Sustainable Diets for Shrimp Aquaculture Utilizing Local By-Products

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Dissertation

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Sustainable Diets for Shrimp Aquaculture Utilizing Local By-Products

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Photograph of the Pacific Whiteleg shrimp on the title page was kindly provided by Mirko Bögner.

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Summary

The global food system, including the aquaculture industry, is challenging the planetary boundaries. Aquaculture has become the fastest-growing food sector in the world. To meet the demand of aquatic protein for a growing human population by 2050, aquaculture production levels will need to increase by more than 50%. Most farmed species depend on external feed supply, but feed drives the majority of the environmental footprint of this sector. Additionally, many of the aquafeed ingredients directly or indirectly compete with human food. Therefore, utilization of by-products as aquafeed ingredients can help to reduce waste, recycle nutrients, add value, and promote circularity of the aquaculture sector.

The objective of this thesis was to identify, characterize, and test by-products as aquafeed ingredients for the high-value Pacific Whiteleg shrimp, *Litopenaeus vannamei*. Under-utilized by-products originating from the three different sectors seafood processing, insect farming, and the cosmetic industry were identified and tested. These include processing remains of the brown shrimp, *Crangon crangon*, (BSPR); exuviae (shed exoskeletons) of growing larvae, cocoons, and dead adult flies (imagines) of the black soldier fly, *Hermetia illucens*; and algae pomace, which remains after aqueous extraction of marine macroalgae, *Saccharina latissima* used to produce skincare products.

Analysis of the chemical composition of these materials revealed high protein contents with favorable amino acid profiles in BSPR and imagines, supporting their potential as a dietary protein source. High levels of chitin and microminerals in cocoons, and potentially bioactive substances in macroalgae suggest their use as feed additives to enhance health and overall performance of *L. vannamei*. Controlled feeding trials were conducted in clear water recirculating aquaculture systems to examine the bioavailability of key nutrients, as well as their effects on growth parameters and health.

Initial experiments with BSPR showed improved growth of shrimp without adverse effects on health when 50% of the conventional fish meal was replaced in the diet. A second, more elaborated controlled feeding experiment revealed that BSPR enhances shrimp growth at a fish meal replacement level of 80%, while concomitantly improving the feed efficiency. Examination of physiological and health parameters (digestive enzymes, phenoloxidase activities, hemolymph parameters) failed to elucidate the underlying mechanism for the enhanced growth performance of shrimp.

The inclusion of defatted meal made from the adult black soldier fly resulted in poor shrimp growth at all tested dietary inclusion levels. Furthermore, diets containing imagines were less attractive for shrimp, resulting in a reduced feed intake. Significant changes in the chemical composition of the muscle and midgut gland of shrimp further indicated malnutrition induced by the replacement of fish meal with imagines.

The suitability of cocoons and algae pomace as feed additives was tested at supplementation levels ranging from 1 to 5% in low fish meal containing diets. Both ingredients enhanced shrimp survival at the highest inclusion level in a controlled feeding trial conducted in a clear water recirculating aquaculture system. However, supplementation of algae pomace resulted in decreased growth performance. Health indices, including the phenoloxidase system and hemolymph parameters, did not show a significant response to the experimental diet treatment. To further validate the suitability of cocoons and algae pomace as feed additives, controlled feeding experiments were conducted in biofloc systems with specifically formulated diets. Dietary effects on gut bacteria counts, hemolymph parameters, and resilience of shrimp to cold temperatures were examined. In the biofloc system experiment, growth was impaired in both diet treatments containing cocoons and algae pomace and no effect on survival was observed. Furthermore, no differences between treatments were detected in any of the analyzed parameters. The potential of cocoons and algae pomace as feed additives thus appears to be limited and likely depends on the rearing system, and applied feed formulation.

To holistically consider the potential of these by-product-based feed ingredients, a variety of factors were evaluated and classified as strengths, weaknesses, opportunities, and threats. This thorough assessment revealed that BSPR originating from a traditional and well-established fishery is characterized by an exceptional nutritional value and a high potential to serve as proteinaceous aquafeed ingredient for shrimp in the current situation. At present, the amount of BSPR that is annually generated from the North Sea regions could cover the entire protein demand required by European shrimp aquaculture. However, limited scalability, as well as potential environmental regulatory constraints restrict future opportunities for this resource. On the contrary, the by-products originating from the novel insect, and cosmetic seaweed industries have a low nutritive value for shrimp, which is the main weaknesses of these resources. Yet, methods exist to improve their suitability as aquafeed ingredients that warrant examination and further development. With the expected growth of these sectors, both of which are capable of addressing many environmental and societal challenges, the role of their by-products as aquafeed ingredient could become more relevant in future.

Zusammenfassung

Das globale System der Lebensmittelproduktion, einschließlich der Aquakultur, belastet massiv die planetaren Grenzen. Die Aquakultur ist der am schnellsten wachsende Lebensmittelsektor der Welt. Um den Bedarf an aquatischem Protein für die wachsende Weltbevölkerung zu decken, müssen die Produktionsmengen der Aquakultur bis 2050 um mehr als 50% steigen. Die meisten Aquakultur-Arten sind auf externe Futtermittel angewiesen. Futtermittel verursachen jedoch den Großteil des ökologischen Fußabdrucks des Sektors. Des Weiteren konkurrieren viele Futtermittelrohstoffe direkt oder indirekt mit menschlichen Lebensmitteln. Die Nutzung von Reststoffen als Rohstoff für Aquakulturfuttermittel kann zu einer Reduzierung von biogenem Abfall, effizienter Nutzung von Nährstoffen, einem erhöhten Mehrwert, sowie zur Entwicklung einer zirkulären Aquakultur beitragen.

Das Ziel dieser Arbeit ist, Reststoffe zu identifizieren und zu charakterisieren, sowie deren Eignung als Futtermittelrohstoff für die Pazifische Weißfußgarnele, *Litopenaeus vannamei*, zu testen. Ungenutzte Reststoffe wurden hierbei aus der Fischerei, der Insektenzucht und der Kosmetikindustrie identifiziert und evaluiert. Im Einzelnen sind das Krabbenpulreste der Nordseegarnele *Crangon crangon;* Häutungen von wachsenden Larven (Exuvien), deren Kokons, und das adulte Stadium (Imago) der Schwarzen Soldatenfliege *Hermetia illucens*; und Algentrester, ein Reststoff der nach wässriger Extraktion der Makroalge *Saccharina latissima* zur Herstellung von Hautpflegeprodukten zurückbleibt.

Die chemische Zusammensetzung dieser Reststoffe zeigten hohe Proteingehalte mit ausgewogenen Aminosäureprofilen in Krabbenpulresten und Insekten-Imagines, was ihre potenzielle Eignung als Proteinquelle nahelegt. Aufgrund des hohen Gehalts an Chitin und Mineralien in den Kokons sowie der potenziellen bioaktiven Substanzen im Algentrester wurden diese Reststoffe als Futtermittelzusatz getestet, um die Gesundheit und das Wachstum von *L. vannamei* zu fördern. Kontrollierte Fütterungsversuche wurden in Klarwasser-Kreislaufanlagen durchgeführt, um die Bioverfügbarkeit von Schlüsselnährstoffen sowie deren Auswirkungen auf Wachstumsparameter und Gesundheit der Garnelen zu evaluieren.

Erste Fütterungsexperimente zeigten ein verbessertes Wachstum von *L. vannamei* bei einer 50%igen Substitution des Fischmehlanteils im Futter mit Krabbenpulresten. Es wurden keine negativen gesundheitliche Auswirkungen auf die Garnelen festgestellt. Ein zweiter umfassender Versuch zeigte ein erhöhtes Garnelenwachstum bei gleichzeitig verbesserter Futterkonversion, wenn 80% des konventionellen Fischmehls durch Krabbenpulreste ersetzt wurde. Die Untersuchung verschiedener physiologischer Parameter (Verdauungsenzymatik, Phenoloxidase-Aktivitäten, Hämolymphparameter) konnte nicht dazu beitragen, die Ursache für die erhöhte Wachstumsleistung näher aufzuklären.

Der Einsatz von entfettetem Mehl aus adulten Schwarzen Soldatenfliegen (Imagines) führte bei allen getesteten Anteilen im Futter zu einem verschlechterten Garnelenwachstum. Futtermittel die Imagines enthielten, waren weniger attraktiv für Garnelen und führten zu einer reduzierten Futteraufnahme. Signifikante Veränderungen in der chemischen Zusammensetzung des Muskelgewebes sowie der Mitteldarmdrüse der Garnelen deuteten auf eine durch Imagines verursachte Mangelernährung hin.

Kokons der Schwarzen Soldatenfliege und Algentrester wurden als Futtermittelzusätze von 1 bis 5% in Futtermitteln mit hohen pflanzlichen Anteilen getestet. Beide Reststoffe verbesserten in der höchsten Konzentration die Überlebensrate der Garnelen in einem Klarwasser-Kreislaufsystem. Die Supplementierung von Algentrester führte jedoch zu geringerem Wachstum der Garnelen. Des Weiteren konnte kein Zusammenhang zwischen der experimentellen Futterbehandlungen und den untersuchten Immun-, und Gesundheitsparametern festgestellt werden. Um die Eignung der Kokons und des Algentresters als Futtermittelzusatzstoff zu validieren, wurde ein weiterer Versuch in Biofloc Systemen durchgeführt mit speziell dafür ausgelegten Futtermitteln. Die Auswirkungen der Futterbehandlung auf die Anzahl und Zusammensetzung der Darmbakterien, Hämolymphparameter sowie die Resilienz der Garnelen gegen niedrige Temperaturen wurden untersucht. In den Biofloc-Systemen wurde das Wachstum der Garnelen durch den Zusatz von Kokons sowie des Algentresters signifikant reduziert und es wurde kein Einfluss auf die Mortalität der Garnelen beobachtet. Die experimentellen Futtermittel zeigten ebenfalls keinen Effekt auf die o.g. Parameter. Das Potenzial von Kokons und Algentrester als Futtermittelzusatzstoffe ist somit begrenzt und wird wahrscheinlich vom Hälterungssystem sowie der angewandten Futtermittelformulierung beeinflusst.

Um das Potenzial der untersuchten Reststoffe als Aquakulturfuttermittel holistisch zu betrachten, wurden verschiedene Faktoren evaluiert und in Stärken, Schwächen, Möglichkeiten und Risiken klassifiziert. Diese ganzheitliche Bewertung verdeutlichte, dass sich Krabbenpulreste aus einer traditionellen und etablierten Fischerei durch einen exzellenten Nährwert und hohes Potenzial als Proteinquelle für *L. vannamei* auszeichnen. Der derzeitige Proteinbedarf der gesamten europäischen Garnelenaquakultur könnte durch die Krabbenpulreste aus der Nordseeregion gedeckt werden. Eine limitierte Skalierbarkeit, sowie umweltrechtliche Einschränkungen könnten jedoch die zukünftige Verfügbarkeit dieses Reststoffes begrenzen. Im Gegensatz dazu bestehen die Hauptschwächen der Reststoffe aus der Insektenzucht und der algenbasierten Kosmetikindustrie in den geringen Nährwerten und den damit verbundenen negativen Auswirkungen auf das Wachstum der Garnelen. Zusätzliche Verarbeitungsmethoden, welche die Nährwerte und somit die Eignung als Futtermittelrohstoffe verbessern könnten, sind möglicherweise anwendbar. Mit dem prognostizierten Wachstum der Insektenzucht und der Nutzung von Meeresalgen z.B. in der Kosmetikindustrie, könnte die Relevanz dieser Reststoffe als Aquakulturfuttermittel in Zukunft steigen und somit auch zur Bewältigung ökologischer und sozialer Herausforderungen beitragen.

List of abbreviations

AA	amino acid
ADC	apparent digestibility coefficient
AP	algae pomace
ARA	arachnoid acid
B2B	business-to-business
BSF	black soldier fly
BSFLM	black soldier fly larvae meal
BSPR	brown shrimp processing remains
CF	correction factor
CFU	colony forming unit
DFI	daily feed intake
DHA	docosahexaenoic acid
DMR	daily molting ratio
EAA	essential amino acid
EAAI	essential amino acid index
EPA	eicosapentaenoic acid
FAO	Food and Agriculture Organization of the United Nations
FCR	feed conversion ratio
FM	fish meal
FO	fish oil
GHG	greenhouse gases
GMO	genetically modified organism
HSI	hepatosomatic index
IM	imago meal
IMTA	integrated multitrophic aquaculture
LC-PUFA	long-chained polyunsaturated fatty acid
MUFA	monounsaturated fatty acid
ndl-PCB	non-dioxin-like polychlorinated biphenyls
NSP	non-starch polysaccharides
PAH	polycyclic aromatic hydrocarbon
PAP	processed animal protein
PCB	polychlorinated biphenyls
PO	phenoloxidase
proPO	prophenoloxidase
PUFA	polyunsaturated fatty acid

RAS	recirculating aquaculture system
RNS	reactive nitrogen species
ROS	reactive oxygen species
sd/SD	standard deviation
SDG	sustainable development goal
SFA	saturated fatty acid
SWOT	strength-weakness-opportunity-threat
TAG	triacylglycerine
THC	total hemocyte count
TRL	technology readiness level
WG	weight gain

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Chapter 1 General Introduction

1.1 Feeding a World of 10 Billion People

1.1.1 The Global Food System in the Anthropocene

Modern human societies have evolved over the past 10 000 years during a relative stable geological period referred to as the Holocene (Manning, 2015). They developed productive agriculture, built large settlements, and established efficient economies, which form the basis of human collaboration. Keystone developments, such as the industrial revolution starting in the second half of the 18th century, supported steady growth of the human population and drove technological innovations. The second half of the 20th century is unique in the history of humankind and marks the beginning of a new age (Steffen et al., 2015). Over the past 70 years, human activities skyrocketed in many areas including population, economy, transportation, and communication. This explosion of human activities is heavily impacting the earth´s biogeophysical features. Since the second half of the 20th century, rapidly increasing levels of various parameters have been observed, including atmospheric carbon dioxide, surface temperatures, and biosphere degradation (Figure 1.1). This situation has encouraged the scientific community to declare that we have transitioned into a new geological era that is mainly influenced by humans: the Anthropocene (Crutzen, 2006; Zalasiewicz et al., 2017).

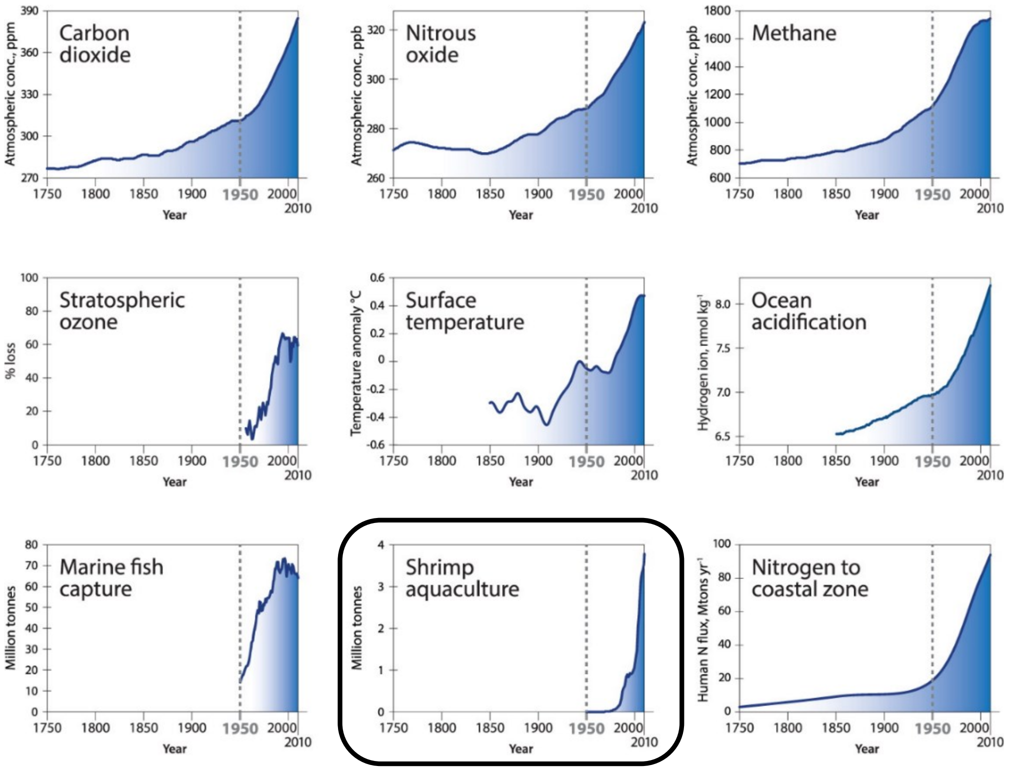

Figure 1.1 Trends from 1750 until 2010 of relevant indicators for the structure and functioning of the Earth system. The framed graphic highlights the massive expansion of the shrimp industry. Dashed lines at 1950 marks the beginning of the Anthropocene. From Steffen et al. (2015) with permission granted by SAGE Publications.

The framework of planetary boundaries refers to processes that are essential to maintain the stability and resilience of the Earth´s system (Rockström et al., 2009). The Holocene was hereby chosen as a reference because it is the only known period on Earth that provides suitable conditions for billions of people. Anthropogenic activities that drive pollution, biodiversity loss, and climate change increasingly perturbate the planetary system. The safe operating space hereby refers to the amount of human perturbations that the system can compensate without changing into "Hothouse Earth" conditions (Steffen, 2022). Exceeding certain thresholds could thus result in disastrous consequences for humanity and all life on Earth. Currently, six of nine identified planetary boundaries are increasingly at risk of being transgressed, including biosphere integrity, biogeochemical flows, land system-, and climate change (Figure 2.2) (Richardson et al., 2023). All of these boundaries are heavily affected by the global food system (Springmann et al., 2018).

Global biogeochemical flows are profoundly influenced by the extensive use of fertilizers and nutrient discharges from agriculture and other food production activities. Conijn et al. (2018) estimated that in 2010 the total nitrogen and phosphorus inputs into the global food system were 100 Mt and 20 Mt, respectively. Half of the nitrogen and 85% of the phosphorus originate from industrial and mineral sources, thereby being newly introduced into the global biogeochemical nutrient cycles. Global food production also consumes large amounts of freshwater. The increasing exploitation and depletion of natural groundwater resources can impair the natural hydrological cycle and related ecosystems (Wada et al., 2010). By 2050, the planetary boundary for water use may exceed by 50% if methods for food production remain unchanged (Springmann et al., 2018). The large demand for arable land leads to clearances of forests and other valuable terrestrial ecosystems that are essential for carbon fixation and biodiversity. These land-use changes are a major cause of the total greenhouse gas (GHG) emissions from the food system, which, in turn, is responsible for one-third of all anthropogenic emissions (Crippa et al., 2021). If the food production system continues operating on a business-as-usual basis, the food system alone could cause a global temperature increase of 1°C by 2100 (Ivanovich et al., 2023).

Should the current food production remain within planetary boundaries, only approximately 40% of the Earth's population could be nourished (Gerten et al., 2020). The global human population is projected to reach 10 billion by the middle of the century. Along with the economic growth of the emerging world, the consumption of resource-intensive animal products may continue to rise (Fróna et al., 2019; Kearney, 2010). Only a transition to more sustainable production and consumption patterns could cover the human nutritional needs by 2050 within the limits of biosphere integrity, land-system change, freshwater use, and nitrogen flows (Gerten et al., 2020).

