ($Welch's F_{(3, 2.67)} = 1587.68$; $p < 0.01$) (Figure 1D).

3.4. Growth Performance of PWS Reared on BSF-Based Feeds. Information on the growth characteristics of the PWS at the end of the 35-day controlled trial is summarized in Figure 2. In the randomized trial, four diets were used for the PWS, which contained BSF larvae raised on plant by-products (i.e., cocoa bean shells, depectinized apple pomace, and potato peelings) or a grain-based control feed.

Shrimp survival rate was found to be consistently high, exceeding 96% on all diets ($\chi^2 = 2.02$; $p = 0.57$; Figure 2A). Final weight ranged from 1.3 to 1.4 g per PWS individual, regardless of the diet ($Welch's F_{(3, 57.77)} = 0.94$; $p = 0.43$; Figure 2B). Average weight gain was 1193% ($Welch's F_{(3, 57.77)} = 0.94$; $p = 0.42$) and the average specific growth rate was 7.2% d^{-1} ($Welch's F_{(3, 57.23)} = 1.43$; $p = 0.24$), with no significant difference between the PWS groups fed the plant by-products and the control feed (Figure 2C,D). Overall, the PWS individuals in the depectinized apple pomace group showed greater variations in final weight, weight gain, and specific growth rate than the other groups.

3.5. Feed Conversion Parameters of BSF-Based Feeds in PWS. Feed conversion parameters and information on the efficiency of conversion of the ingested feed by the PWS are presented in Table 6. Shrimp moisture content varied from 78% to 82% with significant differences among all groups ($F_{(3, 11)} = 17.02$; $p < 0.001$). PWS fed the combined diet with BSF larvae reared on cocoa bean shell had the highest total harvested biomass (FM and DM basis). All PWS fed BSF larvae reared on plant by-products had significantly lower FCR ($Welch's F_{(3, 55.31)} =$

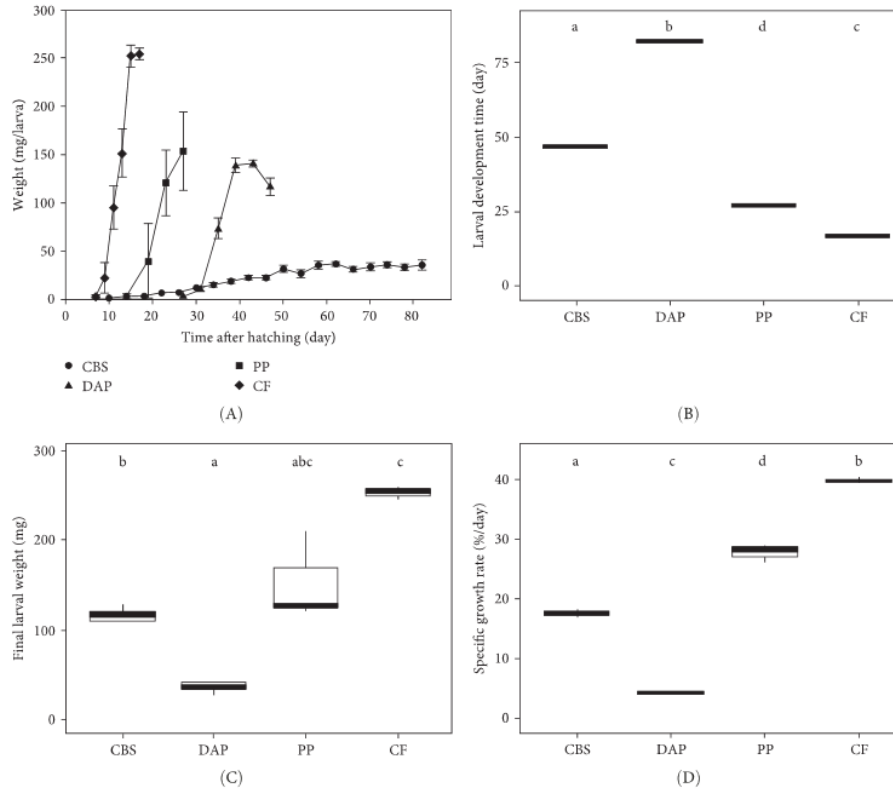


FIGURE 1: Growth curves (A), larval development time (B), final larval weight (C), and specific growth rate (D) of BSF larvae reared on three plant by-products (cocoa bean shells, CBS; depectinized apple pomace, DAP; potato peelings, PP) and a grain-based control feed (CF). Different letters (a–d) indicate statistically significant differences ($p < 0.05$; one-way ANOVA or Welch's ANOVA). Data for (B–D) are presented as box-and-whisker plots. Lines indicate medians and boxes first and third quartiles.

20.25; $p < 0.001$) and significantly higher ECI (Welch's $F_{(3, 56.48)} = 33.76$; $p < 0.001$) values compared to the control feed. ECI and FCR were positively ($r = 0.63$; $p < 0.001$) and negatively ($r = -0.54$; $p < 0.001$) correlated with increasing protein content of BSF larvae, respectively. The fat content of BSF larvae had a comparable effect on ECI ($r = -0.59$; $p < 0.001$) and FCR ($r = 0.48$; $p < 0.001$).