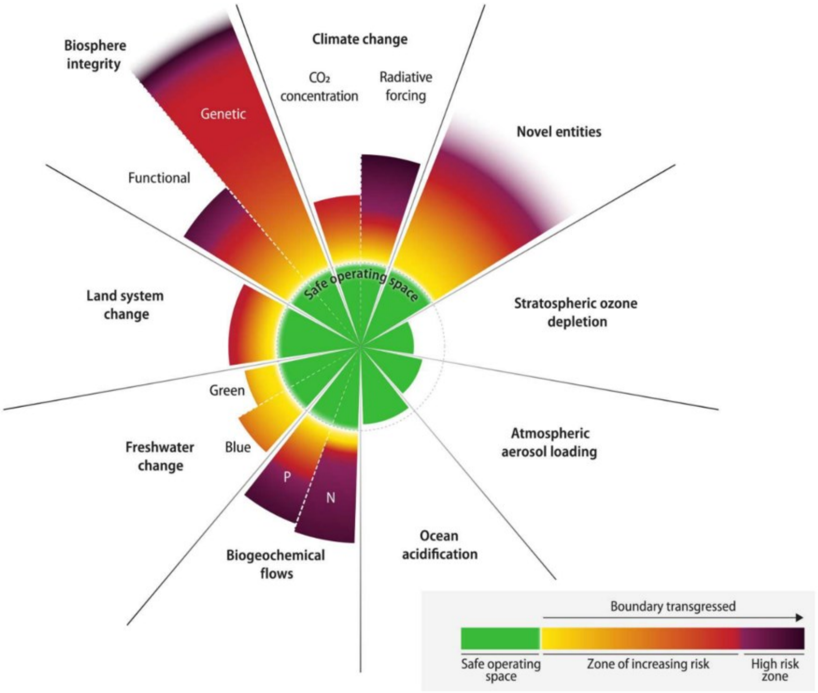

Figure 1.2. Current status of all nine planetary boundaries. The green zone is defined as the safe operating space, whereas the color change from yellow to dark red indicate increasing risk of boundary transgression. Six planetary boundaries are transgressed, which are all affected by the global food production system. From Richardson et al. (2023) with permission from Science Advances under the Creative Commons Non-Commercial license.

The pressing challenge of providing sufficient food for the growing population without exceeding the planetary boundaries has become a core element of international political agendas. The Sustainable Development Goals (SDG) formulated by the United Nations aim to ensure food security by reducing waste and improving resource efficiency (UN General Assembly, 2015). The European green deal calls for protecting the climate and biodiversity by developing a new growth model based on a clean and circular economy (European Commission, 2020a). The "Farm to Fork" strategy of the European Union aims for sustainable food production throughout the entire supply chain to avoid waste and maximize food utilization (European Commission, 2020b). The current socio-ecological issues and their political implications highlight the necessity of transforming the global food production system. As a core segment of the aquatic food producing sector, the development of sustainable aquaculture practices is essential.

1.1.2 The Role of Fisheries and Aquaculture in the Global Food Supply

Food from the aquatic environment including fish, invertebrates (crustaceans, molluscs), and seaweed is highly nutritious and forms an essential part of human food. On average, approximately 15% of the animal protein supply of human diets comes from aquatic sources, while in some coastal areas, this share can reach over 50% (FAO, 2024a). Fish and seafood is characterized by high levels of highly digestible protein and low amounts of saturated lipids (Tacon & Metian, 2013). As the primary source for healthy long chained polyunsaturated fatty acids (LC-PUFAs) and essential minerals, the consumption of aquatic food can greatly benefit human health by counteracting malnutrition and inadequate micronutrient intake (Golden et al., 2021).

Wild capture fisheries previously supplied the bulk of aquatic food for humans. Since the Food and Agriculture Organization of the United Nations (FAO) started to estimate the world fishery production in 1950, landings of wild capture fisheries increased heavily until the late 1980s (FAO, 2018). Since then, the supply of aquatic food from wild captures stagnated at around 80 to 90 million tons per year, as a consequence of fully-, or overexploited natural stocks.

Aquaculture dates back more than 6000 years, but remained a marginal activity until the end of the 20th century (Harland, 2019). Aquaculture production was only a few million tons in 1950, exhibited massive growth throughout the 1990s, and reached 94 million tons in 2022 (FAO, 2024a). The aquaculture sector has since emerged as the fastest growing food-producing sector in the world and production levels exceed landings of wild capture fisheries (FAO, 2024a; Troell et al., 2014). Wild capture fisheries will not increase in future. Therefore, the growing demand for aquatic food can only be met by aquaculture. However, the global supply of aquatic food is currently not keeping up with the human population growth (Tacon & Shumway, 2024). Aquaculture production levels would have to increase by more than 50% within the next 25 years to meet the projected aquatic protein demand for the growing population by midcentury (Boyd et al., 2022).

The massive expansion of aquaculture activities has led to a series of environmental issues such as intensive land and water use, eutrophication and pollution of aquatic ecosystems, and the introduction of non-native and invasive species (Diana, 2009; Naylor et al., 2000; Ottinger et al., 2016). More than 70% of the farmed aquatic animals are fed formulated or otherwise prepared diets (FAO, 2024a). Several life cycle assessments have identified aquaculture feed as the main factor that impacts multiple environmental categories (Bohnes et al., 2019; Cortés et al., 2021; Newton & Little, 2018). Development of sustainable aquaculture diets could thus have far-reaching consequences for the aquaculture sector.

1.2 Aquaculture Diets

1.2.1 Global Demand and Use of Marine Ingredients

Aquaculture production levels are projected to be 111 million tons by 2032, a 22% increase from the average annual levels of 2020 to 2022 (OECD/FAO, 2023). With the high share of cultured species that rely on feed inputs, the demand for aquaculture feeds will rise accordingly. Global aquafeed use was estimated to grow at an annual percentage rate of 4.5% from 2017 to 2025, and may amount to 73 million tons of feed required in 2025 (Tacon, 2019).

The majority of farmed fish and crustaceans require substantial amounts of protein in their diets that can make up to 55% of the dry matter (NRC, 2011). This is markedly higher than the dietary protein requirement of other conventional livestock (e.g. poultry, cattle, swine) which ranges from 10 to 22% (Jia et al., 2022). Fish meal (FM) has long been used as the primary feed ingredient for aquaculture diets due to its high protein content (~ 65%) and historically affordable price (Glencross et al., 2024; Tacon, 2004). Fish meal derives from the rendering process of whole fish and seafood by-products, during which fish oil is also extracted. Fish oil (FO) is the most important source of LC-PUFA including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which are essential nutrients in aquafeeds (Tocher, 2015). Fish meal contains high levels of highly digestible protein with excellent amino acid profiles that match the requirements of most aquatic organisms (Hardy & Tacon, 2002). Low amounts of fibers and the absence of antinutritional factors further increase the nutritional value of this marine-based feed ingredient (Turchini et al., 2019). Fish meal is highly palatable to carnivorous fish and shrimp species, and is a rich source of minerals and trace elements that benefit health and growth (Hardy, 2010). These outstanding nutritive qualities have made fish meal and fish oil the ‘Gold Standards’ in aquafeed formulations.

Fish meal made up 20 to 75% of aquafeeds during the dominant growth phase of the aquaculture sector in the 1990’s (Tacon, 1999). In 1995, the cultivation of carnivorous fish and shrimp consumed 2 to 7.5 times more fish biomass than it actually produced, making aquaculture a net consumer of marine resources (Tacon & Metian, 2008). The immense growth of the industry has positioned aquaculture as the major consumer of fish meal and fish oil globally (Hua et al., 2019). With stagnating landings of wild fisheries and the rising demand, prices of these marine feed ingredients increased drastically (Hardy, 2010). The environmental concerns and the questionable nature of feeding more wild caught fish than is actually being produced has received heavy criticism (Jones et al., 2015; Naylor et al., 2000). Approximately 22% of marine wild capture fisheries, of which 90% is considered suitable for direct human consumption, is used for the production of fish meal (Cashion et al., 2017; FAO, 2024a). To meet the ever-growing demand for aquafeeds, the search for alternative, sustainable, and economically viable feed ingredients has become and will remain a central topic in aquaculture research.

1.2.2 Alternative Feed Ingredients

Major advances in aquaculture nutrition and feed formulations since the beginning of this century have led to improved feed conversion efficiencies and an overall reduction of marine-based ingredients in aquafeeds (Naylor et al., 2021; Tacon & Metian, 2008). In modern feed formulations, the share of fish meal in diets for marine fish and shrimp is estimated to be around 18% and 15%, respectively (Figure 1.3) (Ambasankar et al., 2022; Hua et al., 2019).

Alternative plant-based ingredients, such as legumes, oil seeds, cereal grains, and derived protein concentrates, have become a major source of protein in aquaculture diets (Napier et al., 2020). Legumes such as soy, lupins, and peas are naturally rich in protein and can partly cover the protein requirement of various carnivorous aquatic species (Gatlin III et al., 2007). High shares of fish meal can effectively be replaced by protein concentrates obtained from cereal grains like wheat and barley (Apper-Bossard et al., 2013; Zaretabar et al., 2021). However, the amino acid profile of plant proteins does not resemble that of fish meal. Especially sulfur-containing amino acids are low in plant proteins (Gatlin III et al., 2007). Plant ingredients lack essential minerals that need to be supplemented in high-plant containing aquafeeds (Patrachotpakinkul et al., 2021). Certain sterols and amines which are abundant in fish meal and play important roles in shrimp and fish nutrition are also largely absent in plant derived resources (Morris et al., 2011; NRC, 2011; Salze & Davis, 2015). Furthermore, the presence of anti-nutritional factors, high concentrations of fibers, and low palatability pose limits for their use in aquafeeds (Gatlin III et al., 2007). Nevertheless, plant-based ingredients contribute significantly to the share of proteins in aquaculture diets and support the growth of the industry despite stagnating availability of marine ingredients (Naylor et al., 2021). Further expansion of the agricultural sector is problematic since it already exerts tremendous pressure on the planetary system (Conijn et al., 2018). Approximately 40% of global arable land is currently used to grow crops for animal feed (Mottet et al., 2017). To cover the food demand of the growing human population by midcentury, agricultural products will increasingly have to be used for direct human consumption.

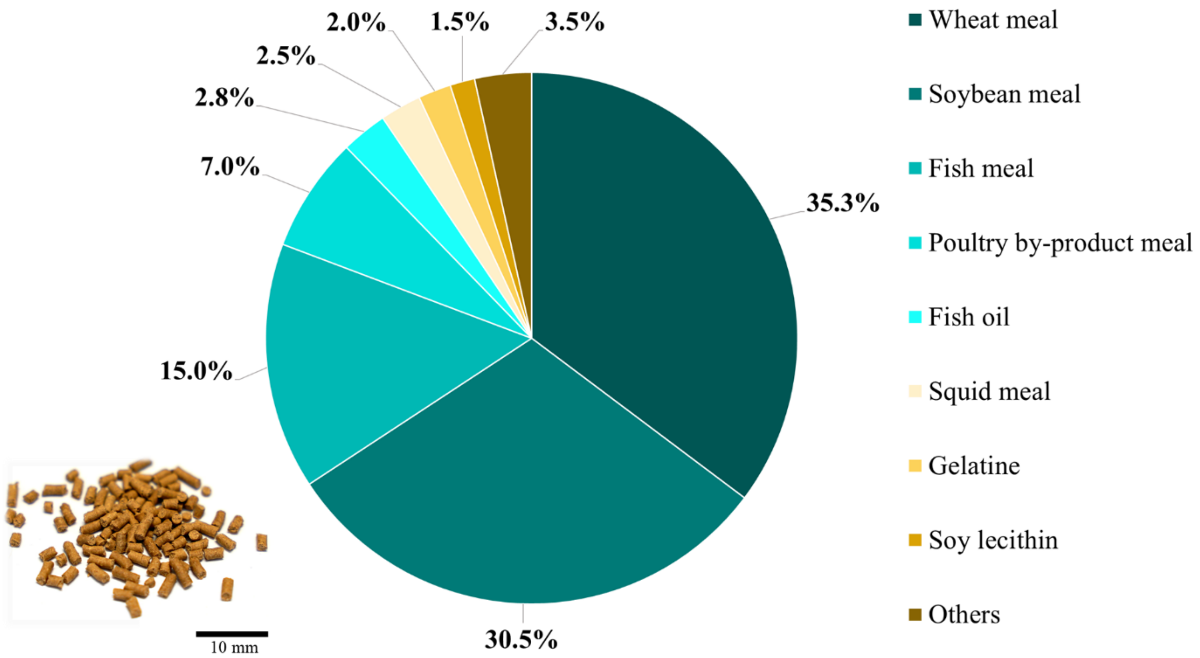

Figure 1.3. Example of a modern feed formulation for shrimp containing 15% fish meal and a range of alternative feed ingredients as it was used in the study of Richardson et al. (2021). The image at the bottom left depicts pelleted shrimp diets with a scale indicating size.

Other alternative feed ingredients include macroalgae, insects and microbial biomass that add to the pool of possible feed ingredients. Approximately 38 million tons of macroalgae biomass were produced globally in 2022 (FAO, 2024a). The protein content of most macroalgae species is moderate (10 - 30% of the dry mass) but of high quality, with a large share of essential amino acids (Hua et al., 2019). Macroalgae are a rich source of bioactive compounds and secondary metabolites that can positively affect health and overall performance of fish and shrimp (Wan et al., 2019). However, the high amounts of fiber, which can reach up to 76% of the dry mass, limit the bioavailability of key nutrients and can negatively affect the overall nutritional value of aquafeeds (Holdt & Kraan, 2011; Hua et al., 2019). Accordingly, macroalgae are only suitable at low dietary inclusion levels (< 10%) for most aquaculture species (Cárdenas et al., 2015; Cruz-Suárez et al., 2009; Ergün et al., 2009).

Seven insect species have recently been approved by the European Union to be used in animal feed (European Commission, 2017). The black soldier fly, (*Hermetia illucens*), the common housefly (*Musca domestica*) and the yellow mealworm (*Tenerbio molitor*) are primarily being considered as aquaculture feed and large-scale production is currently under way (Gold et al., 2018; Hua et al., 2019; Röthig et al., 2023). Meals made from insects are very nutrient dense, containing 40 - 63% protein and up to 36% lipid (Makkar et al., 2014). The digestibility of insect meals in shrimp and fish is high, with apparent nutrient digestibility coefficients exceeding 80% for most insect meals (Gasco et al., 2022; Shin & Lee, 2021). Amino acid profiles largely match the requirements of many cultivated fish and shrimp species, although the composition strongly varies with insect species and their feed substrates (Barroso et al., 2014; Henry et al., 2015). Chitin may negatively interfere with nutrient digestion (Cummins Jr et al., 2017). However, health benefits are also attributed to this nitrogen containing polysaccharide (Mousavi et al., 2020). Insect meals can replace large proportions of fish meal in fish and shrimp diets, but the success depends on the insect species, processing methods, chemical composition, and overall feed formulation (Cummins Jr et al., 2017; English et al., 2021; Nunes et al., 2023).

Microbial biomass derives from bacteria, microalgae, yeasts, and fungi (Jones et al., 2020). These microorganisms can contain high protein levels ranging from 45 - 71% with amino acid profiles that are similar to fish meal (Hua et al., 2019). The large diversity of microorganisms that can be used to produce high-value biomass enables a variety of production methods, resource uses and potential applications. For instance, filamentous fungi and yeast can grow on lignocellulosic material and contain high levels of protein suitable for carnivorous aquaculture species (Karimi et al., 2018; Øverland & Skrede, 2017). Certain microalgae can synthesize essential LC-PUFA and are valuable candidates to substitute fish oil and fish meal (Santigosa et al., 2021; Shah et al., 2018). The nutritional profile of microbial biomass can be influenced and further improved through culture media, growth conditions, and post-harvest treatments unlocking further opportunities to improve the nutritive value (Gamboa-Delgado & Márquez-Reyes, 2018). Despite these promising characteristics of microbial biomass and insect meals, up-scaling and cost-effective production remain critical challenges in the full commercialization of both alternative feed ingredients (Glencross et al., 2024; Jones et al., 2020).

1.2.3 By-Products as Circular Aquafeed Ingredients

The growth of the global aquaculture sector has started to slow down. Highest growth rates of the most dominant commercial species have already been reached (Sumaila et al., 2022). The dependency on feed resources that have reached, or even exceeded their sustainable limits pose a critical challenge for the growth of this sector. The concept of a circular economy offers a growth strategy that enables a 'decoupling' of resource use from growth by reusing co-products from one system as inputs for another (Reike et al., 2018). The circular bioeconomy outlined by the European Commission intends to maximize resource use efficiency and avoid losses by reusing, recycling, and reintegrating biogenic material as much as possible (European Commission, 2018). This concept also aims to minimize the environmental impacts of production systems by addressing multiple categories of the planetary boundary framework (Colombo et al., 2023). Circular production patterns meet the framework of the European Green Deal and contribute to key aspects of the 12th SDG (sustainable consumption and production patterns) of the United Nations (UN General Assembly, 2015; European Commission, 2020a). Applying circular bioeconomic principles on feed use might thus help to develop an aquatic food production system operating within the planetary boundaries.

By-products can be defined as materials that were produced alongside the primary product (Rustad et al., 2011). Other frequently used terms for these production residues are side streams, co-product, or rest raw materials. The quality of by-products can be food grade in some cases, but is usually not intended for human consumption without additional treatment (Lindberg et al., 2021). For instance, the European Commission defines animal by-products as material that is not used for human consumption (European Commission, 2002a). The inclusion of by-products into aquafeeds reintroduces nutrients into human food system, decreases food-feed competition, and avoids the negative environmental impacts that are associated with conventional feed ingredients. Numerous by-products are generated throughout the global food system originating from crop residues, crop processing, livestock, fisheries, aquaculture, and the food and beverage sector (Table S1) Widely available by-products are mostly cost-effective alternatives for feed ingredients and add overall value to material streams that might otherwise go to waste.

The principle of using by-products in aquafeeds has been applied since the early development of formulated diets (D'Abramo & Zeigler, 2022). For instance, the remains of seafood processing can make up between 50 and 70% of the original biomass and are commonly used for fish meal production (Hua et al., 2019; Huntington & Hasan, 2009). Currently, approximately 35% of the total fish meal is made from seafood by-products (FAO, 2024a). By using fish meal made from by-products, aquaculture already contributes to the redirection of marine nutrients into the human food system. By-products from the seafood industry are a diverse group of biogenic materials (bones, skins, head, viscera) and originate from a wide variety of species (Sudaryono, 1996; Nguyen et al., 2017; Rustad et al., 2011). As marine resources, these materials contain nutrients such as EPA, DHA, certain AA, pigments, and trace minerals that are scarce in terrestrial sources. The chemical composition of seafood by-products can differ substantially as does their suitability for use in aquafeeds. A distinctive evaluation of this diverse group of by-products is needed to specifically identify the most suitable and efficient use in aquafeed formulations. With approximately 28 million tons of seafood discards that are accessible for further applications and 12 million tons that are currently going to waste, seafood by-products have tremendous potential to serve as sustainable aquafeed ingredients (Caruso et al., 2020; Jackson & Newton, 2016).

Terrestrial animal by-products that are not suitable for human consumption are also commonly used in modern aquaculture diets. Organs, blood, bones, feathers, and skins are used to produce protein rich meals that usually contain 50 to 80% of protein (Bureau et al., 1999). These ingredients are often referred to as processed animal proteins, since the raw materials are stabilized via heat, steam and /or hydrolysis, and consecutive drying (Glencross et al., 2024). Processed animal proteins (PAP) are primarily made from poultry, pig, and cattle by-products, with over 40 million tons being produced solely in the US and the EU (Toldrá et al., 2016). These meals are well digested by most cultivated fish and shrimp species, presumably due to the absence of anti-nutritional factors and indigestible complex polysaccharides (Bureau et al., 1999; Colombo et al., 2023; Cruz-Suárez et al., 2007). The essential amino acid profiles are well balanced, especially that of poultry by-product meal, which closely resembles that of fish meal (Riche, 2015). PAP is a cost-effective protein source and can substitute large amounts of fish meal in diets for marine fish and shrimp (Chen et al., 2024; Galkanda-Arachchige et al., 2020). The animal derived meals contain vitamins, sterols, and minerals that are not provided by plant-based alternatives (Colombo et al., 2023; Pritchard & Smith, 1957). However, a notable limitation across all PAP meals is the lack of long chained omega-3 fatty acids (Glencross et al., 2024).