4. Discussion

The general goal of this study was to test whether BSF larvae reared on widely available, but underutilized plant by-products may serve as sustainable feeds for PWS aquaculture. Based on randomized controlled feeding trials, we found that (i) the nutritional profiles of the three tested feeds and a grain-based control feed differed in several parameters, (ii) the nutritional profiles of BSF larvae reared on these feeds also differed, (iii)

BSF larvae raised on the plant by-products showed significantly lower growth performances than those raised on the control feed, (iv) growth performance parameters of PWS fed BSF larvae reared on plant by-products or the control feed did not show significant differences between groups, and (v) feed conversion parameters of PWS fed BSF larvae reared on the plant by-products were excellent compared to the control group.

4.1. Nutritional Profiles of Plant By-Products Differ From Grain-Based Control Feed. Most nutritional profiles of the plant by-products used to rear BSF larvae differed from our grain-based control feed in several parameters (Table 4), particularly in moisture, crude fat, and crude protein contents. While moisture content may not be a critical factor in rearing BSF as feed for PWS, previous studies have shown that fat [10, 11] and, to a lesser extent, protein content [31] may be critical.

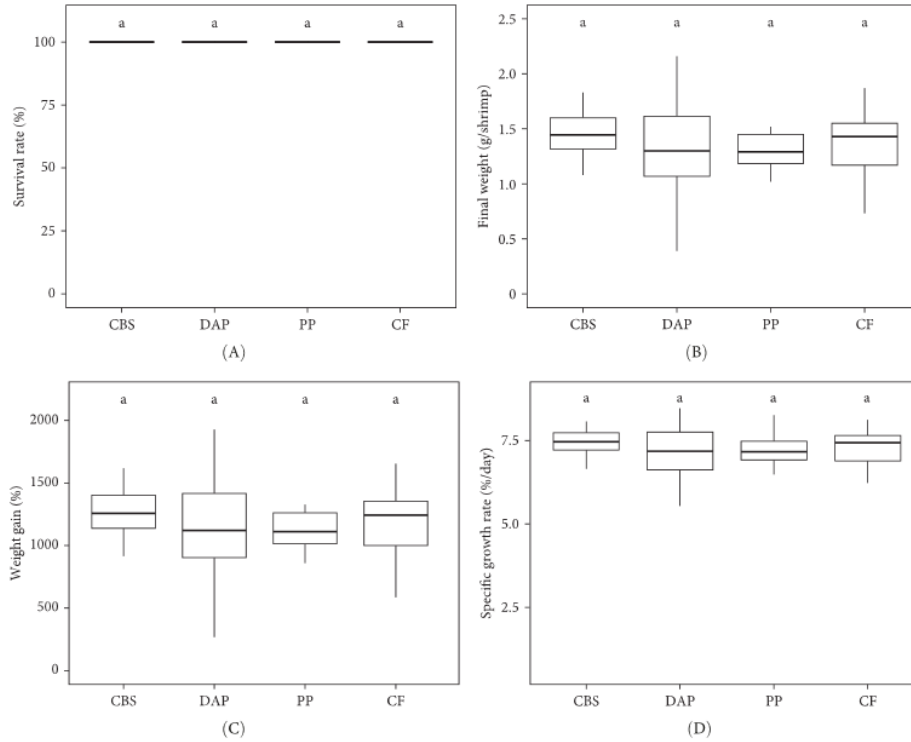


FIGURE 2: Growth characteristics of PWS ((A) survival rate, (B) final weight, (C) weight gain, and (D) specific growth rate) fed a diet containing BSF larvae reared on three plant by-products (cocoa bean shells, CBS; depectinized apple pomace, DAP; potato peelings, PP) or a grain-based control feed (CF). The letters "a" indicate that there were no significant differences between treatment and control groups ($p < 0.05$; KaplanMeier [survival], one-way ANOVA or Welch's ANOVA). Data are presented as box-and-whisker plots. Lines indicate medians and boxes first and third quartiles.

TABLE 6: Feed conversion parameters of PWS fed a combined diet consisting of a compound feed as well as BSF larvae reared on plant by-products or a grain-based control feed.

Parameters	PWS feeding group			
	Cocoa bean shells	Depectinized apple pomace	Potato peelings	Control feed
Initial weight (g FM), $n = 112$		0.1 ± 0.0		
Moisture of shrimp (% FM), $n = 3$	81.9 ± 0.9 ^c	80.7 ± 0.3 ^{ac}	78.4 ± 0.3 ^b	80.3 ± 0.3 ^a
Total harvested biomass (g FM), $n = 28$	40.5	37.3	36.7	37.2
Total harvested biomass (g DM), $n = 28$	7.3	7.2	7.9	7.3
Feed conversion ratio, $n = 28$	1.0 ± 0.2 ^{bd}	0.9 ± 0.5 ^b	0.8 ± 0.1 ^{bc}	1.4 ± 0.3 ^a
Efficiency of conversion of ingested feed, $n = 28$	1.0 ± 0.2 ^c	1.4 ± 0.5 ^b	1.3 ± 0.3 ^b	0.8 ± 0.2 ^a

Note: Data are mean ± SD. Different letters (a–d) within a row indicate statistically significant differences ($p < 0.05$; one-way ANOVA or Welch's ANOVA). Abbreviations: DM, dry matter; FM, fresh matter.

In fact, the rate at which fish meal-based compounds in PWS feeds can be replaced by BSF-based compounds is highly dependent on the fat content of the BSF larvae [18].

Therefore, it could be expected that a low-fat/high-protein diet for BSF would have a positive effect on growth parameters of PWS. Thus, based on nutritional parameters alone,

depectinized apple pomace, and potato peels may be well suited as BSF feeds due to their low fat content, while cocoa bean shells may be suitable due to their high protein content.