The primary agricultural and secondary crop processing industries generate large volumes of by-products, which are mostly untapped resources (Kari et al., 2023). Primary residues from the agricultural sector include leaves, stalks, stems, and roots that directly occur on the farm and fields. By-products from crop processing refer to husks, milling residues, molasses, and extraction residues (Table S1). High quality and nutrient dense by-products regularly find applications in aquaculture diets like oilseed press cakes and milling residues (Agbo et al., 2011; Davies et al., 1990; Fagbenro, 1999). Especially the remains of oil seeds after extraction are rich in protein that can reach up to 45% of the dry matter (Alimon, 2004; Batal et al., 2005). However, many agricultural by-products are of minor nutritive quality and require further processing before they can be used as aquafeed ingredients (Colombo et al., 2023). Nevertheless, the vast diversity of agricultural side streams and crop processing residues provides numerous opportunities for the aquaculture industry that go beyond replacing macro-nutrients. For instance, processing remains of fruit contain high levels of polyphenols, flavonoids, vitamins, minerals, pigments, polysaccharides, and peptides, which can enhance the immune responses of aquatic animals (Dawood et al., 2022). Ingredients that stimulate the immune system of aquaculture species are increasingly relevant in high precision aquafeed formulation used for intensive aquaculture systems.

1.2.4 Feed Additives

In search for alternative materials as aquaculture feed ingredients, the focus has been laid on suitable protein and lipid sources that match the requirements of a variety of farmed species. Today, a great variety of different raw materials have been identified that can be combined and included at different proportions and levels. Feed formulations are usually based on least-cost and availability principles, which means that they are always changing and adapting, depending on current market situations (Glencross et al., 2024). With the decreasing use of the 'Gold Standards' (FM and FO) and the increasing share of suboptimal ingredients, reduced performance and health of the animals is becoming a matter of concern (Kokou et al., 2012; Monteiro et al., 2021). Concomitantly, intensive culture conditions are challenging the health and immune systems of farmed aquatic animals (Hou et al., 2019). Antibiotics have been widely used to prevent the spread of pathogens and boost the overall performance of cultured animals. The extensive use of antibiotics has resulted in the accumulation of these compounds in the environment and increased the risk of multi-resistant bacteria, which ultimately poses a threat to human health. The subtherapeutic use of antibiotics in livestock was banned in the European Union in 2003 (European Commission, 2003).

Feed additives are routinely included in modern feed formulations to mitigate the varying quality of feed ingredients and to improve health and overall performance of fish and shrimp (Dawood et al., 2018; Monteiro et al., 2021). Crystalline amino acids were among the first established feed additives to account for shortages in essential amino acids in diets (Boyd et al., 2022). More recently, compounds that promote feeding behavior, enhance growth and stimulate the immune system have been identified, including organic acids, amines, nucleotides, glycoproteins, peptides, and polysaccharides (NRC, 2011). These substances are either supplemented in the purified form, or raw materials are used that are rich in these bioactive compounds. Feed additives also encompass ingredients that can improve the physical or chemical characteristics of the feed itself, or the quality of the fully grown animal, e.g., fillet quality, coloration, and/ or organoleptic properties (Barrows & Hardy, 2000). These include binding agents, such as alginate, that help stabilize feed underwater, and pigments, such as astaxanthin, which are used to increase the coloration of flesh, skins, and shells of the culture animals (NRC, 2011).

Feed additives are thus a broad class of feed ingredients that can be used for a manifold of purposes. The potential of feed additives to improve the feed quality and ultimately enhance the overall performance of farmed fish and shrimp has been widely recognized by the sector. By-products in particular pose cheap sources for many promising compounds that could be used as feed additives in aquaculture (Dawood et al., 2022). The huge diversity of materials that are co-produced along different processes offers numerous possibilities for application as aquafeed ingredients, which deserve thorough prospection.

1.3 The Pacific Whiteleg Shrimp

1.3.1 Aquaculture of Pacific Whiteleg Shrimp

Marine shrimp are among the most sought-after seafoods worldwide, fetching premium market prices with total global exports reaching 32.3 billon US$ in 2022 (FAO, 2024a). They are among the most relevant animals produced in aquaculture and significantly contributed to the expansion of this sector. The global production of marine shrimp increased by more than 80% in the last decade, with major producing countries in the tropical and subtropical regions of East and Southeast Asia, as well as Latin America (Emerenciano et al., 2022). The Pacific Whiteleg shrimp is by far the most cultured crustacean in the world, accounting for more than half of the total production volume of all crustaceans in 2022 (Figure 1.4, FAO, 2024b). In 2022, production of *L. vannamei* reached an all-time peak of 6.8 million tons (FAO 2024a). Applying a conservative estimate of 1.5 for the feed conversion ratio, this would have theoretically required 10.4 million tons of shrimp feed.

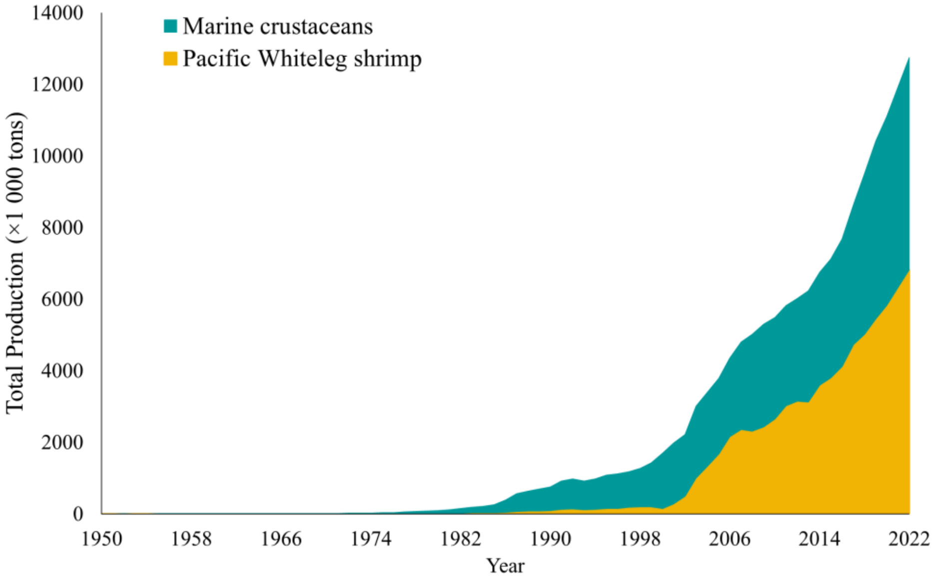

Figure 1.4. Total global aquaculture production of marine crustaceans and Pacific Whiteleg shrimp (*Litopenaeus vannamei*) from 1950 until 2022. Data sourced from FAO, 2024b.

Shrimp aquaculture has primarily developed in coastal areas close to tidal channels, flood plains or lagoons ensuring steady water supply and facilitating water exchange. Artificial ponds are usually constructed for shrimp farms which can be categorized into extensive, semi-intensive, and intensive cultures, depending on stocking rates, yields, as well as feed and water inputs (Ashton, 2008). Over the past 30 years, the growth of the industry was primarily driven by the expansion of extensive and semi-intensive shrimp farms with shrimp stocking densities of approximately 0.35 $kg \cdot m^{-2}$ (Emerenciano et al., 2022). These farming systems require large areas of land, which has led to massive clearances of natural habitats and loss of valuable coastal ecosystems such as mangrove forests (Richards & Friess, 2016). In traditional pond aquaculture, the lack of adequate water treatment causes eutrophication and pollution of the surrounding environment and also limits the effectiveness of biosecurity measures (Herbeck et al., 2013; Lemonnier et al., 2010). Over the last decade, intensive and super-intensive shrimp aquaculture has become more relevant with high density production systems, producing more with less area of land and water input (Emerenciano et al., 2022). Intensive aquaculture systems include recirculating aquaculture systems and biofloc systems which can support shrimp biomasses of up to 3 $kg \cdot m^{-2}$. These systems can be tank-based, permitting shrimp aquaculture to move further inland, reduce pressure on coastal regions, and provide more direct access to urban centers. Indoor farms are now operating in several temperate European countries including the UK, Austria, and Germany (Euroshrimp, 2021).

Recirculating aquaculture systems (RAS) comprise a series of compartments to efficiently remove solid particles (drum filter) and reduce the amounts of dissolved nitrogen compounds in the water (biofilter, denitrification unit) (Figure 1.5). Ozone or UV-treatments can be used for water disinfection to decrease the risk of disease outbreaks. Sophisticated aeration and degassing systems control the amount of dissolved gases such as O_2 and CO_2, while sensors continuously monitor the physiochemical characteristics of the water. RAS reduce nutrient discharges into the environment, require less than 1% of the land area, and use 90 to 99% less water than conventional aquaculture systems (Timmons et al., 2018).

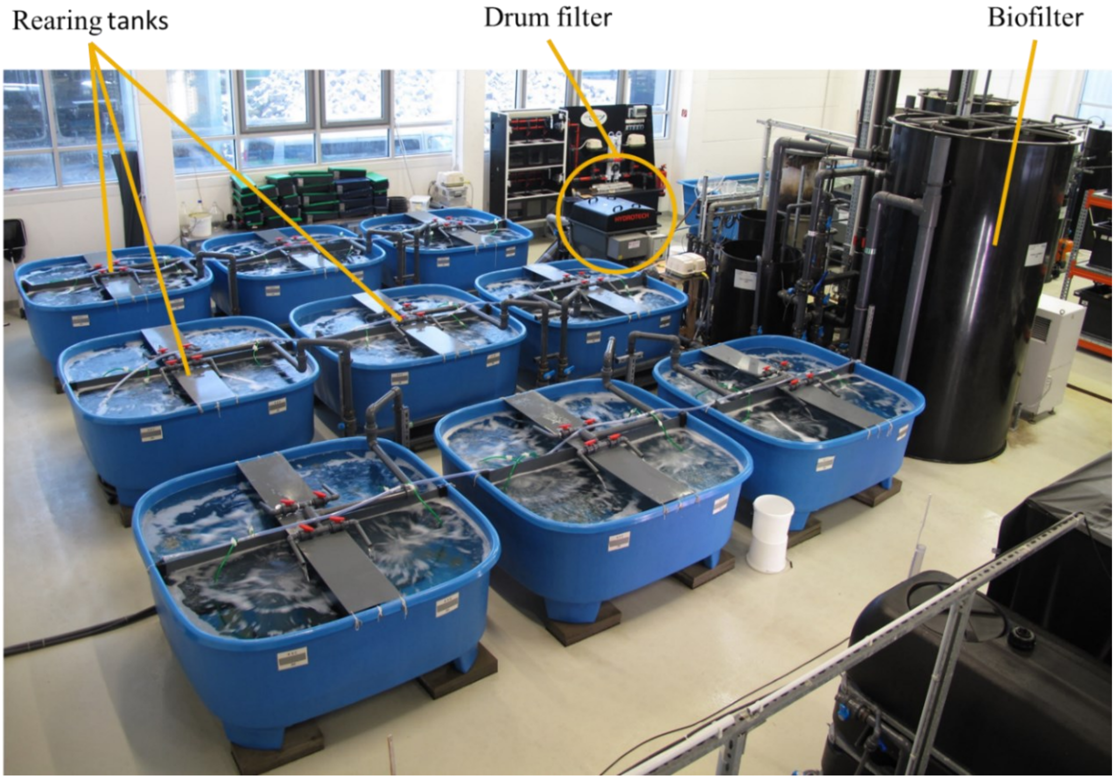

Figure 1.5. Image of a recirculating aquaculture system at the Center of Aquaculture Research of the Alfred-Wegener Institute, Helmholtz Centre for Polar and Marine Research in Bremerhaven, Germany. Orange lines indicate the rearing tanks (500 L), a drum filter and the biofilter unit.

Biofloc systems are also characterized by low water exchange and a microbial-based water treatment. The complex microbial community in the rearing water of these systems thrives on the organic and inorganic nutrients produced by the cultured species and form aggregates. These ‘bioflocs’ present an additional food source for the culture species which enhances nutrient utilization of the whole system (Gamboa-Delgado et al., 2016). The inorganic nitrogen dissolved in the water can hereby be removed by oxidation of chemoautotrophic bacteria, or by assimilation of heterotrophic bacteria (Ebeling et al., 2006). The stability and function of the microbial community is maintained by regulating the C/N ratio and alkalinity of the water, in accordance to the overall feed input. Biofloc technology is commonly used for shrimp culture, presumably because of the specific biology and feeding habit that allow shrimp to thrive in these conditions.

1.3.2 Biology of the Pacific Whiteleg Shrimp

The Pacific Whiteleg shrimp is a decapod crustacean within the suborder of Dendrobranchiata. Shrimp of this suborder are characterized by highly branched gill structures, and unlike other decapods, do not exhibit brooding behavior but release fertilized eggs directly into the water (Bauer, 1998). The Pacific Whiteleg shrimp belongs to the genus of *Penaeu*s, which, along with 40 other genera, form the family of Penaeidae (WoRMS, 2024). Based on morphological and reproductive traits, shrimp species within the *Peneus* genus have been further divided into the subgenera *Farfantepenaeus*, *Fenneropenaeus*, *Litopenaeus, Marsupenaeus*, *Melicertus* and *Penaeus s.s.* (Pérez-Farfante, 1969). The Pacific Whiteleg shrimp was allocated into the subgenus *Litopenaeus* and has since been referred to as *Litopenaeus vannamei*. However, recent findings based on modern molecular techniques revealed that the differences between the subgenera are marginal and do not justify this subgenus classification (Katneni et al., 2021; Ma et al., 2011). The latest review on this topic emphasized that *Penaeus vannamei* is the valid taxonomical name and *Litopenaeus vannamei* is merely to be used as a synonym (Figueredo et al., 2022). The latter publication appeared shortly after acceptance of the first manuscript of this dissertation (Fricke et al., 2022), in which the name *Litopenaeus vannamei* was used. The debate on the taxonomical classification and designation is ongoing. For consistency throughout the chapters, I used the synonymized name *Litopenaeus vannamei* in this work.

The epi-benthic Pacific Whiteleg shrimp naturally occurs in soft-bottom and muddy habitats. It is native to the Eastern Pacific coast of Mexico, Central America, and extends as far south as Northern Peru in areas with water temperatures above 20 °C throughout the year (FAO, 2009). Adult shrimp can reach sizes of 23 cm usually weighing 20 to 30 g. Mature females can reach up to 45 g. They spawn offshore and release 100 000 to 250 000 eggs. Hatching of the larvae takes place in the open water. Freshly hatched nauplius larvae live on yolk reserves for approximately 2 days, until they develop into zoea, mysis, and early postlarvae stages. During the early planktonic life stages, *L. vannamei* feed on phyto-, and zooplankton, and are drifted by tidal currents towards coastal areas (Dugassa & Gaetan, 2018). In the late post larvae stages, shrimp migrate further inshore into coastal estuaries or mangrove areas and begin to feed on detritus and benthic organisms. Juvenile *L. vannamei* are opportunistic omnivores feeding on micro-, and macro algae, annelids, molluscs, and small crustaceans (NRC, 2011; Panutrakul et al., 2010). An aggressive and predatory feeding behavior has been observed in larger specimens (Chavanich et al., 2016).

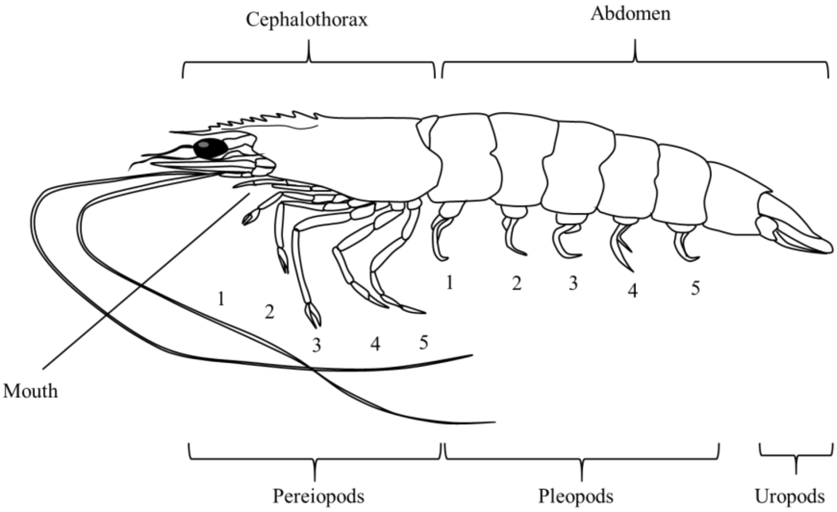

Figure 1.6. Schematic illustration of *Litopenaeus vannamei* showing the main body parts (cephalothorax, abdomen), the mouth, and indicating the 5 pairs of pereiopods and pleopods, as well as the uropods.

Shrimp catch, handle, and preprocess their prey and feed with specialized appendages before ingesting it through their mouth, which is located rostro-ventrally at the cephalothorax (Figure 1.6). The appendages involved in feeding include the first three pairs of walking legs (pereiopods), 3 pairs of mouthparts (maxillipeds), and the jaw structures (mandibles). The first three pereiopods of *L. vannamei* possess chelae with small pincers that are used to take up, hold, and direct feed items to the maxillipeds and mandibles. The three pairs of maxillipeds are morphologically distinct from one another, with the second and third pairs equipped with long setae that form fan-like structures (Figure 1.7).

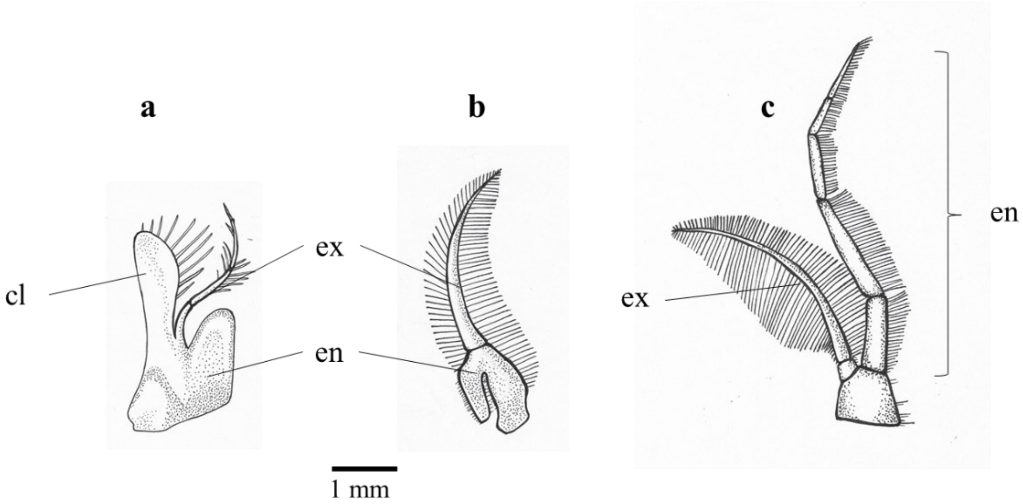

Figure 1.7. Illustration of the three pairs of maxillipeds showing the exopods (ex), endopods (en), and the caridean lobe (cl) of *Litopenaeus vannamei* weighing approximately 12 g.

L. vannamei uses its maxillipeds to hold and convey feed into its mouth. The maxillipeds also play a role in ventilation and in creating a water stream directed to the gill chambers of the shrimp (Kawamura et al., 2018). The terminal ends of the mandibles are highly calcified structures and resemble small teeth used to cut and masticate feed into smaller pieces to be ingested (Zhu et al., 2024).

Unlike fish, which usually take up feed in one single gulp, shrimp require more time to handle and process feed items until they are ingested. Feed fragments and smaller particles are often dropped and lost due to this prolonged handling and nibbling feeding behavior. Feed items are thus precisely inspected and feed with an unpleasant scent, palate, or texture will be rejected (Kawamura et al., 2018). A variety of chemoreceptors are located on the antennae, pereiopods, and maxillipeds, which are involved in the detection and quality assessment of the feeds (Derby & Sorensen, 2008). Ingested feed reaches the stomach through the esophagus where it is further ground and shred by the gastric mill (McGaw & Curtis, 2013). In the posterior end of the stomach, uniformly spaced cuticular hairs form a sieve which allows particles smaller than about 1 µm to pass into the hepatopancreas (Figure 1.8, Pattarayingsakul et al., 2019). The hepatopancreas, or midgut gland, is the main digestive organ of shrimp. It is a multilobate organ with numerous blind ending tubules of varying length that are connected to larger collective ducts which open into the midgut (NRC, 2011). These structures are coated with a single epithelial layer. Unlike the foregut (esophagus and stomach) and the hindgut, the midgut is not covered by a chitinous cuticle, which is part of the shrimp exoskeleton (Dugassa & Gaetan, 2018). The midgut is lined by a peritrophic membrane which encases the digestive material and presumably protects the gut epithelium from mechanical damage and pathogens (Martin et al., 2006). The hepatopancreas is responsible for the production and secretion of digestive enzymes, absorption of digestive material, and nutrient metabolism (McGaw & Curtis, 2013).