However, it should be noted that the plant by-products used in the current study may differ from similar products depending on the place of origin and the plant species or variety used. For example, the protein content of cocoa bean shells can vary worldwide between 10.3% and 27.4% dry weight and the fat content between 1.5% and 8.5% dry weight [20]. Similarly, the fat content of potato peelings may vary between 0.1% and 0.6% of fresh weight (roughly equivalent to 0.6%–2.6% dry weight) and the protein content between 1.2% and 4.4% of fresh weight (roughly equivalent to 7.2%–16.1% dry weight) [32]. These large ranges are not surprising considering, for example, that more than 4000 potato varieties are cultivated [33] and that processing methods for the production of potato peelings may vary.

4.2. Nutritional Profiles of BSF Larvae Reared on Plant By-Products Differ From Control Group. As the nutritional profiles of the plant by-products and the control feed differed in several parameters (Section 4.1 and Table 4), we also expected differences in the nutritional profiles of the BSF larvae reared on these feeds. While we observed significant differences between BSF larvae fed plant by-products vs. the control feed for all parameters tested, the crude fat and crude protein contents are particularly noteworthy (Table 5). Crude fat content was lower and crude protein content was higher in all BSF groups reared on plant by-products. For example, BSF larvae fed potato peelings and depectinized apple pomace had a 91% and 84% lower fat content, respectively, than the control group. Similarly, the protein content was 23% and 28% higher in the former and latter treatment groups, respectively, than in the control group.

Considering the nutritional profile of the BSF larvae from this study (Table 5), both potato peelings and apple pomace appear to be the most promising diets for rearing BSF as a sustainable feed for PWS. These low-fat diets may not only reduce the body fat content of BSF, but also adverse health effects in PWS, such as hepatopancreatic damage [17, 19, 34, 35]. Another advantage of the low fat content of BSF larvae reared on depectinized apple pomace and potato peelings is that further processing steps to reduce the fat content of the BSF-based feed, such as defatting [18], might not be necessary. This may save energy, preserve the nutrients contained in the larvae, and increase their attractiveness for direct feeding in aquaculture, although it may still be necessary to cut the larvae into pieces.

Based on the nutritional parameters examined in the current study, both depectinized apple pomace and potato peelings appear to be very suitable plant by-products for rearing BSF as feed for PWS. Even the cocoa bean shells tested showed a slightly more favorable nutritional profile compared to the control feed.

4.3. Low Growth Performance of BSF Larvae Fed With Plant By-Products. All nutritional profiles of BSF larvae reared on our plant by-products (Section 4.2 and Table 5) were promising in terms of fat and protein contents. That of BSF reared on

cocoa bean shells was even relatively close to that of BSF reared on the grain-based control feed. However, despite these promising nutritional profiles, all growth performance parameters (i.e., larval development time, specific growth rate, and final larval weight) of the BSF reared on plant by-products were significantly inferior to those reared on the control feed (Figure 1). This is particularly evident in the BSF fed depectinized apple pomace and cocoa bean shells, and to a lesser extent in the BSF reared on potato peelings.

These results are broadly consistent with the limited data available in the literature. In a recent study, 12 plant by-products, including apple pomace, were fed to BSF larvae, and their growth performance was recorded [7]. BSF larvae reared on apple pomace showed poor growth rates, resulting in a low final weight (ca. 40 mg). This is in line with our results (final larval weight 27–41 mg; see <https://doi.org/10.22029/jlupub-19060>) and those of other researchers (e.g., 10–30 mg [11], approximately 40 mg [7], and approximately 80 mg [36]).

To the best of our knowledge, there are no experimental data on growth parameters of BSF fed exclusively with cocoa bean shells. However, in a feeding study with cocoa pods [16], BSF larvae also showed decreasing weight gains and final weights with increasing proportions of cocoa pods in the BSF feed.

With regard to potato peelings, we are not aware of any experimental study in which peels of potato (*Solanum tuberosum*) were used as a complete feed for BSF. However, a study using peels of the sweet potato (*Ipomoea batatas*) showed a significantly reduced weight gain and lower final biomass of BSF compared to BSF fed soy bran [37].

The reasons for the reduced growth performance of BSF fed the above mentioned plant by-products are not well understood and may be by-product specific. A review of the health effects of apple pomace [38] concluded that this by-product is generally safe for human consumption. However, the authors raised concerns about the presence of natural toxins and pesticides when used as animal feed. Of particular relevance is the potent insecticide acetamiprid. In addition, the high content of tannins and anti-nutritional phenolic compounds in apple pomace could also have health implications for BSF [11].

As for cocoa beans, a review [20] raised concerns about heavy metal intake and insecticide contamination. The latter may accumulate particularly in the outer part of the cocoa bean—the cocoa bean shell. The authors also stressed the high concentration of polycyclic aromatic hydrocarbons, which may accumulate during the industrial drying of cocoa beans. They are known toxic chemicals that have been shown to slightly increase the development time of BSF larvae [39].

Finally, potatoes, like many other vegetables, often contain high levels of insecticides, which accumulate particularly in the peel [40]. In addition, potato peelings may have high levels of steroidal alkaloids such as α -solanine and α -chaconine, which are natural toxins with insecticidal properties [41].

Besides these properties of specific plant by-products potentially affecting the growth characteristics of BSF larvae, there may also be general nutritional limitations. For example, studies have shown that fruit and vegetable by-products are low in essential amino acids [42]. This may contribute to a prolonged developmental time of BSF larvae reared on plant by-products

[7]. Therefore, supplementation with specific essential amino acids that are lacking in plant by-products may be a key to optimizing plant by-products as feed for BSF larvae and increasing growth performance of BSF.

4.4. No Difference in Growth Performance of PWS Fed With BSF Larvae Reared on Plant By-Products. Our analyses of the nutritional profiles of the BSF larvae reared on plant by-products showed significant differences between these feeds and the grain-based control feed (Section 4.2). In addition, our analyses of the growth performance of BSF larvae reared on these by-products showed significantly delayed developmental processes and reduced final weights (Section 4.3). Therefore, we also expected lower growth performance in our PWS reared on by-product-based combined diets (i.e., 50% conventional fish meal-based compound feed and 50% BSF larvae reared on by-products) compared to our control feed (i.e., 50% conventional fish meal-based compound feed and 50% BSF larvae reared on a grain-based feed).

However, our randomized controlled trial showed no significant differences in growth parameters between the PWS treatment groups or between the treatment and control groups (Figure 2). Survival rates of PWS were greater than 96% for all feeds tested (Figure 2A). In addition, final weights, weight gains, and specific growth rates (Figure 2B–D) were very similar. This suggests that potential nutritional deficiencies or contaminants and toxins of the plant by-products may affect the BSF (Section 4.3), but not the PWS fed these BSF larvae. We can only speculate as to the reasons for these findings. Possibly, BSF larvae do not bioaccumulate some of these contaminants and toxins, but rather degrade them, as recently proposed for polycyclic aromatic hydrocarbons [39] and for the toxic polyphenolic compound gossypol [14]. Furthermore, BSF may partially compensate for the deficiency of some essential amino acids in plant by-products [43]. Accordingly, the authors found that BSF fed different vegetable by-products all had essential amino acid compositions close to the FAO nutritional requirements, at least for humans.

These results may have important economic implications for commercial shrimp aquaculture, as BSF larvae reared on plant by-products may not require defatting or supplementation of essential amino acids to be used as PWS feeds.

4.5. Good Feed Conversion Parameters in PWS Fed With BSF Larvae Reared on Plant By-Products. The results of our feed conversion analyses are given in Table 6 and are rather unexpected. Our experimental feeds based on BSF reared on plant by-products showed excellent FCRs, ranging from 0.8 (BSF fed depunctinized apple pomace) to 1.0 (BSF fed cocoa bean shells). These values were also better than the PWS control group, which had an FCR of 1.4.

In well-managed PWS farms, FCR ranges from 1.3 to 1.5, and in average PWS farms from 1.6 to 1.8. In poorly managed farms, the FCR can be as high as 2.5 (reviewed in [44]). Furthermore, in experimental studies where defatted BSF meal was used to rear PWS, FCR ranged from 1.1 to 1.4 [18, 19]. FCR also depends on the age of the PWS, with young individuals or larvae often having better values, which can be as low as 1.1 [31].

The FCR determined in our study has important implications, as it shows that the low specific growth rates of BSF fed plant by-products (Figure 1 and Section 4.3) may be fully (potato peelings) or partially (cocoa bean shells and depunctinized apple pomace) compensated for by the better FCR of the PWS reared on these BSF. The excellent FCR in our treatment groups may also help to reduce the amount of feed required for BSF-based aquaculture, with potentially positive side effects on water quality and nutrient pollution.

The reasons for these exceptional FCRs are not known to us. However, our empirical observations suggest that the BSF larvae reared on plant by-products provide better feeding stimuli to the PWS than those reared on the control feed. This may be due to the often higher protein and lower fat contents of the BSF reared on plant by-products. In addition, the excellent FCR could also be related to differences in the composition of macro- and micronutrients, which vary depending on the feed and rearing conditions [45].

Our finding of outstanding FCR could also be of economic importance for commercial shrimp farming, as it suggests that some as yet unspecified characteristics of BSF reared on plant by-products could significantly improve feed conversion parameters.

4.6. Limitations and Outlook. In our study, we used BSF-based feeds to investigate nutritional and growth-performance parameters in PWS. Both the treatment and control feeds consisted of 50% fresh BSF larvae. Therefore, our results of similar growth performance and excellent FCR of PWS fed with BSF larvae reared on plant by-products may be primarily applicable to shrimp aquaculture systems, where BSF larvae are fed directly to the PWS.

In large-scale aquaculture facilities, PWS are typically reared on extruded feed pellets, which would require processing of BSF. On the one hand, these processing steps may alter the macro- and micronutritional profiles of the BSF and lead to a reduction in feeding stimuli. On the other hand, previous studies have suggested that processing of BSF larvae (e.g., drying, cuticle removal) may also increase nutrient availability and/or acceptability of the BSF [45].

Therefore, we suggest future studies using pelleted feed with meal of the BSF, which were reared on plant by-products. We also recommend controlled trials aimed at improving the growth parameters of BSF fed with plant by-products, for example by adding essential amino acids. The fact that the nutritional profiles of BSF reared on plant by-products are very promising in terms of protein and fat contents would also be of interest for future research. In particular, the low fat content could make it possible to replace more than 50% of the fish meal-based components in PWS feeds with BSF-based compounds. Finally, the excellent FCR found in PWS fed with BSF reared on plant by-products call for future studies on the drivers of these improved parameters, for example through specific micro- and macronutritional analyses.