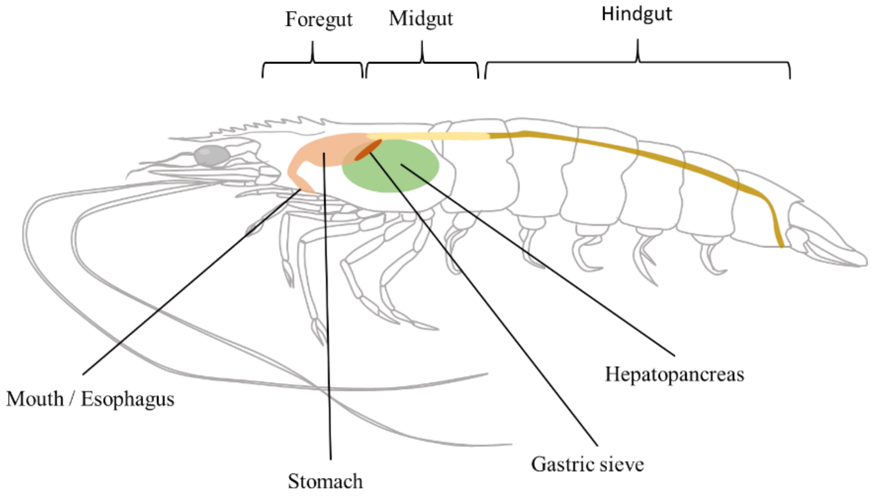

Figure 1.8. Schematic illustration of the digestive system of *L. vannamei.*

Digestive enzymes are synthesized in specialized cells of the hepatopancreas and are released into the collective ducts towards the stomach for extracellular digestion (Ceccaldi, 1989). *Litopenaeus vannamei* possess a set of digestive enzymes to break down the major feed components, grossly grouped into proteases, lipases, and carbohydrases. Shrimp digestive enzymes have a wide substrate specificity and act together to disintegrate larger and complex compounds into subunits of smaller molecular weight. The resulting chyme passes through the gastric filter into the hepatopancreas, where the nutrients can be resorbed by specific (R-)cells (Saborowski, 2015).

Proteases can be distinguished according to the position where they cleave their substrate and by the amino acid at the active site of the enzyme itself. Endopeptidases hydrolyze peptide bonds within a protein chain, while exopeptidases act on the terminal ends of peptides. Exopeptidases can further be classified into aminopeptidases and carboxypeptidases, depending on the terminal end of the polypeptide chain they attack (amino or carboxylic). The most relevant endopeptidases in penaeids are the serine proteases trypsin and chymotrypsin, which can be responsible for up to 60% of the total protein digestion (Lemos et al., 2000; Tsai et al., 1986). Trypsin selectively cleaves polypeptide chains at arginyl- and lysyl-bonds, while chymotrypsin preferably hydrolyses peptide bonds formed by aromatic amino acids (Navarrete del Toro & García-Carreño, 2019). Three isoforms of trypsin and two isoforms of chymotrypsin have been identified in *L. vannamei*, each with slightly different biochemical characteristics and hydrolytic capabilities (Aguiñaga-Cruz et al., 2019; Muhlia-Almazán & Fernández-Gimenez, 2022). Other digestive endo- and exopeptidases that have been characterized in decapods include metallo-, cysteine-, and aspartate proteases (Maytorena-Verdugo et al., 2017; Navarrete del Toro et al., 2006; Teschke & Saborowski, 2005). The aspartic protease cathepsin D is usually found in lysosomes and is known to have intracellular functions (Benes et al., 2008). However, in several decapod species, including *L. vannamei*, this protease is commonly found in the gastric fluid, suggesting its relevance as a digestive enzyme (Martínez-Alarcón et al., 2018). These diverse proteolytic enzymes, each employing different catalytic mechanisms and biochemical characteristics, presumably act synergistically to efficiently hydrolyze and digest proteins (Navarrete del Toro & García-Carreño, 2019).

Lipases are glycerol ester hydrolases, which primarily degrade triacylglycerides (TAG) into monoacylglycerides and free fatty acids. Non-specific esterases also have the ability to hydrolyze TAGs. For long time, it remained unclear, whether the lipolytic activities in the hepatopancreas of shrimp originates from true lipases (Carrillo-Farnes et al., 2007). Rivera-Pérez et al. (2011) were the first to isolate and characterize a lipase from the midgut gland of *L. vannamei*. It is a glycoprotein with properties typical for serine lipases. Del Monte et al. (2003) showed that *L. vannamei* also has the capability to hydrolyze polar phospholipids. Penaeid shrimp lipases preferably hydrolyze substrates containing long chained n-3 and n-6 fatty acids (NRC, 2011).

Carbohydrases are a group of enzymes that break down complex polysaccharides into oligosaccharides which are then further hydrolyzed into monosaccharides. Polysaccharides differ in the composition of the sugar monomers and the glycosidic linkages, which require specific enzymes for their cleavage. Extracts of the hepatopancreas from *L. vannamei* showed amylase, maltase, and laminarinase activities (Omondi & Stark, 1995). *L. vannamei* thus possesses enzymes that are capable of hydrolyzing α- and β-glycosidic bonds. Chitinolytic enzymes that degrade chitin, a β-1,4 linked amino-polysaccharide, have also been detected in the digestive organs of penaeid shrimp (Huang et al., 2010; Kono et al., 1995). Additional carbohydrases that were characterized in the hepatopancreas of the penaeid shrimp *Marsupenaeus japonicus* and *Penaeus monodon* include β-mannosidase, α-fucosidase and β-galactosidase (Chuang & Yang, 1991a; Chuang et al., 1991b; Chuang et al., 1991c). Cellulase activity has also been reported to occur in *L. vannamei* (Moss et al., 2001). However, it remains unclear whether the enzymes are endogenous or derive from exogenous sources. Exogenous enzymes may originate from the digestive organs of ingested prey, liberated from lysed microbes, or from bacteria inhabiting the gut of shrimp. A microbiome is present throughout the digestive tract of Pacific Whiteleg shrimp. Many strains are capable of producing exogenous enzymes including proteases, amylases, lipases/esterases and chitinases (Tzuc et al., 2014). The microbial community of the digestive tract of shrimp therefore has the potential to contribute to the degradation of dietary components.

1.3.3 Nutritional Requirements

The main dietary components in the feed of *L. vannamei* are protein, lipids, and carbohydrates. Protein is the most relevant nutrient for shrimp, used to generate energy and increase biomass. The protein requirement of *L. vannamei* changes throughout the different life stages. Higher amounts of protein are needed to feed post larvae (~54%) than adult shrimp (~35%) (Barreto et al., 2023; NRC, 2011). The recommended protein levels should be considered as approximate, since the dietary quality of protein is a function of the availability and composition of the proteinogenic amino acids. Amino acids can be divided into essential and non-essential amino acids. Essential amino acids (EAA) cannot be synthesized by shrimp and must be supplied with the feed. The ideal protein concept follows Liebig's law of the minimum and defines the optimal dietary EAA quantities without excess or deficit (Wang & Fuller, 1989). The ideal amino acid profile is usually expressed as the ratio of each EAA to lysine given the arbitrary value of 100 (Table 1). However, the non-essential amino acids are also important building blocks of proteins and needed for a balanced diet. As stated by Albert Tacon in the publication of Turchini et al., 2019, "non-essential dietary nutrients may in fact be so essential that the cell/body actually produces them". In penaeid shrimp, amino acids are the major substrates for gluconeogenesis, used to synthesize glucose, thus making protein the preferred energy source (NRC, 2011). However, the use of protein as metabolizable energy in aquafeeds is undesirable due to the high cost and increasing amounts of ammonia excreted into the environment. Thus, aquafeeds are formulated to precisely meet the protein requirements of cultured species, to ensure optimal growth, and to minimize the catabolic loss of nitrogen.

Table 1. Ideal proportions of essential amino acids (EAA) for penaeid shrimp provided by NRC, 2011 and the calculated ideal EAA profile for *L. vannamei* based on the lysine requirements defined by Fox et al. (1995).

Amino Acid	Ideal EAA proportions	Ideal EAA profile (% protein)
Lysine (Reference)	100	4.5
Arginine	95	4.3
Histidine	38	1.7
Isoleucine	48	2.2
Leucine	81	3.6
Methionine	48	2.2
Phenylalanine	55	2.5
Threonine	67	3.0
Tryptophan	10	0.5
Valine	65	2.9

Lipids and carbohydrates are other energy yielding nutrients that can be metabolized by shrimp. Aside from providing metabolizable energy, lipids also supply important compounds such as polar lipids, sterols, and long chained polyunsaturated fatty acids. These components are crucial for cell membrane structure, act as precursors for hormones, and play vital roles in various metabolic processes (Glencross, 2009b; Niu et al., 2011; Palacios et al., 2004; Teshima, 1997). Cholesterol plays a crucial role in shrimp nutrition, since crustaceans cannot synthesize this sterol de novo but rely on this compound for membrane structures and as a precursor for several hormones (Morris et al., 2011; Teshima, 1997). Shrimp do not tolerate high amounts of lipid in their diets and levels higher than 10% can result in stunted growth (D'Abramo, 1989). The poor capability to utilize lipids in shrimp may be related to the limited ability to emulsify dietary lipids due to the lack of bile acids (Glencross, 2009b; Li et al., 2023). However, acyltaurines have been found in the gastric fluids of some decapods that show emulsifying properties (Dall & Moriarty, 1983). It is difficult to accurately define the absolute dietary lipid requirements for shrimp. These depend on various nutritional factors, such as the contents of dietary protein and carbohydrates, as well as the composition and fatty acid profile of the lipid source (NRC, 2011). Penaeid shrimp preferably utilize long chained polyunsaturated fatty acids (LC-PUFA), while saturated fatty acids are less bioavailable (Glencross et al., 2002). The most relevant LC-PUFA required for many essential metabolic and physiological processes are arachidonic acid (ARA, 20:4(n-6)), eicosapentaenoic acid (EPA, 20:5(n-3)), and docosahexaenoic acid (DHA, 22:6(n-6)). It is generally assumed that marine shrimp are unable to convert and elongate C_{18} PUFAS into C_{20} and C_{22} LC-PUFAs, making the dietary uptake essential (NRC, 2011). These essential fatty acids have endocrine functions and influence several metabolic processes. They play important roles in cell synthesis, neural development, ion regulation, immune function and reproduction (Glencross, 2009).

Carbohydrates are not essential nutrients for shrimp, as protein and lipids can provide the necessary metabolic energy. However, carbohydrates are routinely included into diets because they are the least expensive energy source available for aquafeeds. The ability to utilize carbohydrates depends on the type of carbohydrate and the set of available digestive enzymes suitable for their hydrolyzation (ref. section 1.3.2). Monosaccharides and disaccharides are generally not well tolerated in high amounts by penaeid shrimp. High dietary levels of glucose and maltose (> 20%) can induce hyperglycemia in *P. monodon*, negatively affecting the overall performance and survival of shrimp (Niu et al., 2012; Piedad-Pascual et al., 1983). Polysaccharides, such as starch are more suitable for crustaceans and acceptable dietary levels range between 20 and 30% (Wang et al., 2016). Non-starch polysaccharides (NSP) are generally poorly digested by shrimp and therefore play a minor role as energy source in shrimp nutrition (NRC, 2011). Due to their physicochemical properties, which influence digesta viscosity and the capacity to bind organic and inorganic molecules, higher levels of NSPs can negatively influence the digestive process (Gatlin III et al., 2007). At lower inclusion rates, however, certain polysaccharides can stimulate the immune system of shrimp and thus may be applied as additives in aquafeeds (Mohan et al., 2019; Rajan et al., 2023).

Shrimp need minerals for various biological functions, including structural components for the exoskeleton, maintaining intra-, and extracellular homeostasis, nerve impulse transmission, and as cofactors for enzymes (NRC, 2011). Marine shrimp can take up minerals from the surrounding water, but some minerals must be provided by the diet (Davis et al., 1993). Dietary requirements of phosphorous, magnesium, copper, manganese, selenium, and zinc have been defined for *L. vannamei* (NRC, 2011).

Vitamins are organic compounds that are also distinct from the macro-nutrients and need to be supplied with the diet. These include the fat-soluble vitamins A, D, and E, which can lead to poor appetite, lower growth, and increased mortality if deficient in diets for *L. vannamei* (He et al., 1992). Water-soluble vitamins often function as important cofactors for enzymes such as thiamin, riboflavin, and pantothenic acid. Deficiencies are often difficult to determine (NRC, 2011). Diets supplemented with the water-soluble vitamins ascorbic acid, choline, pantothenic acid, and niacin resulted in higher growth rates and improved feed conversion ratios in *Penaeus indicus* (Reddy et al., 2001). To ensure a steady dietary supply of minerals and vitamins and avoid deficiencies, premixes that contain generous amounts of these organic and inorganic compounds are commonly added to aquafeeds.

1.4 Objectives of this Thesis

The need to transform the production patterns of aquaculture is imperative. Feed is the most critical factor associated with the environmental footprint of this sector. At the same time, the growth of the industry heavily relies on readily available and low-price feed ingredients to cover the increasing global demand for aquatic foods (FAO, 2024a; Tacon & Metian, 2015). To address this issue, the aim of this thesis is to identify by-products and their potential as sustainable feed ingredients for *L. vannamei* raised under intensive culture conditions.

Following research questions are addressed in this thesis to tackle the afore mentioned challenges:

1. Are local by-products available that are suitable as aquafeed ingredients for *L. vannamei*?

2. How and to what extent can these by-products be integrated into well-balanced diets for shrimp?

3. What is the potential of these by-products in view of circular bioeconomic principles in the aquatic food production system?

Industries in northern Germany were evaluated to identify side streams that may be suitable as aquafeed ingredients. Professional networks and B2B platforms of the food producing sector (NaGeb e.V., Bremen; Leroma GmbH, Essen; BaMS e.V., Kiel) hereby provide insights into the vast diversity of industries that generate residual material streams. The most promising materials are subjected to detailed chemical characterizations. Controlled digestibility trials enable to quantify the bioavailability of key nutrients *in vivo*. This information is needed to precisely formulate aquaculture feeds that meet the nutritional requirements of *L. vannamei*. Feeding experiments in intensive culture conditions (RAS and Biofloc) further elucidate the suitability of the raw materials as aquafeed ingredients.

In the following chapters, promising by-products from seafood processing, insect rearing, and cosmetical industries are presented. Based on their chemical composition, materials are tested as either protein source, or as feed additives. Finally, considerations that go beyond the biological assessment are made to fully characterize the potential of the investigated by-products as sustainable and circular feed ingredients for shrimp aquaculture.

A detailed description of the materials and methods applied, as well as the results generated in this thesis, is provided in Chapter 2.

Chapter 2 Publications and Manuscripts

2.1 Contributions

Three publications and two manuscripts have been prepared within the frame of this work and are included in this dissertation. The contributions of individual authors to the following scientific works are provided in this chapter and are based on the definitions by Brand et al. (2015). My own work contribution is further given in more detail and is described as percentage of the total workload grouped according to the relevant tasks.

Chapter 2.2 (Publication I)

Brown shrimp (*Crangon crangon*) processing remains as ingredient for *Litopenaeus vannamei* feeds: Biochemical characterization and digestibility

Enno Fricke, Marie Koch, Heiko Dietz, Matthew James Slater, Reinhard Saborowski

EF: conceptualization, methodology, investigation, formal analysis, writing – original draft, visualization; **MK**: Investigation, writing – review and editing; **HD**: resources, writing-review and editing; **MJS**: resources, writing - review and editing, supervision; **RS**: conceptualization, resources, writing – review and editing, supervision, project administration, funding acquisition.

My contribution described in % of the total workload:

Experimental concept and design:	**ca. 90%**
Experimental work and data acquisition:	**ca. 80%**
Data analysis and interpretation:	**ca. 90%**
Preparation of figures and tables:	**ca. 100%**
Drafting of the manuscript:	**ca. 90%**

Chapter 2.3 (Publication II)

Brown shrimp (*Crangon crangon*) processing remains enhance growth of Pacific Whiteleg shrimp (*Litopenaeus vannamei*)

Enno Fricke, Matthew James Slater, Reinhard Saborowski

EF: conceptualization, methodology, investigation, formal analysis, writing – original draft, visualization; **MJS**: resources, writing - review and editing, supervision, funding acquisition; **RS**: conceptualization, resources, writing – review and editing, supervision, project administration, funding acquisition.

My contribution described in % of the total workload:

Experimental concept and design:	**ca. 90%**
Experimental work and data acquisition:	**ca. 70%**
Data analysis and interpretation:	**ca. 90%**
Preparation of figures and tables:	**ca. 100%**
Drafting of the manuscript:	**ca. 80%**

Chapter 2.4 (Publication III)

Utility of by-products of black soldier fly larvae (*Hermetia illucens*) production as feed ingredients for Pacific Whiteleg shrimp (*Litopenaeus vannamei*)

Enno Fricke, Reinhard Saborowski, Matthew James Slater

EF: conceptualization, methodology, investigation, formal analysis, writing – original draft, visualization; **RS**: resources, writing – review and editing, supervision; **MJS**: conceptualization resources, writing - review and editing, supervision, funding acquisition, project administration.

My contribution described in % of the total workload:

Experimental concept and design:	**ca. 90%**
Experimental work and data acquisition:	**ca. 80%**
Data analysis and interpretation:	**ca. 90%**
Preparation of figures and tables:	**ca. 100%**
Drafting of the manuscript:	**ca. 90%**

Chapter 2.5 (Manuscript I)

Defatted black soldier fly (*Hermetia illucens*) imago meal as replacement for fish meal in diets for Pacific Whiteleg shrimp *Litopenaeus vannamei*

Enno Fricke, Henry Udeh, Tabea Galonska, Celina Behrendt, Reinhard Saborowski, Matthew James Slater

EF: conceptualization, methodology, investigation, formal analysis, writing – original draft, visualization; **HU**: investigation; **TG**: methodology, investigation; **CB**: methodology, investigation; **RS**: resources, writing – review and editing, supervision; **MJS**: conceptualization resources, writing - review and editing, supervision, funding acquisition, project administration.

My contribution described in % of the total workload:

Experimental concept and design:	ca. **80%**
Experimental work and data acquisition:	ca. **60%**
Data analysis and interpretation:	ca. **90%**
Preparation of figures and tables:	ca. **100%**
Drafting of the manuscript:	ca. **90%**

Chapter 2.6 (Manuscript II)

Evaluating diets for Pacific Whiteleg shrimp, *Litopenaeus vannamei*, supplemented with algae pomace (*Saccharina latissima*) and cocoons (*Hermetia illucens*) in clear water recirculating-, and biofloc systems

Enno Fricke, Ramon Felipe Siqueira Carneiro, Flavia Banderó Hoffling, Mateus Aranha Martins, Felipe do Nascimento Vieira, Walter Quadros Seiffert; Reinhard Saborowski, Matthew James Slater

EF: conceptualization, methodology, investigation, formal analysis, writing – original draft, visualization, funding acquisition; **RFSC**: investigation; **FBH**: investigation, writing - review and editing; **MAM**: investigation, writing - review and editing; **FDNV**: resources, supervision, writing - review and editing; **WQS**: resources, project administration; **RS**: resources, writing – review and editing, supervision; **MJS**: resources, writing - review and editing, supervision, funding acquisition, project administration.