5. Conclusions

Our study shows that the nutrient profiles of three plant by-products, selected for their low economic value and high

availability (i.e., cocoa bean shells, depectinized apple pomace, and potato peelings), differ in several parameters, particularly in crude fat and crude protein contents.

The nutritional profiles of BSF larvae reared on these three experimental feeds also differed from each other and from the grain-based control feed, particularly in crude fat, crude protein, and ash contents. Based on these nutritional parameters, both depectinized apple pomace and potato peelings appear to be very suitable plant by-products for rearing BSF as a feed for PWS, possibly eliminating the need for defatting the BSF.

However, all BSF reared on the plant by-products showed significantly lower growth performances than those reared on the control feed. The reasons for this are largely unknown. Though, by-product-specific parameters (e.g., specific toxins and insecticides) and general parameters (e.g., the low content of some essential amino acids in plant by-products) may play a role.

In contrast to the varying growth performances of the BSF, PWS fed with BSF larvae reared on plant by-products or the control feed showed no significant differences in growth parameters. The reasons for the consistent growth performance of the PWS also require future investigation. It is possible that BSF do not bioaccumulate some of the contaminants and toxins contained in the plant by-products. BSF may also partially compensate for the deficiency of some essential amino acids in the plant by-products.

Finally, we found excellent FCR for our PWS fed with BSF larvae reared on plant by-products, well below published values for commercial shrimp farming and experimental studies with BSF. These excellent FCR may fully (potato peelings) or partially (cocoa bean shells and depectinized apple pomace) compensate for the lower growth performance of the BSF reared on these by-products. The reasons for these superior FCR are still unknown, but the high feeding stimuli provided by our experimental feeds and specific micro- and macronutritional profiles may play a role.

Our results could be of great economic importance for future shrimp aquaculture, confirming that BSF may be an important link in the conversion of low-cost plant by-products into high-quality and valuable protein sources for sustainable shrimp farming in a circular economy.

To further facilitate the industrial application of plant by-products for insect and shrimp production, we recommend several future research directions using BSF reared on plant by-products: (i) studies with BSF meal, (ii) studies to improve growth parameters in BSF, (iii) studies with BSF replacement rates in shrimp feeds >50%, and (iv) studies to decipher the factors responsible for the excellent FCR in PWS.

Data Availability Statement

The data that support the findings of this study are openly available in JLUpub at <https://doi.org/10.22029/jlupub-19060>, reference number 19703.

Disclosure

The funder had no role in the study design, data collection and interpretation, or the decision to submit the work for publication.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Annalena Barth, Patrick Schubert, Thomas Wilke, Patrick Klüber, and Dorothee Tegtmeier designed the study. Annalena Barth, Patrick Klüber, and Daniel Kreft performed the experiments with assistance from Slim Bendag and Patrick Schubert. Annalena Barth and Patrick Klüber collected and analyzed the data and prepared drafts of the figures and tables. Thomas Wilke and Annalena Barth drafted the manuscript. All authors contributed to writing, reviewing, and editing of the final manuscript.

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3 Appendix

3.1 Supplementary tables of the 2nd Paper

Table S1: GenBank accession numbers and haplotype information for the cytochrome c oxidase subunit I (COI) sequences used for the haplotype network analyses of the black soldier fly (BSF) and the Pacific white shrimp (PWS). GenBank accession numbers for the specimens used in the current feeding study are in **bold**; sequences from cultured specimens are in italics. Note that many literature studies or GenBank data entries do not provide information on the origin of the specimens (wild caught vs. cultured). Therefore, the number of haplotypes found in cultured PWS may be higher.