My contribution described in % of the total workload:

Experimental concept and design:	ca. **70%**
Experimental work and data acquisition:	ca. **60%**
Data analysis and interpretation:	ca. **90%**
Preparation of figures and tables:	ca. **100%**
Drafting of the manuscript:	ca. **90%**

Seafood Processing Industry

The following two chapters (2.2 and 2.3) describe brown shrimp (*Crangon crangon*) processing remains as underutilized by-product and its use as feed ingredient for *Litopenaeus vannamei*

Dried processing remains of the brown shrimp, *Crangon crangon*. The black line indicates 10 mm

2.2 Publication I

Brown shrimp (*Crangon crangon*) processing remains as ingredient for *Litopenaeus vannamei* feeds: Biochemical characterization and digestibility

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Brown shrimp (*Crangon crangon*) processing remains as ingredient for *Litopenaeus vannamei* feeds: Biochemical characterisation and digestibility

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ABSTRACT

Processing remains of brown shrimp, *Crangon crangon*, account for up to 60 % of the catch while only the small muscle fraction is used for human consumption. Incorporation into aquafeeds for high-valued species would reduce waste, create by-product value and promote sustainable aquaculture development. A detailed chemical characterisation of the remains from mechanically peeled brown shrimp was made and apparent nutrient digestibility coefficients in *Litopenaeus vannamei* were investigated. Brown shrimp processing remains (BSPR) contain substantial amounts of key nutrients (521 g·kg^{-1} crude protein, 74 g·kg^{-1} total lipid, 15 MJ·kg^{-1} gross energy) and valuable functional ingredients were detected (cholesterol, astaxanthin). Apparent energy (82 %) and protein (86 %) digestibility coefficients reveal good bioavailability of these nutrients. Dry matter digestibility was lower (64 %) presumably due to the high ash content (244 g·kg^{-1}). The amino acid profile meets dietary requirements of penaeid shrimp with high apparent lysine and methionine digestibility coefficients. Analysis of macro- and micro minerals showed reasonable levels of required dietary minerals (phosphorus, magnesium, copper, manganese, selenium, zinc) and apparent copper digestibility was high (93 %). Contamination levels present in BSPR were below European standards acceptable for human consumption. Processing remains of brown shrimp have a high potential as alternative feed ingredient in sustainable diets for *L. vannamei* in recirculating aquaculture systems.

1. Introduction

Aquaculture has been identified as one of the key food producing sectors with a need for a sustainable development in the European Union (EU) (The European Commission, 2013). While capture fisheries yields in the EU have decreased over the last two decades, aquaculture production continues to grow and is increasingly important in seafood supply (STECF, 2021). The majority of aquaculture production in Europe is based on fed aquaculture species (FAO, 2020) that require high levels of dietary protein and lipid often originating from marine sources. Gephart et al. (2021) estimated that aquaculture feeds are responsible for more than 60 % of the industry's greenhouse gas emissions, with highest values for crustacean aquaculture. Shrimp aquaculture is still a niche business in Europe but land-based production is gaining relevance and production levels are growing (Euroshrimp, 2021). By applying circular economy approaches such as incorporation of biogenic sidestreams in aquafeeds, environmental impacts could potentially be reduced drastically (Regueiro et al., 2021).

Brown shrimp (*Crangon crangon*) is an intensively fished species in the southern North Sea with annual landings of more than 30,000 tons and high market values (BLE, 2019; ICES, 2022). Only the abdominal muscle of brown shrimp is used for human consumption. The remains after processing, consisting of cephalothorax, internal organs, ovaries, and exoskeleton account for up to 60 % of the total catch and are mainly discarded (R. Saborowski, personal communication). Brown shrimp processing remains (BSPR) are rich in valuable biomolecules such as proteins, long chained polyunsaturated fatty acids, and glucosamines (Synowiecki and Al-Khateeb, 2000). Therefore, it could be a sustainable by-product based feed ingredient for penaeid aquaculture.

Different meals made from shrimp by-products have been tested as feed ingredients. These meals were made from remains of *Litopenaeus vannamei, Penaeus monodon, Palaemonetes varians* or were not further defined. Despite differences in proximate compositions, the tested shrimp meals showed high nutrient bioavailability and led to good growth performances in *L. vannamei* and *P. monodon* (Fox et al., 1994; Salas-Leiton et al., 2020; Terrazas-Fierro et al., 2010; Yang et al., 2009).

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In this study we provide a detailed description of mechanically processed brown shrimp remains and measure the contents of relevant macro- and micronutrients. To assess possible bioaccumulation of contaminants in BSPR, levels of heavy metals and persistent organic pollutants are reported and discussed. Furthermore, a controlled feeding trial was conducted to define the apparent digestibility of key nutrients for *L. vannamei*.

2. Material and methods

2.1. *Brown shrimp processing remains*

Frozen remains of mechanically processed brown shrimp, *C. crangon*, were obtained from the shrimp trading company Alwin & Siegfried Kocken GmbH, Germany. The automated peeling process by machine utilizes pneumatic sorting and peeling of cooked brown shrimp, which have been boiled in sea water on board ship immediately after the catch. The brown shrimp processing remains were separated into cephalothorax, abdominal shell, muscle, and eggs, then gross composition by mass was determined. The cephalothorax includes the appendages such as antennae, maxillipeds, pereiopods, and the internal organs including stomach, midgut gland, and ovaries. The abdominal shell comprises mainly the abdominal cuticle, the pleopods and uropods, but not the abdominal muscle (Fig. 1.). The abdominal muscle refers to meat fragments, which have not been completely removed by the mechanical peeling process.

For biochemical analyses and feed production, the pooled shrimp remains were oven dried in glass dishes at 60 °C for 48 h. The dried shrimp remains were ground in a two-step process using a blender (HR2094, Philips, Germany) and a knife mill (GM 200, Retsch, Germany). To avoid overheating during the grinding process, the remains were ground for 30 s followed by a rest of another 30 s. The grinding process lasted for 3 min. The meal was then passed through a 500-μm sieve. The particles remaining on the sieve were repeatedly ground until all of them passed through the sieve. The resulting homogenous powder was stored at 4 °C until further use.

2.2. *Biochemical analysis*

Moisture and ash content was analysed according to the standard methods of the Official Analytical Chemists AOAC (2010) (method 934.01 and 942.05). Energy content was determined using a bomb calorimeter (Parr 6100, Parr Instrument Company, USA). Chitin was extracted after Percot et al. (2003) and quantified gravimetrically. Nitrogen was determined through combustion of samples and detection of the resulting gaseous oxides using an elemental analyzer (Euro Elemental Analyzer, Eurovector SPA, Italy). Protein was determined by multiplying the nitrogen content with the factor 6.25 (Dumas, 1831). The nitrogen content of BSPR was corrected for chitin bound nitrogen to calculate the protein content. Total lipids were extracted after Folch et al. (1957) and determined gravimetrically. Cholesterol was measured with a commercial test-kit (Boehringer, Germany). Amino acids, fatty acids, heavy metals, PAHs, and PCBs were analysed by certified laboratories (LUFA Nord-West, Germany). The mineral and yttrium content was measured using inductively coupled plasma-optical emission spectrometry (ICP-OES Thermo iCAP, Thermo Fisher Scientific, USA) based on the European standardized method for determination of trace elements in foodstuffs (DIN: EN 13805:2014, German version). Prior to ICP-OES analysis, samples were digested with nitric acid and a Mars Xpress microwave digestion system (CEM GmbH, Germany). Astaxanthin was determined by the SGS Institute Fresenius GmbH, Germany. The effect of drying method on the astaxanthin yield was addressed by comparing lyophilized to oven-dried samples.

2.3. *Digestibility trial*

The apparent dry matter, energy, protein, lysine, methionine, and copper digestibility coefficients of BSPR were determined using a test diet, and a reference diet as described by Glencross et al. (2007). The reference diet was formulated to meet nutritional requirements of *L. vannamei* in the grow-out phase (NRC, 2011). The test diet was prepared by adding 3 parts of BSPR to 7 parts of the reference diet mash on weight basis (Table 1). Yttrium oxide was added as an inert marker. To maintain a homogenous particle size of the feed mixtures, all ingredients were ground and passed through a 500 μm sieve as described above. The ingredients were mixed thoroughly and water was added to reach a moisture content of approximately 15 % to achieve a dough suitable for

Table 1
Ingredient composition of the reference diet and test diet used in the digestibility trial with *Litopenaeus vannamei*.

Ingredient	Reference diet	Test diet
	($g \cdot kg^{-1}$)	($g \cdot kg^{-1}$)
Fishmeal[a]	360	252
Brown shrimp processing remains[b]	–	300
Soymeal[c]	220	154
Wheatmeal[c]	319	223.3
Fishoil[a]	20	14
Lecithin (soy)[d]	20	14
Gluten (wheat)[c]	50	35
Vitamin and mineral premix[e]	5	3.5
Yttrium oxide[f]	5	3.5
Cholesterol[f]	1	0.7
Proximate composition ($g \cdot kg^{-1}$)		
Dry matter	916	942
Crude protein (N·6.25)	392	436
Gross Energy ($MJ \cdot kg^{-1}$)	19.3	18.3

[a] Bioceval GmbH & Co. KG (Cuxhaven, Germany)
[b] Kocken & Ehlerding Krabbenhandels-GmbH (Bremerhaven, Germany)
[c] Mühle Schlingemann e.K. (Waltrop, Germany)
[d] Alexander Müller GbR (Herzlake, Germany)
[e] Research Diet Services (Wijk bij Duurstede, Netherlands)
[f] Merck KGaA (Darmstadt, Germany)

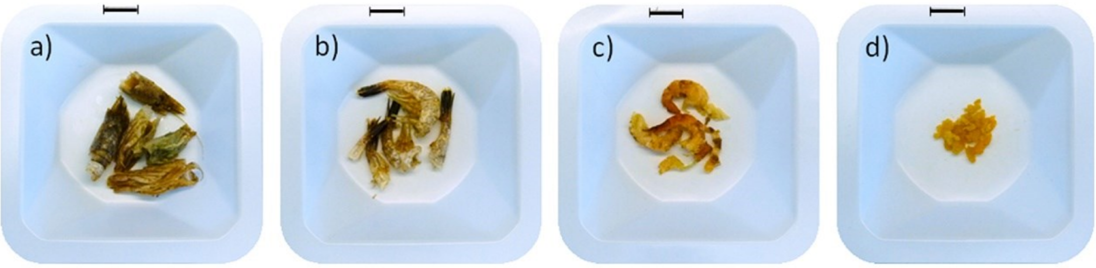

Fig. 1. Different body parts of *C. crangon* that remain after mechanical processing: a) cephalothorax, b) abdominal shell, c) abdominal muscle, and d) eggs. The scale indicates 1 cm.

pelleting. A pellet machine (PP200, Cissonius, Germany) with a die hole diameter of 2.5 mm was used to produce the feeds. Feed pellets were left to cool and dry for 24 h at room temperature and then stored in air tight casks at 4 °C until usage.

The digestibility trial was conducted at the Centre for Aquaculture Research of the Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research in Bremerhaven, Germany. Juvenile white leg shrimp (*Litopenaeus vannamei*) with an average weight of 9.4 ± 1.8 g were obtained from a local farm (Förde Garnelen GmbH & Co. KG, Germany) and acclimatized to experimental conditions for two weeks. The experiment took place in a recirculating aquaculture system (RAS) with 12 separated 50-liter aquaria. The RAS consisted of a mechanical filter, protein skimmer, biofilter, and a bypass for ozone and UV-treatment. Water inflow was set to be approximately 50 $l{\cdot}h^{-1}$ per tank and aeration of all aquaria was kept similar. Temperature, pH, conductivity, and dissolved oxygen concentration were measured constantly via sensors (Senect GmbH & Co. KG, Germany) immersed in the effluent water of the aquaria and recorded once a day. Manual measurements were done twice a week to verify the continuous sensor measurements. Water samples were taken twice a week to monitor ammonia, nitrite, and nitrate levels using an automated analyser (QuAAtro39 AutoAnalyzer, SEAL Analytical GmbH, Germany). Mean values and standard deviations of water parameters were: temperature 27.2 ± 0.4 °C, pH 7.97 ± 0.04, electrical conductivity 34.0 ± 0.9 $mS{\cdot}cm^{-1}$, dissolved oxygen 73.1 ± 3.8 %, ammonia 0.16 ± 0.03 $mg{\cdot}l^{-1}$, nitrite 0.16 ± 0.02 $mg{\cdot}l^{-1}$, and nitrate 87.43 ± 10.91 $mg{\cdot}l^{-1}$.

Each aquarium was stocked with 15 shrimp reaching a total biomass of 140.3 ± 3.3 g. The reference and test diets were randomly assigned to 6 replicate tanks. Shrimp were fed four times a day (9:00, 11:00, 14:00, 17:00) at a daily feeding rate of 4.5 % of the biomass as suggested by Tacon et al. (2013) for shrimp at this size and rearing temperature. The feeding rate was adjusted weekly assuming an average individual weight gain of 2 $g{\cdot}week^{-1}$, which was the maximum weight gain observed for shrimp in previous growth trials and this particular RAS. Shrimp were fed the experimental diets for one week prior to faeces collection. One hour after each feeding event, uneaten feed remains, exuviae, faeces, or dead shrimp were removed from the aquaria and discarded if present. Fresh and intact faeces were collected shortly before the next feeding using a fine meshed hand-net. Faeces were not collected for analysis prior to the morning feeding at 09:00 as they might have spent several hours overnight in the water and may have lost indefinite amounts of nutrients due to leaching.

If dead shrimp were present in aquaria and cannibalism could have occurred, faeces were not collected for analysis that day but discarded. Faeces of two aquaria with the same diet treatment were pooled in 50-ml centrifugation tubes and immediately stored at − 20 °C, which resulted in 3 replicates (n = 3) per treatment. Three weeks of feeding and faeces collection yielded enough material for analysis (~30 g wet weight per replicate) and the feeding trial was stopped. Faeces samples were lypholized (Christ Alpha 1–4 LSC, Martin Christ Gefriertrocknungsanlagen GmbH, Germany), ground to a fine powder using a manual mortar and stored in a desiccator until further analysis.

The apparent digestibility coefficients (ADC) of the tested nutrients of each diet were calculated after Cho and Slinger (1979):

$$ADC \quad (\%) = 100 - \left[100\left(\frac{Y_{diet}}{Y_{faeces}}\right)\cdot\left(\frac{N_{faeces}}{N_{diet}}\right)\right] \tag{1}$$

with Y being the yttrium, and N the considered nutrient concentration in diet and faeces samples based on the dry matter.

The apparent nutrient digestibility of the BSPR was then calculated following the equation of Bureau and Hua (2006):

$$ADC_{(BSPR)} = ADC_{test\ diet} + \left[\left(ADC_{test\ diet} - ADC_{ref\ diet}\right)\cdot\left(\frac{0.7\cdot N_{ref}}{0.3\cdot N_{BSPR}}\right)\right] \tag{2}$$

where N_{ref} and N_{BSPR} are the nutrient concentrations of the reference diet mash and shrimp processing meal (as is).

To determine the amount of digestible nutrients in BSPR, the apparent digestibility coefficients (ADC) was multiplied with the respective nutrient content.

2.4. *Data analysis*

Data compilation and calculations were made with Microsoft Excel. Comparisons of amino acid compositions of different shrimp meals were made with linear regressions using R (R Core Team, 2019) and the graphical illustration was made using GraphPad Prism.

3. Results

3.1. *Chemical composition of Brown Shrimp Processing Remains (BSPR)*

Based on dry weight, the majority of the remains consists of the cephalothorax with approximately 44 %. The abdominal shell makes up 35 %, whereas abdominal muscle contributes 18 % to the total remains. Eggs were also present and comprised 3 % of the brown shrimp processing remains.

After drying at 60 °C for 48 h, the moisture content decreased from 70 % to approximately 33 %, resulting in a dry matter content of 966 $g{\cdot}kg^{-1}$ of the BSPR. The main nutrient in the BSPR was protein followed by ash and chitin (Table 2). The total lipid content of 74 $g{\cdot}kg^{-1}$ included 9 $g{\cdot}kg^{-1}$ cholesterol. The astaxanthin content in the oven dried BSPR accounted for only 62 % of the astaxanthin content of the lyophylized BSPR, with 1.8 $mg{\cdot}kg^{-1}$. The caloric energy content was 15 $MJ{\cdot}kg^{-1}$.

The predominant amino acids were glutamine, asparagine, arginine, glycine, lysine, and leucine, which together account for approximately 55 % of the total amino acids (Table 3). Cysteine, tryptophan, methionine, and histidine were present in smaller amounts, ranging from 1.3 % to 2.7 % of the total amino acids. Comparison of BSPR and amino acid compositions of different shrimp meals reported in literature showed correlation coefficients (r^2) from 0.04 to 0.93 (Table 4).

The fatty acid composition of BSPR was dominated by monounsaturated fatty acids (MUFA) at 42 % of the total fatty acid content (10 $mg{\cdot}kg^{-1}$, Table 5). Saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) were detected at similar levels, 7.1 and 6.8 $mg{\cdot}kg^{-1}$, respectively. Eicosapentaenoic acid (EPA, C20:5(n-3)), oleic acid (C18:1 (n-9c)), palmitoleic acid (C16:1(n-7c)) and palmitic acid (C16:0) showed highest concentrations, contributing 12–20 % to the total fatty acids. Vaccenic acid (C18:1(n-7)), docosahexaenoic acid (DHA, C22:6(n-3)) and stearic acid (C18:0) were present in moderate amounts ranging from 3 % to 7 % of the total fatty acids.

Calcium, phosphorus, sodium, potassium, and magnesium were the most abundant minerals (Table 6). Calcium showed the highest concentration with 90 $g{\cdot}kg^{-1}$, followed by phosphorus and sodium. Potassium and magnesium were present in smaller amounts. Zinc, manganese, copper, and selenium were present at concentrations in the $mg{\cdot}kg^{-1}$ range.

Table 2

Gross nutrient composition of mechanical processed *Crangon crangon* remains (BSPR) (values expressed as $g{\cdot}kg^{-1}$ "as is" unless otherwise indicated).

Nutrient ($g{\cdot}kg^{-1}$, unless otherwise indicated)	BSPR
Dry matter	966
Crude protein (N·6.25)	521
Gross energy ($MJ{\cdot}kg^{-1}$)	15
Total lipid	74
Ash	244
Chitin	90
Cholesterol	9
Astaxanthin (lyophilized) ($mg{\cdot}kg^{-1}$)	2.9
Astaxanthin (oven dried) ($mg{\cdot}kg^{-1}$)	1.8

Table 3
Amino acid content of mechanical processed *Crangon crangon* remains (BSPR).

Amino acid (g·kg^{-1})	BSPR
Arginine	37
Histidine	12
Isoleucine	21
Leucine	31
Lysine	33
Phenylalanine	19
Methionine	12
Threonine	20
Valine	23
Asparagine	48
Glutamine	67
Alanine	26
Cysteine	6
Glycine	36
Serine	22
Proline	21
Tyrosine	21
Tryptophane	6

3.2. *Contaminants*

The concentrations of heavy metals mercury and cadmium were less than 0.2 mg·kg^{-1}. Likewise, the lead content was below the detection limit of 0.2 mg·kg^{-1}. Arsenic concentration was highest, reaching an average value of 12.8 mg·kg^{-1} (Table 7).

The sum of polyaromatic hydrocarbons (PAHs) in BSPR was below the detectable threshold of 10 µg·kg^{-1}. The polychloride biphenyls 28 (2,4,4’-Trichlorobiphenyl), 52 (2,2’,5,5’-Tetrachlorobiphenyl) and 101 (2,2’,4,5,5’-Pentachlorobiphenyl) were not detectable, whereas the PCB 138 (2,2’,3,4,4’,5’-Hexachlorobiphenyl), 153 (2,2’,4,4’,5,5’-Hexachlorobiphenyl) and 180 (2,2’,3,4,4’,5,5’-Heptachlorobiphenyl) had values of 6.2, 2.5, and 1.8 µg·kg^{-1} (Table 7).

3.3. *Digestibility of BSPR by Litopenaeus vannamei*

The apparent dry matter digestibility reached mean values of 64 %. Related to the dry matter content in BSPR, 615 g kg^{-1} is available for digestion (Table 8). The apparent energy digestibility was 82 % and reached 12 MJ·kg^{-1} in BSPR. The ADC for protein showed higher values of 86 % which results in a protein digestibility of about 450 g·kg^{-1} in BSPR. The ADCs for the essential amino acids methionine and lysine reached values exceeding 100 %. To calculate the digestible levels of these amino acids in BSPR, a complete bioavailability was assumed, leading to 33.1 g·kg^{-1} and 11.5 g·kg^{-1} for methionine and lysine, respectively. Apparent copper digestibility coefficients showed mean values of 93 %. The total bioavailable copper content in BSPR therefore resulted in 0.038 g·kg^{-1}.

4. Discussion

4.1. *Chemical composition of brown shrimp processing remains*

Brown shrimp processing remains are a heterogeneous mixture of different body parts and organs. Beside the chitinous cuticle and substantial amounts of internal organs and muscle tissue, a small amount of eggs is present as well. This gross composition is reflected in the nutrient profile of the BSPR. The chitin content merely made up 90 g·kg^{-1}, whereas the amount of protein exceeded 500 g·kg^{-1}. Synowiecki and Al-Khateeb (2000) investigated *C. crangon* processing remains and isolated almost twice as much chitin (178 g·kg^{-1}) but reported a lower protein content of 406 g·kg^{-1}. These differences can be explained by the different way of shrimp processing, i.e. mechanical peeling vs. manual peeling. The mechanical peeling is less accurate than the manual peeling. It shreds the shell to a higher degree. Smaller chitin particles are washed away along the further separation process and lack in the total chitin calculation. On the other hand, mechanical peeling leaves a higher share of partially squashed muscle tissue within the remains. Manual peeling separates the abdominal muscle from the shell and the cephalothorax more completely. Therefore, the amount of protein is higher in the mechanically peeled remains. The protein value is in the same range as in other shrimp by-products. Shrimp head meals from *L. vannamei* or *P. monodon* contain protein in the range of 371–566 g·kg^{-1} (Fox et al., 1994; Liu et al., 2013; Terrazas-Fierro et al., 2010; Villarreal et al., 2006; Yang et al., 2009). The ash content of shrimp meals reported in these studies ranged from 190 to 453 g·kg^{-1}, with higher values in meals containing smaller amounts of protein.

The amino acid composition of BSPR resembles that of other shrimp meals of either undefined origin or of meals from *L. vannamei* and *P. varians*, respectively (Liu et al., 2013; Salas-Leiton et al., 2020; Terrazas-Fierro et al., 2010). Prevalent amino acids of these shrimp meals were asparagine, arginine, glutamine, lysine, and leucine. Lower contents were reported for phenylalanine, cysteine, methionine, and histidine. The amino acid values of these meals correlated closely (r^2 = 0.82–0.90). In contrast, shrimp meals investigated by Nwanna (2003)

Table 4
Amino acid compositions of shrimp head meals from the literature used for linear regression comparisons with brown shrimp processing remains.

Amino Acid (g 100 g Protein^{-1})	Liu et al. (2013)	Terrazas-Fierro et al. (2010)	Fox et al. (1994)	Salas-Leiton et al. (2020)	Nwanna (2003)
Arginine	7.4	7.1	4.2	11.7	2.3
Histidine	2.2	2.5	1.3	2.8	0.6
Isoleucine	2.5	5.1	2.5	3.5	1.5
Leucine	4.6	8.1	4.4	6.4	2.4
Lysine	8.0	8.5	4.3	6.8	2.4
Phenylalanine	1.3	5.9	16.1	4.6	5.8
Methionine	4.9	1.6	0.4	2.4	1.1
Threonine	4.7	4.2	2.9	4.1	2.4
Valine	4.8	5.6	3.2	3.9	1.8
Asparagine	12.3		7.3	10.6	
Glutamine	12.1		10.0	13.7	
Alanine	6.1		5.1	6.1	
Cysteine	0.7	0.9	0.4	1.1	
Glycine	7.2		5.0	8.2	
Serine	4.6		3.2	4.4	
Proline	2.7		4.0	5.7	
Tyrosine	3.2		2.6	4.1	0.5
Tryptophan					
r^2	0.82	0.87	0.24 (0.93)*	0.90	0.04 (0.52)*

* r^2 values if phenylalanine is not considered.

Table 5
Fatty acid contents in mechanically processed *Crangon crangon* remains (BSPR).

Fatty acids	BSPR (%)	BSPR (g·kg^{-1})
Saturated fatty acids (SFA)		
C12:0	0.3	0.1
C14:0	2.8	0.7
C15:0	1.3	0.3
C16:0	19.9	4.8
C17:0	0.8	0.2
C18:0	3.1	0.8
C20:0	0.3	0.1
C22:0	0.2	0.1
C24:0	0.3	0.1
Total SFA	29.2	7.1
Monounsaturated fatty acids (MUFA)		
C16:1(n-7c)	15.8	3.8
C16:1(n-7t)	2.0	0.5
C18:1(n-9c)	14.1	3.4
C18:1(n-9 t)	1.8	0.4
C18:1(n-7)	7.4	1.8
C20:1(n-9)	0.6	0.1
C24:1(n-9)	0.4	0.1
Total MUFA	42.4	10.3
Polyunsaturated fatty acids (PUFA)		
C18:2(n-6)	1.3	0.3
C20:2(n-6)	0.4	0.1
C22:2(n-6)	0.3	0.1
C18:3(n-3)	1.5	0.4
C20:4(n-6)	2.2	0.5
C22:4(n-6)	1.2	0.3
C20:5(n-3)	11.9	2.9
C22:5(n-3)	3.6	0.9
C22:6(n-3)	5.1	1.2
Total PUFA	27.8	6.8

Table 6
Mineral content of mechanical processed *Crangon crangon* remains (BSPR).

Mineral	BSPR
Macrominearls (g·kg^{-1})	
Calcium	90
Phosphorus	16
Potassium	7
Magnesium	3
Microminerals (mg·kg^{-1})	
Zinc	101
Copper	41
Manganese	10
Selenium	2

Table 7
Concentration of contaminants measured in mechanically processed *Crangon crangon* remains (BSPR).

Contaminants	BSPR
Heavy metals (mg·kg^{-1})	
Arsenic	12.8
Led	< 0.2
Cadmium	0.16
Mercury	0.15
EPA-PAHs (μg·kg^{-1})	< 10
NDL-PCBs (μg·kg^{-1})	
PCB 28	< 1.0
PCB 52	< 1.0
PCB 101	< 1.0
PCB 138	6.2
PCB 153	2.5
PCB 180	1.8
Sum of NDL-PCBs	13.5

Table 8
Apparent digestibility coefficients (ADC) for dry matter, energy, protein, methionine, lysine, and copper in brown shrimp processing remains (BSPR) and the calculated amounts of the digestible nutrients.

Nutrient	ADC (%)	Digestible nutrient in BSPR (g·kg^{-1})
Dry matter	63.7 ± 0.5	615
Energy (MJ kg^{-1})	81.5 ± 4.4	12.2
Protein	86.2 ± 3.0	449
Methionine	108.9 ± 3.5	33.1*
Lysine	109.0 ± 1.6	11.5*
Copper	92.6 ± 2.7	0.038

* Methionine and lysine digestibility in BSPR is calculated with an ADC of 100 %.

and Fox et al. (1994) showed different amino acid profiles than the BSPR meal (r^2 = 0.04 and 0.23). The differences rest primarily upon the phenylalanine content. If this amino acid is omitted from the correlation, the coefficients increases to 0.52 and 0.93, respectively. Therefore, despite single differences between species or processing method, shrimp meals are largely similar in their amino acid profiles. Likewise, whole body amino acid compositions in fish of different species and sizes are largely the same (NRC, 2011; Kaushik and Seiliez, 2010; Wilson and Cowey, 1985). Moreover, the amino acid composition of BSPR meets the ideal dietary essential amino acids (EAA) requirement of penaeid shrimp (Fig. 2.). About 41 % of the total amino acids present in BSPR are EAA. The amount of EAA exceeds the recommended dietary concentrations by 25 % for threonine, to up to 156 % for tryptophan. This supports the suitability of BSPR as a valuable nutritive dietary protein and amino acid source for *L. vannamei* aquafeeds.

The energy content of the BSPR is similar to that of the head meal made of *L. vannamei* and the 'unspecified' shrimp head meal investigated by Terrazas-Fierro et al. (2010) and Liu et al. (2013), with values of 12.2 and 16.3 MJ·kg^{-1}. The shrimp by-product meal analysed by Yang et al. (2009) showed a higher energy content of 21 MJ·kg^{-1}, but it was not further described what shrimp species the meal was made from.

Shrimp meals show a high variation in total lipid content, ranging from 17 to 100 g·kg^{-1} (Fox et al., 1994; Liu et al., 2013; Nwanna, 2003; Salas-Leiton et al., 2020; Terrazas-Fierro et al., 2010; Villarreal et al., 2006; Yang et al., 2009). This variability might be related to differences in shrimp species, body parts used, processing methods, and seasonal variations. Lipid metabolism and energy storage is known to differ between crustacean taxa due to species specific life strategies and evolutionary adaptations (Lee et al., 2006; Martínez-Alarcón et al., 2019). The BSPR meal contains relatively high lipid levels and is similar to meals

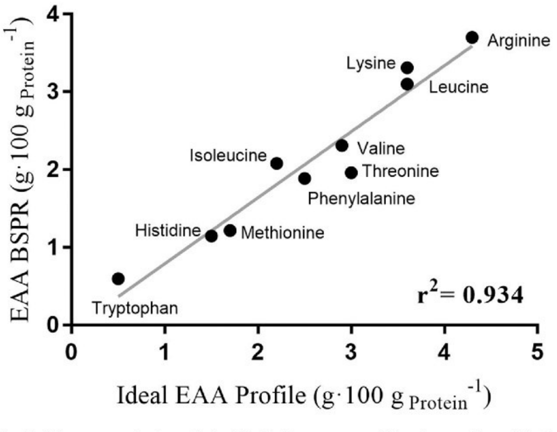

Fig. 2. Linear correlation of the ideal dietary essential amino acid profile for penaeid shrimp (NRC, 2011) and the essential amino acid content of brown shrimp processing remains (BSPR), expressed as g·100 g$^{-1}_{\text{Protein}}$.

made from *P. varians* and shrimp head meals made from *P. monodon* and *L. vannamei* (Fox et al., 1994; Salas-Leiton et al., 2020; Villarreal et al., 2006). Interestingly, the lipid content in BSPR meal comprises higher levels of polar phospholipids and free fatty acids which are more readily digested than triacylglycerols (Martínez-Alarcón et al., 2019; Mika et al., 2014).

Analysis of the fatty acid profile showed a balanced spectrum of saturated, monounsaturated and polyunsaturated fatty acids. Together, mono-and polyunsaturated fatty acids account for about 70 % of the total fatty acid content. On a percentage basis, this is similar to the *P. monodon* head meal investigated by Fox et al. (1994). The long chained polyunsaturated fatty acids EPA, DHA, and arachidonic acid were also detected at moderate amounts, but were not high when compared to meals made from *P. varians*, *P. monodon*, and snow crab (*Chinoecetes opilio*) (Shahidi and Synowiecki, 1991; Fox et al., 1994; Salas-Leiton et al., 2020).

Crustaceans are not capable of synthesizing sterols de novo (Teshima, 1997). Therefore, cholesterol is an essential nutrient, which has to be accounted for in aquafeed formulations for penaeid shrimp. This is especially important when feed formulations are based on plant-derived raw materials, which are naturally low in cholesterol (Cheng and Hardy, 2004) and costly cholesterol supplementation is needed. Krzynowek and Panunzio (1989) investigated the cholesterol content in shrimp muscles of different species and geographical origin and found average cholesterol levels of 7.5–9.5 g·kg^{-1} on a dry matter basis. The cholesterol content of BSPR is thus in the upper range with 9 g·kg^{-1}. Most studies investigating shrimp derived raw materials did not report cholesterol contents. However, in view of varying total lipid contents (Liu et al., 2013; Terrazas-Fierro et al., 2010; Yang et al., 2009), distinct cholesterol concentrations are likely. Cholesterol requirements in diets for *L. vannamei* are satisfactory at levels between 0.13 % and 0.35 %, depending on dietary phospholipid concentrations (Gong et al., 2000). Addition of BSPR in *L. vannamei* diets could therefore provide sufficient cholesterol for optimal growth, without the need of any further supplementation.

The astaxanthin content of BSPR varied between 1.8 and 2.9 mg·kg^{-1}, depending on the drying method. Astaxanthin is a powerful antioxidant (Cahú et al., 2012) and seems to be vulnerable to the constant hot air treatment during the oven drying process. Fox et al. (1994) investigated *P. monodon* derived shrimp head meals and also found reduced astaxanthin levels when meals were oven dried. Hu et al. (2019) even found an almost 10-fold reduction in astaxanthin recovery when *P. borealis* shells were dried in ventilation, compared to fresh material. Freeze drying is therefore a more suitable treatment if astaxanthin has to be preserved in BSPR. Cooking has also been reported to decrease the astaxanthin content of shrimp shells (Hu et al., 2019). This might explain the generally low astaxanthin level in BSPR, as brown shrimp are directly cooked on board the fishing vessel after the catch.

The two main macro-minerals in BSPR are calcium and phosphorus with 90 g·kg^{-1} and 16 g·kg^{1}, respectively. The high calcium content probably derived from the exoskeleton present in the processing remains. Crustacean exoskeletons are primarily mineralised with calcium carbonate (Conklin, 1982), but also small amounts of magnesium and phosphorus have been reported. Nwanna (2003) investigated fermented shrimp head waste meal (originating from 4 different penaeid species) and found almost identical calcium and phosphorus concentrations of 87.2 g·kg^{-1} and 16.8 g·kg^{-1}. Since marine crustaceans can take up minerals from the ambient seawater (calcium, potassium, and magnesium), dietary supplementation is often dispensable or hard to determine (NRC, 2011). But minimum dietary requirements for phosphorus, magnesium, copper, manganese, selenium, and zinc for *L. vannamei* have been summarised by the NRC (2011). All of these minerals are present at reasonable concentrations in BSPR.

4.2. *Contaminants*

A crucial step in raw material characterisation is the assessment of related risks that might be inherent in the resource (Glencross et al., 2020). Marine organisms are known to accumulate heavy metals and persistent organic pollutants depending on species, trophic level, and geographic distribution (Costa and Fattori, 2010).

The heavy metal concentrations of cadmium, lead and mercury in BSPR were all markedly below the maximum levels defined by the European Union for foods (The European Commission, 2006). On wet weight basis, the concentrations did not exceed 10 % of the official guidelines defined acceptable for human consumption. Marx and Brunner (1998) measured heavy metal concentrations in brown shrimp caught in German mud flats area of the southern North Sea and reported similar results. These findings indicate that the metal contents in brown shrimp did not alter dramatically over the past two decades in the German Bight.

The arsenic content in the BSPR is higher than that of the other analysed heavy metals. Seafood is known to contain high amounts of arsenic, which is primarily bound in organic compounds such as arsenobetaine, arsenosugars, and arsenolipids (Taylor et al., 2017). The toxicity of arsenic is attributed to the inorganic forms (Ahsan et al., 2006) while the organic bound arsenic appears to cause no or minor toxic effects (Arnold et al., 2003; Cano et al., 2001). Ruttens et al. (2012) analysed arsenic compounds in shrimp and found the contribution of inorganic forms to be minor (less than 1.5 %). The arsenic content in BSPR is therefore in the range typical for marine organisms and can be considered innocuous.

According to the regulations of the European Union (The European Commission, 2011), the levels of ndl-PCBs reported in our study are within the permitted range and are acceptable for human consumption. Raemaekers et al. (2006) monitored the concentrations of polychlorinated biphenyls and organochlorine pesticide in brown shrimp over 11 years and observed a steady-state at low levels as well. Based on these findings, brown shrimp and brown shrimp processing remains originating from the North Sea are a safe marine resource suitable as raw material for aquaculture feeds.

4.3. *Digestibility*

To evaluate new feed ingredients, it is important to know the amount of nutrients present and also their bioavailability to the consuming species. This is commonly estimated via the indirect measurement of digestibility proposed by Cho and Slinger (1979) and modified by Bureau and Hua (2006).

The apparent dry matter digestibility of BSPR is within the range of 50–84 % that have been reported for different shrimp by products meals in *L. vannamei* feeds (Liu et al., 2013; Yang et al., 2009, Terrazas-Fierro, 2010). Dry matter and energy in shrimp by product meals were shown to be less well digested than in high quality fishmeal (Yang et al., 2009; Liu et al., 2013). Authors suggested this might be related to the high ash and chitin content present in crustacean derived meals. On the other hand, Terrazas-Fierro et al. (2010) reported excellent dry matter digestibility in shrimp head meals, exceeding values of various tested fishmeals. The apparent digestible energy coefficients of BSPR also exceeded values reported by Liu et al. (2013) and Yang et al. (2009) by about 10–20 %. This points out varying nutritional qualities of shrimp meals of different origin and processing method.

The apparent protein digestibility was high. The ADC protein might even be slightly underestimated, since the nitrogen content of the peritrophic membrane surrounding the faecal strings could not be quantified. Protein in shrimp by-product meals was demonstrated to be highly digestible for *L. vannamei* and reached ADCs from 79 % to 98 % (Yang et al., 2009; Liu et al., 2013; Terrazas-Fierro et al., 2010). The methionine and lysine digestibility measured in this study exceeded values of 100 %. Digestibility values exceeding 100 % are not uncommon

(Cruz-Suárez et al., 2009; Rivas-Vega et al., 2009; Terrazas-Fierro et al., 2010) and can have several reasons related to the indirect digestibility measurement in aquatic nutritional studies, as explained by Glencross et al. (2007). As pointed out by Cruz-Suárez et al. (2009) the amino acids methionine and lysine are highly soluble in water and leaching from the faeces is probably one reason. Nevertheless, our results suggest that BSPR are a good source of highly available protein and essential amino acids.

Copper is a central element in many fundamental metabolic processes (NRC, 2011; O'Dell, 1976; White and Rainbow, 1985) and is especially relevant for crustaceans. In crustaceans, copper is needed for the respiratory pigment haemocyanin, which can account for up to 40 % of the total body copper content (Depledge, 1989). The copper-dependent enzymes superoxide dismutase and phenoloxidase play key roles in the crustacean immunological defence system (Culotta et al., 2006; Sritunyalucksana and Söderhäll, 2000). There is evidence that reproduction and molting behaviour in shrimp is also affected by copper (Rao and Anjaneyulu, 2008; Shan et al., 2019). The bioavailability of minerals depends on their chemically bound form and other constituents present in formulated diets such as anti-nutritional factors (Lin et al., 2013; NRC, 2011). The almost complete apparent copper digestibility of 0.038 $g \cdot kg^{-1}$ in BSPR by *L. vannamei* might be explained by the organically bound copper present in BSPR which seem to facilitate its uptake and bioavailability.

4.4. Resource utilization

Assuming annual brown shrimp landings of up to 30,000 tons in the North Sea, dried processing remains would account for 5400 tons per year. Land based shrimp production in Europe is an emerging and dynamic sector with an estimated production of 447 tons in 2020 and a growing tendency (Euroshrimp, 2021). Brown shrimp processing remains could theoretically cover the entire nutritive protein demand of the current land based penaeid culture in Europe. Through creating further application as a by-product, value is added to the brown shrimp industry, which in turn could promote local processing. The establishment of local value chains and circular economy approaches could lead to substantial reduction of biological waste, transport routes, and associated CO_2 emissions.

5. Conclusions

Brown shrimp processing remains are a valuable by-product, containing substantial amounts of essential dietary macro- and micronutrients needed for penaeid aquafeeds. The apparent digestibility of key nutrients is excellent and provides the necessary baseline information for adequate diet formulations. The processing remains of brown shrimp, therefore, represent an underutilized marine resource with great potential as alternative and sustainable aquafeed ingredient, particularly for local application in the European market.