Black soldier fly (BSF)			Pacific white shrimp (PWS)		
Haplotype #	GenBank accession #	Country of origin	Haplotype #	GenBank accession #	Country of origin
1	<i>PQ187607</i>	Germany	1	<i>PQ187640</i>	Austria
	<i>PQ187608</i>	Germany		<i>PQ187641</i>	Austria
	<i>GQ465783</i>	unknown		<i>JN165700</i>	India?
	<i>LR792262</i>	Switzerland		<i>JN165701</i>	India?
	<i>LR812715</i>	Switzerland		<i>JN165702</i>	India?
	<i>MT178496</i>	Italy		<i>JN165703</i>	India?
	<i>MT178512</i>	Poland		<i>JN165704</i>	India?
	<i>MT520675</i>	Uganda		<i>KJ879293</i>	Egypt
	<i>MT520676</i>	Uganda		<i>KJ879294</i>	Egypt
	<i>MT520677</i>	Uganda		<i>KJ879295</i>	Egypt
	<i>MT520678</i>	Uganda		<i>KJ879314</i>	Egypt
	<i>MT520679</i>	Uganda		<i>KJ879315</i>	Egypt
	<i>MT520680</i>	Uganda		<i>KJ879316</i>	Egypt
	<i>OP164686</i>	Vietnam		<i>KP976242</i>	China
	<i>OP164687</i>	Vietnam		<i>KP976244</i>	China
	<i>OP164689</i>	Vietnam		<i>KP976245</i>	China
	<i>OP164692</i>	Vietnam		<i>KP976246</i>	China
	<i>OP164696</i>	Vietnam		<i>KP976247</i>	China
	<i>OP164697</i>	Vietnam		<i>KY018725</i>	unknown
	2	<i>LR778162</i>		Thailand	<i>KY018738</i>
<i>LR792242</i>		Thailand	<i>KY018739</i>	unknown	
3	<i>OP164695</i>	Vietnam	<i>KY018741</i>	unknown	
4	<i>LR792261</i>	Switzerland	<i>KY018742</i>	unknown	
	<i>LR812714</i>	Switzerland	<i>KY018745</i>	unknown	
5	<i>HM381282</i>	USA	<i>KY018750</i>	unknown	
	<i>KY679159</i>	unknown	<i>KY018752</i>	unknown	
	<i>KY817115</i>	Russia	<i>KY018791</i>	unknown	
	<i>LR778157</i>	Kenya	<i>KY018793</i>	unknown	
	<i>LR778160</i>	Bhutan	<i>KY018795</i>	unknown	
	<i>LR792227</i>	Australia	<i>KY018802</i>	unknown	
	<i>LR792258</i>	Kenya	<i>KY018828</i>	unknown	
	<i>LR792259</i>	Kenya	<i>KY018872</i>	unknown	
	<i>LR792260</i>	Spain	<i>KY018873</i>	unknown	
	<i>LR792263</i>	South Africa	<i>KY018874</i>	unknown	
	<i>LR792264</i>	South Africa	<i>KY018875</i>	unknown	
	<i>LR792265</i>	South Africa	<i>KY018881</i>	unknown	
	<i>LR792266</i>	South Africa	<i>KY018882</i>	unknown	
	<i>LR792267</i>	USA	<i>KY018883</i>	unknown	
	<i>LR812711</i>	Kenya	<i>KY018898</i>	unknown	
	<i>LR812712</i>	Kenya	<i>KY018910</i>	unknown	
	<i>LR812713</i>	Spain	<i>KY018913</i>	unknown	
	<i>LR812716</i>	South Africa	<i>KY656473</i>	USA	
	<i>LR812717</i>	South Africa	<i>LC121765</i>	Japan	
	<i>LR812718</i>	South Africa	<i>MF752470</i>	India	
<i>LR812719</i>	South Africa	<i>MF752471</i>	India		
<i>LR812720</i>	USA	<i>MH194437</i>	Peru		
<i>MN868749</i>	Portugal	<i>MH194528</i>	Peru		
<i>MN868766</i>	Portugal	<i>MK000245</i>	unknown		

MN868787	Portugal		MK301236	unknown
MT079205	Bangladesh		MK301239	unknown
MT178467	Bhutan		<i>MK792401</i>	India
MT178481	Australia		MN863506	India
MT178483	China		MT072759	USA
<i>MT178490</i>	USA		MT072760	USA
<i>MT178493</i>	France		MT072763	USA
<i>MT178494</i>	France		MT072764	USA
<i>MT178495</i>	France		MT072765	USA
<i>MT178497</i>	Indonesia		MT072767	USA
<i>MT178498</i>	China		MT072771	USA
<i>MT178503</i>	Vietnam		MT072772	USA
MT178507	South Africa		MT072774	USA
<i>MT178511</i>	China		MT072777	USA
MT181121	South Africa		MT072778	USA
MT433999	Malaysia		MT072779	USA
MT483914	China		MT072780	USA
MT483915	China		MT072781	USA
MT483916	China		MT072782	USA
MT483917	China		MT072783	USA
MT483920	Australia		MT072784	USA
MT483921	Australia		MT072786	USA
MT483922	Australia		MT607566	Peru
MT483923	Australia		MT607568	Peru
MT483924	Australia		MT607592	Peru
MT483925	Australia		MW027142	unknown
MT483932	Netherlands		MW027158	unknown
MT483933	Netherlands		MW916386	unknown
MT483934	Netherlands	2	MT072766	USA
MT483935	Netherlands	3	KY018915	unknown
MT483936	Netherlands		MF490133	Brazil
MT483937	Kenya		MG001172	USA
MT483938	Kenya		MT072775	USA
MT483939	Kenya		MT072776	USA
MT483940	Kenya		MT607524	Peru
MT483941	Kenya		ON263409	Indonesia
MT483942	Kenya	4	JN165705	unknown
MT520651	South Africa	5	OM672408	Brazil
MT520652	South Africa	6	<i>KJ879297</i>	Egypt
MT520653	South Africa	7	DQ534543	unknown
MT520654	South Africa		HQ700931	unknown
MT520655	South Africa	8	AY781297	unknown
MT520656	South Africa		KJ679914	unknown
MT520671	USA		KJ679916	unknown
MT520672	USA		<i>KJ879296</i>	Egypt
MT520673	USA		<i>KJ879313</i>	Egypt
MT520674	USA		KP976243	China
MT520681	USA		KP976248	China
MT520682	USA		KP976249	China
MT520683	USA		KP976250	China
MT520684	USA		KU324658	Egypt
MT520685	USA		<i>KY018748</i>	unknown
MT520686	USA		<i>KY018751</i>	unknown
MW173681	India		<i>KY018911</i>	unknown
MW278893	India?		MH300665	unknown
MW278894	India?		MK301237	unknown
MZ148804	India		MK301238	unknown
MZ148805	India		MT072769	USA
MZ148806	India		MT072770	USA
NC_035232	unknown	9	KJ679911	unknown
OP537078	unknown		KJ679912	unknown
OP537079	unknown		KJ679913	unknown