CRediT authorship contribution statement

Enno Fricke: Investigation, Methodology, Data curation, Writing – original draft, **Marie Koch:** Methodology, Writing – review & editing, **Heiko Dietz:** Methodology, Validation, **Matthew James Slater:** Conceptualization, Resources, Writing – review & editing, Supervision. **Reinhard Saborowski:** Conceptualization, Validation, Resources, Writing – review & editing, Supervision, Project administration, funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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2.3 Publication II

Brown shrimp (*Crangon crangon*) processing remains enhance growth of Pacific Whiteleg shrimp (*Litopenaeus vannamei)*

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Brown shrimp (*Crangon crangon*) processing remains enhance growth of Pacific Whiteleg shrimp (*Litopenaeus vannamei*)

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ABSTRACT

Underutilized seafood by-products bear considerable potential to reduce economically and environmentally unsustainable fish meal and oil use in aquaculture feeds. The current study investigated the suitability of brown shrimp, *Crangon crangon*, processing remains (BSPR) as feed ingredient for Pacific Whiteleg shrimp, *Litopenaeus vannamei*. Two controlled feeding trials with juvenile *L. vannamei* were conducted to determine growth performance, feed utilization, digestive enzyme activities, and haematological parameters. In a preliminary feeding trial, shrimp showed best weight gain with diets replacing 50% of fishmeal with BSPR when compared to the control ($p = 0.003$). Muscle tissue (moisture, protein, lipid) and digestive enzyme activities were not affected by diet. The daily molting ratio increased significantly ($p = 0.005$) with high BSPR inclusion levels and a higher molting synchronicity was observed. The second feeding trial with diets representing 0, 20, 40, 60, 80, and 100% replacement of fishmeal with BSPR showed significantly enhanced growth when replacement exceeded 60%. Concomitantly, feed conversion ratios improved by up to 37% when BSPR was included in higher proportions than fishmeal. Shrimp haematology (protein, haemocyanin, phenoloxidase activity, prophenoloxidase activity) was not affected by dietary treatment, suggesting good health and nutritional status of the shrimp. Brown shrimp processing remains are a nutritive valuable, growth promoting feed ingredient for *L. vannamei*.

1. Introduction

Driven by the steadily growing aquaculture sector, aquafeed producers are continuously searching for new sources of economically viable and sustainable feed ingredients. The global demand for commercial aquaculture feed increased from <20 million metric t in the year 2000, to >50 million t in 2017, and is expected to exceed 70 million t by 2025 (Tacon, 2019). Prior to 2000, aquaculture feeds contained large amounts of relatively inexpensive fishmeal and fish oil, derived from small pelagic species (Naylor et al., 2000). However, the number of overexploited fish stocks continues to increase globally and most are fished at their biological limits (FAO, 2020). Consequently, fishmeal production has stagnated over the last decades and prices continue to increase along with the rising demands (Cottrell et al., 2020).

These ecological and economic concerns have created a need for alternative ingredients as protein and lipid sources for aquaculture feeds. Among possible alternatives, terrestrial plant-based ingredients are widely studied and applied in feeds (Bartley, 2022). Issues related to proteinaceous plant ingredients are the presence of antinutritional factors (e.g. protease inhibitors, phytate, non-starch polysaccharides) and amino acid profiles that are suboptimal for most aquaculture species (Alarcón et al., 2001; Francis et al., 2001; Glencross, 2009). Advances in research and feed formulations have helped to overcome many of these nutritional challenges and commercial aquafeeds now routinely include significant amounts of plant-based ingredients (Kaushik et al., 2004; Klinger and Naylor, 2012; Napier et al., 2020). Still, most cultured carnivorous species rely on a minimum of marine sourced ingredients for healthy growth (Naylor et al., 2021). The increased demand for terrestrial crops for aquafeeds may also further intensify industrial agriculture practices, leading to higher land and fresh water usage, deforestation and environmental pollution (DeLonge et al., 2016; Fry et al., 2016). Furthermore, aquafeed ingredients such as soy and wild forage fish are suitable for direct human consumption rather than being processed as feedstuff. Therefore, aquafeed raw materials based on residual biogenic material that do not divert human food resources are favourable from multiple perspectives.

Responsible utilization of natural resources is one key element of the UN sustainable development goals and reduction of food loss throughout

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the supply chain has a high priority (UN General Assembly, 2015). Following the principles of circular economy, political agendas such as the European Green Deal encourage recycling and up-valorisation of underutilized resources (The European Commission, 2020). By-products, side-streams, or rest raw materials are terms applied to materials which are left over following the preparation of a 'main' product. These can be recycled or used for further applications (Rustad et al., 2011). The inclusion of by-products into aquaculture feeds reduces waste, recycles valuable nutrients, and can markedly decrease their environmental impact (Afreen and Ucak, 2020; Leyva-López et al., 2020). By-products applicable for aquaculture feeds range from agricultural, livestock, and seafood industries (Pelletier et al., 2018). Caruso et al. (2020) estimated that on a global scale, the seafood industry produces approximately 27.85 million t of discards which are accessible for further applications. By-products from seafood processing comprise a huge variety of biological materials (bones, skins, meat-residue, viscera) originating from a broad range of marine taxa including fish, molluscs, and crustaceans (Nguyen et al., 2017; Rustad et al., 2011; Sudaryono et al., 1996). Although it is common practice to convert seafood remains into fishmeal used for aquaculture feeds (Huntington and Hasan, 2009), substantial volumes of marine by-products remain unused (Jackson and Newton, 2016). Taking into account the large diversity of seafood by-products, a more distinctive evaluation of the different raw materials helps to identify most suitable and efficient utilization.

Approximately 300 different shrimp species are commercially exploited worldwide (Gillet, 2008). Shrimp products are primarily traded without the cephalothorax and exoskeleton, leaving 50–70% of the processed biomass as remains or discards (Ramírez, 2013; Saborowski et al., 2022). By-products from shrimp processing industries contain substantial amounts of valuable biomolecules such as chitin, protein, lipids, and astaxanthin (Fox et al., 1994; Shahidi and Synowiecki, 1991). Depending on species origin and processing method, shrimp waste meal can differ quite substantially in their chemical composition (Fox et al., 1994; Fricke et al., 2022).

Crustacean meals were shown to be suitable feed ingredients for several penaeid species (e.g. *Litopenaeus stylirostris, Penaeus monodon, Litopenaeus vannamei*) (Amar et al., 2006; Ambasankar et al., 2022; Córdova-Murueta and García-Carreño, 2002; Sabry-Neto et al., 2017; Smith et al., 2005; Suresh et al., 2011; Villarreal et al., 2006; Williams et al., 2005). Tested meals were made from different taxa (e.g. Penaeidae, Munididae, and Euphausidae), different raw materials (whole animal, offal, heads) and were processed in different ways (oven-dried, lyophilized, cooked, fermented, enzymatically hydrolyzed).

The brown shrimp, *Crangon crangon,* is the most important commercial fishery in the southern North Sea with annual landings of 30,000 t and more (ICES, 2022). Only the muscle tissue of the pleon is used for human consumption. The processing remains account for up to 21,000 t (Saborowski et al., 2022). When crustacean-based meals are included in diets for shrimp feeds, sanitary risks and regulations should to be taken into account to prevent possible disease transmissions. Previous disease outbreaks led to EU legislations strictly regulating the use of animal-based feed ingredients (The European Commission, 2001). Despite most restrictions being lifted, intra-species recycling remains prohibited in the European Union (The European Commission, 2009b). In this case cold water brown shrimp belongs to the taxonomic suborder of Pleocyemata and are genetically distinct from L. *vannamei*, (suborder Dendrobranchiata) (WoRMS Editorial Board, 2022). No regulatory limitation to commercial use in future exist in the current regulatory framework.

The remains contain substantial amounts of key nutrients (521 $g \cdot kg^{-1}$ crude protein, 15 $MJ \cdot kg^{-1}$ gross energy, 74 $g \cdot kg^{-1}$ total lipid) which meet the nutritional demands of *L. vannamei* (Fricke et al., 2022). Yet information is lacking on how brown shrimp processing remains (BSPR) affect growth and physiology of *L. vannamei* when included in formulated diets. While growth and survival are the parameters of primary concern in aquaculture production, metabolic and immunological parameters provide further insights into the functionality of the ingredient (positive or negative) and the nutritional condition of the studied species (Lemos et al., 2000; Pascual et al., 2003; Weiss et al., 2020). In the present study, feed acceptability was assessed and two consecutive feeding trials were conducted to evaluate the suitability and appropriate inclusion level of BSPR in diets for *L. vannamei.* Along with growth performance traits, various parameters (molting frequency, digestive enzyme activities, haematological characteristics) were investigated.

2. Material and methods

2.1. Brown shrimp processing remains (BSPR)

Approximately 100 kg of frozen, mechanically peeled brown shrimp remains were obtained from a local shrimp trading company (Alwin & Siegfried Kocken GmbH, Spieka-Neufeld, Germany). The frozen remains were oven-dried in several batches using glass dishes (25 cm × 36 cm × 5 cm (w x l x h)) at 60°C for 48 h until the moisture content dropped from about 70%, to <5%. This drying process resulted in a dry BSPR meal recovery of 25%. The dried remains were ground with a knife mill in alternate 30 s intervals of milling and rest to avoid excessive heat development (GM 200, Retsch, Germany) and passed through a 500-μm sieve using a vibrational sieving machine (Analysette, Fritsch GmbH, Germany). This process was repeated up to six times, until all particles of the BSPR passed through the sieve. The resulting BSPR meal was stored in air-tight casks at 4°C until the experimental feeds were produced.

2.2. Acceptability and preliminary-trial

2.2.1. Diet preparations

The commercially available fishmeal used to produce the experimental diets in this study was made from a mixture of by-products originating from wild and cultured fish species (*Gadus morhua, Pollachius virens, Scomber scombrus, Clupea harengus, Oncorhynchus mykiss, Cyprinus carpio*). A control diet with fishmeal and two experimental diets, replacing 50 and 100% of the fishmeal with BSPR, were used to determine the acceptability of the diets (Table 1). The same feeds were then used for a 39-day trial to assess effects of BSPR on survival, growth, molting frequency, gross chemical composition of muscle tissue, digestive enzyme activities, and hemocyanin levels in *L. vannamei.* Experimental feeds were formulated to meet the nutritional requirements of *L. vannamei* (NRC, 2011). All diets were formulated to be similar in their digestible protein and energy levels, assuming an apparent protein and energy digestible coefficient of 86.2% and 81.5% for BSPR (Fricke et al., 2022). To maintain a homogenous particle size of the feed mixtures, all ingredients were processed to a homogenous meal (<500 μm) as described above.

To prepare the feed mixtures, dry ingredients needed in smallest amounts were mixed first, and stepwise raw materials were added that were needed in larger quantities. Fish oil and lecithin were emulsified before they were added to the diet mixtures. The resulting dough was mixed thoroughly and water was sprayed onto the mixture to achieve a moisture content of approximately 15%. The dough was then conditioned at 40°C for 30 min. After conditioning, the feed mixtures were repeatedly passed through a pelleting machine (die hole diameter 2.5 mm, PP120, Cissonius, Zehdenick, Germany) three consecutive times. The resulting feed pellets were dried for 24 h at room temperature (approximately 22°C) and stored in air-tight casks at 4°C until the start of the experiment two weeks later.

2.2.2. Experimental system and design

All feeding experiments were conducted at the Centre for Aquaculture Research of the Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research in Bremerhaven, Germany. Experiments were conducted in compliance with official animal experiment regulations and registered at designated authorities (Altfeed; TA21–04). The

Table 1

Ingredients, chemical composition, and water stability of diets prepared for the acceptability- and preliminary-trial. Values expressed as g·kg^{-1} "as is", unless otherwise indicated. BSPR – Brown shrimp processing remains.

Ingredient	Control	BSPR 50	BSPR 100
Fishmeal [1]	360	180	0
BSPR [2]	0	180	360
Soymeal [3]	220	220	220
Wheatmeal [4]	319	320	320
Fishoil [5]	20	20	20
Lecithin (soy) [6]	20	20	20
Gluten (wheat) [7]	0	20	50
Alginate [8]	50	30	0
Vitamin and mineral premix [9]	5	5	5
Met-Met [10]	5	5	5
Cholesterol [11]	1	0	0
Chemical composition & water stability			
Dry matter	925 ± 15 ^{a}	926 ± 10 ^{ab}	936 ± 4 ^{b}
Protein (N·6.25)	368 ± 4	366 ± 11	363 ± 5
Gross Energy (MJ·kg^{-1})	17.76 ± 0.06 ^{c}	17.53 ± 0.02 ^{b}	17.12 ± 0.04 ^{a}
Total lipid	72.4 ± 3.2	79.8 ± 8.6	71.3 ± 11.0
Crude fibre	13	30	46
Ash	97.2 ± 0.1 ^{a}	106.2 ± 0.4 ^{ab}	124.6 ± 0.4 ^{b}
Water stability (% DM loss)	87.2 ± 5.6 ^{b}	78.1 ± 13.3 ^{ab}	54.1 ± 9.1 ^{a}

[1] Protein 64%, lipid 9%, ash 21%; Bioceval GmbH & Co. KG (Cuxhaven, Germany).

[2] Protein 52%, lipid 7%, ash 24%, chitin 9%; Kocken & Ehlerding Krabbenhandels-GmbH (Bremerhaven, Germany).

[3] Protein 48%, lipid 3%, ash 6%; Agravis Raiffeisen AG (Bremerhaven, Germany).

[4] Protein 12%, lipid 2%, ash 1%; Alexander Müller GbR (Herzlake, Germany).

[5] Bioceval GmbH & Co. KG (Cuxhaven, Germany).

[6] Alexander Müller GbR (Herzlake, Germany).

[7, 8, 11] Merck KGaA (Darmstadt, Germany).

[9] vitamin and mineral premix (mg·kg^{-1} diet): retinyl acetate (750 IU), cholecalciferol (750 IU), dl-a-tocopherol 75, menadione 10, thiamine 7.5, riboflavin 7.5, pyridoxin-HCL 15, cyanocobalamin 0.0375, nicotinic acid 17.5, D-pantothenic acid 17.5, choline chloride 250, folic acid 1.5, biotin 0.125, vitamin C 31.25, inositol 75, iron 12.5, copper 10, manganese 5, zinc 18.75, iodine 0.5, selenium 0.075, cobalt 0.015, magnesium 75; Research Diet Services (Wijk bij Duurstede, Netherlands).

[10] Evonik Industries AG (Essen, Germany).

Values in the same row with different superscript letters indicate statistical significant differences.

acceptability trial and the preliminary-trial were both run in a recirculating aquaculture system (RAS) with 12 separate 50-L fish tanks (50 cm × 25 cm × 40 cm (w × l × h)). The RAS consisted of a mechanical filter, a protein skimmer, a bio-filter, an ozone supply, and a reservoir of approximately 360 L artificial seawater. Inflow of seawater was maintained at approximately 50 L·h^{-1} and continuous aeration of the tanks was provided. Following the illumination of the facility, the system was exposed to a 12:12 h light/dark cycle at low light intensity (lights on 7:30; lights off 19:30; at approx. 2 μmol m^{-2} s^{-1}).

Throughout the trials, the water parameters temperature (27. 6 ± 0.7°C), dissolved oxygen (DO, 83.1 ± 7.4%), pH (7.4 ± 0.4), and conductivity (32.9 ± 2.3 mS·cm^{-1}) were monitored constantly by electrodes (Senect GmbH & Co. KG, Landau, Germany) immersed in the effluent water of the tanks and noted daily. Additionally, manual measurements were made twice a week to verify the automated measurements. Water samples were also analysed twice a week using a QuAAtro39 Continuous Segmented Flow Analyzer (SEAL Analytical GmbH, Norderstedt, Germany) for ammonia (0.10 ± 0.05 mg·L^{-1}), nitrite (0.24 ± 0.19 mg·L^{-1}), and nitrate (270.14 ± 81.19 mg·L^{-1}).

A stock of approximately 500 juvenile shrimp was purchased from a commercial shrimp farm (Förde Garnelen GmbH & Co. KG, Strande, Germany) and acclimatized to the experimental system and facility conditions for two weeks prior the start of the experiments. In this period, shrimp received a commercial grow-out feed (38% gross protein, 11% gross fat; Le Gouessant, France) three times a day.

To evaluate the acceptance of the experimental diets, the time was recorded that shrimp needed to approach and start to ingest a pellet after it was placed in the tank. Therefore, 180 randomly selected shrimp weighing 6.10 to 14.40 g were allocated to 12 tanks, resulting in an average total biomass of 140.33 ± 3.33 g and 15 individuals per tank. Prior to weighing to the nearest 0.01 g, excess water was carefully removed from each shrimp with a moist paper tissue. The acceptability experiment started three days after weighing the shrimp to avoid post-handling effects. During this period, shrimp received the commercial grow-out feed mentioned above, three times per day (9:00, 12:00, 16:00). On the morning of the fourth day at 9:00, instead of feeding the commercial feed, the acceptance of the experimental diets was tested. Therefore, one feed pellet was placed into a tank and the time was stopped until any of the 15 shrimp in the tank approached and grabbed the pellet. This procedure was repeated 10 times per tank. Only one diet was tested per tank, resulting in four replicate tanks per feed.

For the preliminary-feeding trial, 16 new randomly taken shrimp with an average weight of 6.54 ± 1.31 g were stocked per tank. The average total initial biomass was 104.63 ± 0.92 g per tank. Each experimental feed was tested in quadruplicate and was randomly allocated to the tanks. Due to the low hydro-stability of pelleted feeds, the feed ration was provided four times a day (10:00, 12:00, 14:00, 17:00) and uneaten feed was recollected using a fine meshed net after 1.5 h, if present. Feed remains were oven-dried over night at 80°C and the weight was recorded the following day. Shrimp received the experimental diets at 4.5% of the biomass per day as recommended by Tacon et al. (2013). The feeding rate was adjusted weekly assuming a growth rate of 2 g per week. If mortalities occurred, the related feeding rate was adjusted accordingly.

The hard carapace structure of an exuvia was counted as one molting event and noted on a daily basis for each tank. To calculate the daily molting ratio (DMR), the number of carapaces (N_c) found each day was related to the number of shrimp (N_s) per tank as reported by Shan et al. (2019):

$$DMR = N_c / N_s \bullet 100$$

To describe the synchronism of molting, days were counted and grouped together when the daily molting ratio was >10% (DMR > 10%).

At the end of the feeding trial (day 39), all shrimp were removed from the tanks and weighed again. The parameters survival and weight gain (WG) were calculated as follows:

$$\text{Survival } (\%) = \text{number of shrimp survived} \div \text{number of shrimp stocked} \times 100$$

$$\text{WG } (\%) = (\text{final body weight (g)} - \text{initial body weight (g)}) \div \text{initial body weight (g)} \times 100$$

The dry matter loss of the feeds caused by leaching was determined by placing 2 g of the experimental diets in the tanks without the presence of shrimp. Following the feeding protocol, feed pellets were recollected after 1.5 h, oven dried overnight, and reweighed. The weight difference was used to calculate the correction factor (CF):

$$\text{CF} = \text{dry weight feed input (g)} \div \text{dry weight recovered feed (g)}$$

the water stability of the diets (DM loss (%)):

$$\text{DM loss } (\%) = 100 - \text{dry weight recovered feed (g)} \div \text{dry weight feed input} \times 100$$

and the daily feed intake (DFI) as described by Smith et al. (2005):

$$\text{DFI} = [\text{feed applied (g)} - (\text{feed recovered (g)} \times \text{CF})] \div (\text{BW}_{\text{mean}} \text{ (g)} \times \text{days of the feeding trial}) \times 100$$

Where BW_{mean} is the mean biomass of shrimp in the respective tank during the time of the trial.