6	ON738663	India		KJ679915	unknown
7	MG682545	India	10	MT072785	USA
8	HM433911	USA	11	MT072768	USA
	LR792229	USA	12	KF418241	unknown
	MT178459	USA	13	KJ679907	unknown
	MT178460	USA		KJ679909	unknown
	MT178461	USA	14	KJ679906	unknown
	<i>MT178462</i>	USA		KJ679908	unknown
	<i>MT178463</i>	USA		KJ679910	unknown
	MT178470	USA		KY564433	India
	MT178492	USA			
	MT181122	USA			
9	MT178491	USA			
10	MT178458	USA			
	MT178488	USA			
	MT178489	USA			
	MT181123	USA			
11	HM399363	Australia			
	KM928149	Canada			
	KM967419	Canada			
	LR778159	Bhutan			
	LR778161	Thailand			
	LR792226	USA			
	<i>LR7922254</i>	Australia			
	<i>LR7922256</i>	Australia			
	LR812707	Australia			
	LR812709	Australia			
	MT178464	Australia			
	MT178465	Australia			
	MT178466	Bhutan			
	MT178474	Thailand			
	MT178478	Samoa			
	MT178485	Singapore			
	<i>MT178487</i>	Singapore			
	MT178504	Thailand			
	MT178505	Thailand			
	<i>MT178506</i>	Thailand			
	MT178508	Australia			
	MT178509	Singapore			
	MT186669	Singapore			
	MT520687	Thailand			
	MT908920	Malaysia			
	MT908967	Malaysia			
	<i>OP164688</i>	Vietnam			
	<i>OP164690</i>	Vietnam			
	OP164691	Vietnam			
	OP164693	Vietnam			
	<i>OP164694</i>	Vietnam			
12	<i>MT178502</i>	Malaysia			
13	MT520663	Thailand			
	MT520664	Thailand			
	MT520665	Thailand			
	MT520666	Thailand			
14	LR792233	Panama			
	MT178473	Panama			
15	MT483918	Costa Rica			
16	LR778200	Bolivia			
17	ON783031	Ecuador			
	ON783032	Ecuador			
	ON783033	Ecuador			
	ON783034	Ecuador			
	ON783035	Ecuador			

	ON783036	Ecuador
	ON783038	Ecuador
	ON783039	Ecuador
18	LR792234	French Guiana
	MT178477	Guyana
19	LR778199	Bolivia
20	LR792231	Peru
	MT178471	Peru
21	LR778202	Bolivia
22	JN308280	Papua New Guinea
	JN308281	Papua New Guinea
	JN308282	Papua New Guinea
	JN308283	Papua New Guinea
	JN308284	Papua New Guinea
	JN308285	Papua New Guinea
	JN308286	Papua New Guinea
	JN308287	Papua New Guinea
	JN869986	Malaysia
	KX053393	French Polynesia
	KX053394	French Polynesia
	LR585074	unknown
	LR778154	Madagascar
	LR778155	Madagascar
	LR778156	Zambia
	LR778195	Bolivia
	LR778207	Switzerland
	LR792223	Malaysia
	LR792224	Vietnam
	LR792225	Australia
	LR792253	Australia
	LR792255	Australia
	LR792257	Australia
	LR812706	Australia
	LR812708	Australia
	LR812710	Australia
	MG733996	India
	MT151288	Réunion
	MT178475	Madagascar
	MT178479	Malaysia
	MT178480	Vietnam
	MT178484	Singapore
	MT178486	Singapore
	MT178499	Australia
	MT178500	Australia
	MT178501	Singapore
	MT178510	Australia
	MT520667	USA
	MT520668	USA
	MT520669	USA
	MT520670	USA
	MW881502	India
	OP218410	Indonesia
	OP218413	Indonesia
	OP218414	Indonesia
23	OM909154	India
24	LR778204	Colombia
25	LR792239	Paraguay
26	LR778193	Bolivia
	LR792230	Peru
	LR792232	Peru
	LR792236	Brazil
	LR792241	Paraguay

	MT178469	Peru
	MT178472	Peru
27	LR778158	Ghana
	MN868751	Portugal
	MT151287	Benin
	MT483926	Ghana
	MT483927	Ghana
	MT483928	Ghana
	MT483929	Ghana
	MT483930	Ghana
	MT483931	Ghana
	MT520657	Nigeria
	MT520658	Nigeria
	MT520659	Nigeria
	MT520660	Nigeria
	MT520661	Nigeria
	MT520662	Nigeria
28	LR778213	Peru
29	LR778194	Bolivia
	LR792238	Bolivia
	LR792240	Paraguay
	MT178476	Bolivia
30	LR778201	Bolivia
31	LR778203	Bolivia
32	LR778196	Bolivia
33	LR778206	Brazil
34	LR778212	Peru
35	LR778197	Bolivia
36	LR778198	Bolivia
37	LR778205	Brazil
	LR792235	Brazil
	LR792237	Brazil
38	LR778192	Bolivia
39	ON783037	Ecuador
40	LR778208	Mexico
41	LR792228	USA
	MT178468	USA
42	LR778210	Venezuela
43	LR778209	Venezuela
44	LR778211	Venezuela
	MT178482	Venezuela

Table S2: Effects of fishmeal- and black soldier fly (BSF)-based feeds on amino acid contents in % dry matter (mean values \pm standard deviations) of individual Pacific white shrimp (PWS) and statistical differences between treatments (DM = dry matter).

Amino acid	Amino acid content of PWS (% DM)		Statistical differences
	PWS control group	PWS treatment group	
	(Fishmeal-based feed) (n = 8)	(BSF-based feed) (n = 8)	
Alanine	0.65 \pm 0.07	0.56 \pm 0.03	p = 0.004
Arginine	0.79 \pm 0.05	0.76 \pm 0.05	p = 0.281
Aspartic acid	1.13 \pm 0.19	1.13 \pm 0.07	p = 0.336
Cysteine	0.13 \pm 0.01	0.12 \pm 0.01	p = 0.574
Glutamic acid	1.73 \pm 0.08	1.63 \pm 0.11	p = 0.121
Glycine	0.74 \pm 0.05	0.71 \pm 0.04	p = 0.336
Histidine	0.88 \pm 0.07	0.77 \pm 0.08	p = 0.014
Isoleucine	0.33 \pm 0.03	0.33 \pm 0.03	p = 0.779
Leucine	0.71 \pm 0.03	0.66 \pm 0.05	p = 0.054
Lysine	0.86 \pm 0.05	0.81 \pm 0.05	p = 0.152
Methionine	0.16 \pm 0.01	0.15 \pm 0.01	p = 0.442
Phenylalanine	0.40 \pm 0.02	0.38 \pm 0.03	p = 0.336
Proline	0.58 \pm 0.03	0.55 \pm 0.04	p = 0.121
Serine	0.45 \pm 0.03	0.41 \pm 0.02	p = 0.014
Threonine	0.41 \pm 0.03	0.39 \pm 0.03	p = 0.281
Tryptophan	0.07 \pm 0.03	0.08 \pm 0.01	p = 0.397
Tyrosine	0.28 \pm 0.01	0.25 \pm 0.02	p = 0.009
Valine	0.47 \pm 0.03	0.48 \pm 0.04	p = 1.000

Table S3: Effects of fishmeal- and black soldier fly (BSF)-based feeds on life-history and physiological traits (mean values \pm standard deviations) of Pacific white shrimp (PWS) and statistical differences between treatments (DM = dry matter). Statistical comparisons are based on the data from the 4 control and 4 treatment tanks.

Parameter	Life-history and physiological traits of PWS		Statistical differences
	PWS control group (Fishmeal-based feed)	PWS treatment group (BSF-based feed)	
Growth parameters			
Initial weight (g)	0.37 \pm 0.16		N/A
Final weight (g)	8.01 \pm 1.13	6.78 \pm 0.88	p < 0.001
Weight gain (%)	2050 \pm 302	1720 \pm 236	p < 0.001
Specific growth rate (% per day)	7.28 \pm 0.33	6.89 \pm 0.30	p < 0.001
Length (cm)	10.83 \pm 0.76	10.12 \pm 0.85	p = 0.124
Survival rate (%)	80.0 \pm 12.0	85.0 \pm 4.0	p = 0.513
Production index (g)	6.20 \pm 0.94	5.62 \pm 0.33	p = 0.375
Crude protein (% DM)	69.59 \pm 0.71	67.7 \pm 0.72	p < 0.01
Crude fat (% DM)	5.58 \pm 0.78	6.97 \pm 0.39	p < 0.01

3.2 Additional publications

Wilke, T.; Bendag, S.; **Barth, A.**; Reinold, T.; Schubert, P. (2025). Individual Shrimp Rearing Increases the Power of Experimental Trials. *Aquaculture Journal*, 5, 2.
<https://doi.org/10.3390/aquacj5010002>

Bendag, S.; **Barth, A.**; Nitzsche, J.; Schubert, P.; Wilke, T. (2026). Wood as alternative tank construction material in sustainable shrimp aquaculture. *Aquaculture Research*, accepted for publication

3.3 Conference contributions

Barth, A.; Schubert, P., Tschirner, M. & Wilke, T. (2020): Sustainable insect feed for a closed shrimp aquaculture. ICMS International Conference on Marine Science: tropical ocean for the future 2020, Colombia online, Poster.

Barth, A.; Tschirner, M. & Wilke, T. (2021): Effects of different protein sources on the nutrient profile and growth of whiteleg shrimp (*Litopenaeus vannamei*). Aquaculture Europe 2020: AE2020 ONLINE, online, Poster.

Barth, A.; Tschirner, M., & Wilke, T. (2021): Effects of different protein sources on the nutrient profile and growth of whiteleg shrimp (*Litopenaeus vannamei*). 14th GGL Annual Conference, Poster.

Barth A., Bendag S., Tschirner M., Schubert P., & Wilke T. (2022): Innovative Health Index for Shrimps. 15th GGL Annual Conference, Poster.

Barth A., Bendag S., Tschirner M., Schubert P., & Wilke T. (2023): Black soldier fly as a sustainable shrimp feed. ICMS International Conference on Marine Science: Working today for the oceans of tomorrow, Poster.

Barth A., Bendag S., Schubert P., & Wilke T. (2023): Black soldier fly as sustainable aquafeed for whiteleg shrimp. 16th GGL Annual Conference, Talk.

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5 Affidavit

“I declare that I have completed this dissertation single-handedly without the unauthorized help of a second party and only with the assistance acknowledged therein. I have appropriately acknowledged and cited all text passages that are derived verbatim from or are based on the content of published work of others, and all information relating to verbal communications. I consent to the use of an anti-plagiarism software to check my thesis. I have abided by the principles of good scientific conduct laid down in the charter of the Justus Liebig University Giessen „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ in carrying out the investigations described in the dissertation.”

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Gießen, 06.02.2026

Annalena Barth (Authorin | *author*)