The feed conversion ratio (FCR) was determined as:

$$\text{FCR} = [\text{feed applied (g)} - (\text{recovered feed (g)} \times \text{CF})] \div \text{corrected biomass gain (g)}$$

using a corrected biomass gain to account for the biomass of dead shrimp as described by Kitabayashi et al. (1971):

$$\text{Corrected biomass gain (g)} = \text{final total weight} + [0.5 \times (\text{average initial weight} + \text{average final weight}) \times \text{number of dead shrimp}] - \text{initial total weight}$$

2.2.3. Sampling and biochemical analysis

Sampling was conducted in the morning at the end of the preliminary trial, before feeding. Three shrimp in the intermolt stage were selected per replicate. The molting stage was determined on the setal development of the uropod as proposed by Robertson et al. (1987). Haemolymph was sampled directly from the ventral sinus using a sterile 1-mL plastic syringe filled with 100 μL cold, citric-acid based anticoagulant (27 mmol·L^{-1} trisodium citrate, 385 mmol·L^{-1} sodium chloride, 115 mmol·L^{-1} glucose, pH 7.5) as described by Huang et al. (2010). Prior to sampling, the puncture site between abdomen and cephalothorax was disinfected with 75% ethanol. Depending on the volume of withdrawn haemolymph, samples were diluted with cold anticoagulant to reach a final dilution of 1:2. Haemolymph samples were immediately shock frozen in liquid nitrogen and stored at −80°C. Midgut glands were dissected and weighed to the nearest 0.01 g to determine the hepatosomatic index (HSI):

$$\text{HSI} = \text{weight hepatopancreas (g)} \div \text{body weight (g)} \times 100$$

The midgut glands were then shock-frozen in liquid nitrogen and stored at −80°C until digestive enzyme activities were analysed. The abdominal muscles of the sampled shrimp were dissected and stored at −20°C for proximate composition analysis.

Haemolymph samples were used to determine the haemocyanin levels using UV-wavelength spectroscopy at a wavelength of 335 nm (Chen and Cheng, 1993; Pascual et al., 2003). Based on the functional subunit of haemocyanin, an extinction coefficient of $\varepsilon_{335} = 17.26$ L·mol^{-1}·cm^{-1} was used to calculate haemocyanin concentrations.

Crude enzyme extracts of midgut gland tissues were prepared with a Precellys lysing kit (Bertin Instruments, France) in ultrapure water (Milli-Q, Merck, Darmstadt, German) at a ratio of 1:10 (weight to volume). After homogenization, samples were centrifuged at 16.800 *g* for 30 min and 4°C. The supernatant was aliquoted and stored at −80°C until further analysis of digestive enzyme activities. The protein content of the crude enzyme extracts was measured after Bradford (1976) using a commercial protein kit (Bio-Rad Laboratories GmbH, Feldkirchen, Germany). Total proteolytic activity was measured after García-Carreño (1992) using azocasein as substrate (1% in 0.1 mol·L^{-1} Tris-HCL buffer, pH 8). The reaction was stopped after 30 min at room temperature, by addition of 10% trichloroacetic acid. Samples were then centrifuged at 16.500 *g* for 5 min and 4°C and the absorbance of the supernatant was measured at 366 nm. Specific protease activity (U) is defined as the change of extinction in one minute per mg protein ($\text{dE}_{366}\cdot\text{min}^{-1}\cdot\text{mg}_{\text{protein}}^{-1}$).

The digestive chitinase, esterase, and lipase activities were measured using substrates releasing fluorogenic 4-methylumbelliferyl (MUF). Substrates based on derivates of 4-methylumbelliferyl are suitable to measure digestive enzyme activities in small crustaceans (Knotz et al., 2006). The substrate 4-methylumbelliferyl *N*-acetyl-β-D-glucosaminide was used for chitinase, 4-methylumbelliferyl butyrate for esterase, and 4-methylumbelliferyl oleate for lipase activity (Merck, Darmstadt, Germany). Substrates were first diluted in dimethylsulfoxide (DMSO) and then mixed with 0.1 mol·L^{-1} Tris/HCL pH 7 to reach a final substrate concentration of 0.1 mmol·L^{-1} and a DMSO content of 2% in the reaction. Assays were conducted at room temperature in black 96-well microplates (Brand, Wertheim, Germany) using a plate fluorometer (Fluoroskan Ascent FL, Thermo Fischer, Germany) at $\lambda_{\text{ex}} = 355$ nm and $\lambda_{\text{em}} = 460$ nm. The total reaction time was 10 min with reads taken every 30 s. A calibration curve was prepared with 4-methylumbelliferol covering concentrations up to 50 μmol·L^{-1}. Specific enzyme activity (U) was expressed as the release of one μmol·L^{-1} MUF in one minute per mg protein ($\text{U}\cdot\text{mg}_{\text{protein}}^{-1}$).

Biochemical characterisations were made of the experimental feeds and shrimp muscle tissues. Moisture and ash were determined following the standard methods of the Official Analytical Chemists AOAC (2010). Protein was determined after Dumas (1831), using an elemental analyzer (Euro Elemental Analyzer, Eurovector SPA, Italy) to measure the nitrogen content. The nitrogen content of BSPR was corrected for chitin bound nitrogen. Gross energy content was determined via combustion with a calorimeter (Parr 6100, Parr Instrument Company, USA). Total lipids were measured gravimetrically following to the protocol described by Folch et al. (1957), using dichloromethane and methanol for extraction. Determination of crude fibre was made by a certified

Table 2

Ingredients, chemical composition, and water stability of extruded diets used for the performance trial with stepwise replacement of fishmeal with brown shrimp processing remains (BSPR). Values expressed as g·kg^{-1} "as is", unless otherwise indicated.

Ingredient	Control	BSPR 20	BSPR 40	BSPR 60	BSPR 80	BSPR 100
Fishmeal [1]	360	288	215	144	72	0
Brown shrimp processing remains [2]	0	72	144	216	288	360
Soymeal [3]	220	220	220	220	220	220
Wheatmeal [4]	313	314	314	314	299	279
Fishoil [5]	20	20	20	20	20	20
Lecithin (soy) [6]	20	20	20	20	20	20
Gluten (wheat) [7]	0	15	30	50	65	85
Alginate [8]	50	35	20	0	0	0
Vitamin and mineral premix [9]	10	10	10	10	10	10
Met-Met [10]	5	5	5	5	5	5
Cholesterol [11]	1	0	0	0	0	0
Yttriumoxide [12]	1	1	1	1	1	1
Chemical composition & water stability						
Dry matter	953 ± 0.6 ^{a}	956 ± 1.1 ^{ab}	963 ± 1.4 ^{b}	963 ± 2.1 ^{b}	963 ± 1.0 ^{b}	963 ± 1.1 ^{b}
Protein (N·6.25)	389 ± 9	389 ± 13	398 ± 39	395 ± 11	389 ± 6	411 ± 11
Gross energy (MJ·kg^{-1})	18.9 ± 0.37 ^{ab}	19.70 ± 0.01 ^{b}	19.44 ± 0.20 ^{ab}	19.55 ± 0.11 ^{ab}	19.10 ± 0.04 ^{ab}	18.74 ± 0.02 ^{a}
Total lipid	137 ± 16 ^{c}	117 ± 5 ^{bc}	127 ± 21 ^{bc}	106 ± 8 ^{ab}	100 ± 4 ^{a}	95 ± 9 ^{a}
Crude fibre	20	25	34	36	45	47
Ash	95.0 ± 0.4 ^{a}	99.3 ± 0.6 ^{b}	106.2 ± 0.8 ^{c}	111.2 ± 0.3 ^{d}	120.6 ± 0.6 ^{e}	130.4 ± 0.3 ^{f}
Water stability (% DM loss)	20.7 ± 1.3	22.6 ± 2.9	20.5 ± 1.1	20.2 ± 2.4	18.7 ± 2.1	18.4 ± 2.8

[1] Protein 64%, lipid 9%, ash 21%; Bioceval GmbH & Co. KG (Cuxhaven, Germany).

[2] Protein 52%, lipid 7%, ash 24%, chitin 9%; Kocken & Ehlerding Krabbenhandels-GmbH (Bremerhaven, Germany).

[3] Protein 38%, lipid 21%, ash 6%; Mühle Schlingemann e.K. (Waltrop, Germany).

[4] Protein 11%, lipid 2%, ash 1%; Mühle Schlingemann e.K. (Waltrop, Germany).

[5] Bioceval GmbH & Co. KG (Cuxhaven, Germany).

[6] Alexander Müller GbR (Herzlake, Germany).

[7] Mühle Schlingemann e.K. (Waltrop, Germany).

[8] Salandis GbR (Greifswald, Germany).

[9] vitamin and mineral premix (g·kg^{-1} diet): retinyl acetate (3000 IU), cholecalciferol (3000 IU), dl-a-tocopherol 0.3, menadione 0.04, thiamine 0.03, riboflavin 0.03, pyridoxin-HCL 0.06, cyanocobalamin 0.15 (mg·kg^{-1}), nicotinic acid 0.07, D-pantothenic acid 0.07, choline chloride 1, folic acid 6 (mg·kg^{-1}), biotin 0.5 (mg·kg^{-1}), vitamin C 0.125, inositol 0.3, iron 0.05, copper 0.04, manganese 0.02, zinc 0.075, iodine 2 (mg·kg^{-1}), selenium 0.3 (mg·kg^{-1}), cobalt 0.06 (mg·kg^{-1}), magnesium 0.3; Spezialfutter Neuruppin GmbH & Co KG (Neuruppin, Germany).

[10] Evonik Industries AG (Essen, Germany).

[11,12] Merck KGaA (Darmstadt, Germany).

Values in the same row with a different superscript letters indicate statistical significant differences.

laboratory for animal feed analyses (LUFA Nord-West, Germany) according to the standard method defined by the European Commission Regulation (EC) No 152/2009 (The European Commission, 2009a).

2.3. *Performance trial*

2.3.1. *Diet preparations*

Based on the results of the preliminary-trial, a second, 43-day feeding experiment was conducted using highly nutritious extruded experimental diets to simulate commercially produced feeds and to obtain robust performance data (Table 2). Based on digestible levels, isocaloric and isoproteinaceous feeds were formulated to meet nutritional requirements of *L. vannamei* (NRC, 2011). The fishmeal content was replaced by BSPR in steps of 20% resulting in five experimental diets and one control. Diets were produced by hot extrusion using a DNDL44 co-rotating twin-screw extruder (Bühler GmbH, Braunschweig, Germany). After extrusion, the 2 mm pellets were dried at 140°C for 15 min to achieve a moisture content of <7%. In a last step, the extruded feeds were vacuum coated with fish oil using a PG-10VC LAB (Dinnissen BV, Sevenum, The Netherlands). Biochemical characterisation of the feeds was conducted as described in the previous section.

2.3.2. *Experimental system and design*

The performance trial was conducted in a RAS comprising 36 separated tanks (50 cm × 45 cm × 45 cm (w × l × h)) with a water volume of 100 L. All tanks were supplied with the same water within the RAS, which consisted of a biofilter, mechanical drum filter, a protein skimmer, an ozone supply, and UV-treatment. Water inflow of individual tanks was approximately 100 L·h^{-1} and all tanks were equipped with an air stone for sufficient aeration. Light exposure of the system was the same as mentioned in Section 2.2.2. The physiochemical water parameters were monitored and measured as described above under Section 2.2.3 (°C: 26.9 ± 0.6; DO: 81.0 ± 7.0%; pH: 8.1 ± 0.1; mS·cm^{-1}: 33.1 ± 1.1; NH_4^+: 0.11 ± 0.08 mg·L^{-1}; NO_2^-: 0.23 ± 0.17 mg·L^{-1}; NO_3^-: 85.13 ± 35.14 mg·L^{-1}).

About 1000 live juvenile shrimp (*L. vannamei*) were purchased from an indoor shrimp farm (Neue Meere, Gronau, Germany) and acclimatized to the experimental conditions for two weeks. During this time, shrimp were fed with the commercial grow-out feed mentioned in Section 2.2.2. At the start of the feeding trial, 720 shrimp weighing 4.31 ± 1.03 g were distributed equally to the tanks resulting in 20 individuals and a total biomass of 86.21 ± 1.06 g per tank. Care was taken to keep shrimp size distributions similar across all tanks with coefficients of variation ranging from 23.3% to 25.0%. The six experimental diets were randomly allocated to the tanks in six replicates. The high water stability of extruded feed pellets allowed to leave the feeds in the tanks for a longer period of time (~2 h), without losing excessive amounts of feed due to leaching. Thus, a different feeding regime was applied to accurately monitor feed intakes of the shrimp and define feed conversion ratios. Experimental feeds were supplied at 10% of the biomass per day, in three equal rations (08:00, 12:00,16:00). Two hours after each feeding, uneaten feed remains were carefully siphoned out of each tank into a separated bucket. Faeces were removed if present and discarded. The clean uneaten feed remains were collected in a fine-meshed net, oven dried over night at 80°C and reweighed the next morning. If the recovered uneaten feed remains were <10% of the feed input, the feeding rate was increased to ensure a surplus of feed availability for the shrimp.

The quantification of the feed pellet dry matter loss in the water and corresponding correction factor were made in the same manner as described for the preliminary-trial (Section 2.2.2.). Following the feeding protocol of the performance trial, the feed remains were collected from the tanks after 2 h.

After 43 days all shrimp were removed from the tanks and weighed. Survival, weight gain, daily feed intakes, feed stabilities and feed conversion ratios were calculated as described above.

2.3.3. *Sampling and haemolymph analysis*

In the morning of the last day of the feeding trial, haemolymph of 3 shrimp in the intermolt stage were sampled per tank as described in Section 2.2.3. All consumables were autoclaved to avoid pyrogenic effects on phenoloxidase activity. To obtain enough sampling material for the analyses, haemolymph samples were diluted with cold anticoagulant to reach a final concentration of 1:5. Between working steps, individual samples were kept on ice. A subsample of haemolymph was centrifuged for 10 min at 4°C and 800 g and the supernatant constituted the plasma. Both, haemolymph and plasma samples, were aliquoted, shock frozen in liquid nitrogen, and stored at −80°C until further use. Phenoloxidase activity was determined in plasma after Huang et al. (2010). Formation of dopachrome from L-DOPA was measured at 490 nm, using a substrate concentration of 3 mg·mL^{-1} in 0.1 mol·L^{-1} potassium phosphate buffer, pH 6.6. The method was adapted for microplates as described by Weiss et al. (2020) using an Infinite 200 PRO spectrophometer (Tecan, Männedorf, Switzerland). Prophenoloxidase activity was measured in haemolymph samples which were incubated with trypsin (1 mg·mL^{-1}) for 5 min at 27°C. Activity was then detected using L-Dopa as described above. One unit of enzyme activity is expressed as a linear increase in absorbance of 0.001 per min per mL sample. Haemolymph protein content was determined after Bradford (1976).

2.4. *Statistics*

Normal distribution and homoscedasticity of data was analysed using the Shapiro-Wilks and the Bartletts test. When normal distribution and homoscedasticity of data was given, statistical comparison of treatments was done by one-way analysis of variance (ANOVA) at a significance level of 0.05. A Tukey's post-hoc test was used when differences between treatment groups were significant. If assumptions for normality failed, data was square root or log transformed. When transformed data met parametric assumptions, statistical tests were done as described above. When data did not meet the requirements for parametric tests after transformations, a non-parametric Kruskal-Wallis test was applied, followed by a Nemenyi post-hoc test if significant differences were present. Percentage data of shrimp survival was arcsine transformed prior to statistical analysis. Direct comparisons to the control group were made with unpaired *t*-tests for final weight and weight gain data of the preliminary-trial. To describe the relationship between inclusion of brown shrimp processing remains and growth of the performance trial, a third polynomial regression was fitted to the weight gain data. To identify the optimal inclusion rate of BSPR the first derivative was used to define the local maximum. Data analysis was made with R (R Core Team, 2019).

3. Results

3.1. *Acceptability & preliminary-trial*

The Pacific Whiteleg shrimp, *L. vannamei*, readily accepted all pelleted experimental diets. No rejection was observed, e.g. dropping of pellets once they were grabbed. The time shrimp needed to start to ingest feed pellets ranged from 18 to 25 s on average (Table 4). Feeds containing 50% fishmeal and 50% BSPR (BSPR 50) were approached most rapidly (18 ± 4 s), followed by the control (20 ± 4 s) and BSPR 100 (25 ± 5 s). The differences in accessing time were not statistically significant (one-way ANOVA, $p = 0.075$).

The overall survival of shrimp in the preliminary-trial was 90%, indicating proper rearing conditions (Table 4). Growth was lowest in shrimp feeding on the control diet with a mean weight gain of 66 ± 7%. Average weight gain was slightly higher in the BSPR 100 treatment (68 ± 24%), but showed high variation. Growth was highest in shrimp feeding on BSPR 50 (94 ± 9%), thus exceeding the performance of the control group by almost 30%. Due to the high variation in the BSPR 100 treatment, one-way ANOVA showed no statistical differences when all means were compared against each other ($p = 0.062$). However, when comparing directly against the control, the final weight and weight gain of shrimp feeding on BSPR 50 was statistically significant (unpaired t-test, $p = 0.003$).

The daily feed intake was highest in the control treatment with 4.3 ± 0.4% of shrimp biomass per day (Table 4). It accounted for 3.9 ± 0.1% in the BSPR 50 treatment and was significantly lower in the BSPR 100 treatment with 3.7 ± 0.3% (one-way ANOVA, $p = 0.033$). The lower feed intake and higher growth in the BSPR 50 treatment led to a significantly reduced feed conversion ratio of 2.3 ± 0.2 when compared to the control (one-way ANOVA, $p = 0.02$).

The daily molting ratio (DMR) increased with higher BSPR inclusion rates and differed significantly between the BSPR 100 and the control treatment (one-way ANOVA, $p = 0.007$, Table 4). A similar trend was observed in number of days with DMR >10%, though statistically not significant (Kruskal-Wallis, $p = 0.077$).

The hepatosomatic index (HSI), ranging from 3.2 ± 0.6 to 3.6 ± 0.7, did not vary significantly between shrimp feeding on the different experimental diets. Chemical analysis of abdominal muscle tissue did not show significant effects related to the diet. Average muscle moisture content was 764 ± 16 g·kg^{-1} across all treatments and the protein content ranged from 812 ± 28 g·kg^{-1} to 821 ± 20 g·kg^{-1}. The lipid content showed a decreasing trend from 58 ± 9 g·kg^{-1} to 45 ± 6 g·kg^{-1} in shrimp muscle tissue with increasing BSPR content but no significant differences between treatments (one-way ANOVA, $p = 0.083$).

Haemocyanin levels of the different treatment groups were similar, ranging from 1.1 ± 0.2 nmol·L^{-1} to 1.3 ± 0.3 nmol·L^{-1}. Statistical comparisons confirmed that hemocyanin levels were not significantly affected by the diet (Kruskal-Wallis, $p = 0.309$).

The activities of the tested digestive enzymes were not affected by the diet (Table 4). Total protease and lipase activities were low with values ranging from 0.05 ± 0.01 $\text{U·mg}^{-1}_{\text{protein}}$ to 0.06 ± 0.01 $\text{U·mg}^{-1}_{\text{protein}}$ and 0.04 ± 0.01 $\text{U·mg}^{-1}_{\text{protein}}$ to 0.07 ± 0.03 $\text{U·mg}^{-1}_{\text{protein}}$. Chitinase activities were more variable with average values ranging from 29.1 ± 13.0 $\text{U·mg}^{-1}_{\text{protein}}$ to 57.5 ± 13.5 $\text{U·mg}^{-1}_{\text{protein}}$ with high individual variation indicated by large standard deviations within the treatment groups. Average esterase activities were very similar across treatments with mean levels of 103 ± 25 $\text{U·mg}^{-1}_{\text{protein}}$ to 110 ± 43 $\text{U·mg}^{-1}_{\text{protein}}$. High variability was also present in esterase activity values.

3.2. *Performance trial*

The extruded experimental diets were all well accepted by *L. vannamei* during the feeding trial. The shrimp showed no abnormalities or signs of malnutrition. A trend towards a better survival with increasing BSPR in diets was observed, but group comparisons with a non-parametric Kruskal-Wallis test showed no significant relationship ($p = 0.065$).

The weight gain of shrimp was significantly higher at BSPR substitution values above 60% (Table 5). Best growth appeared in shrimp feeding on the BSPR 80 diet with a mean weight gain of 189 ± 17% and average body mass of 12.4 ± 0.7 g.

The polynomial regression of fishmeal substitution levels with BSPR and the weight gain of *L. vannamei* showed a good fit with a coefficient of correlation of 0.8 (Fig. 1). A local maximum was identified at a BSPR

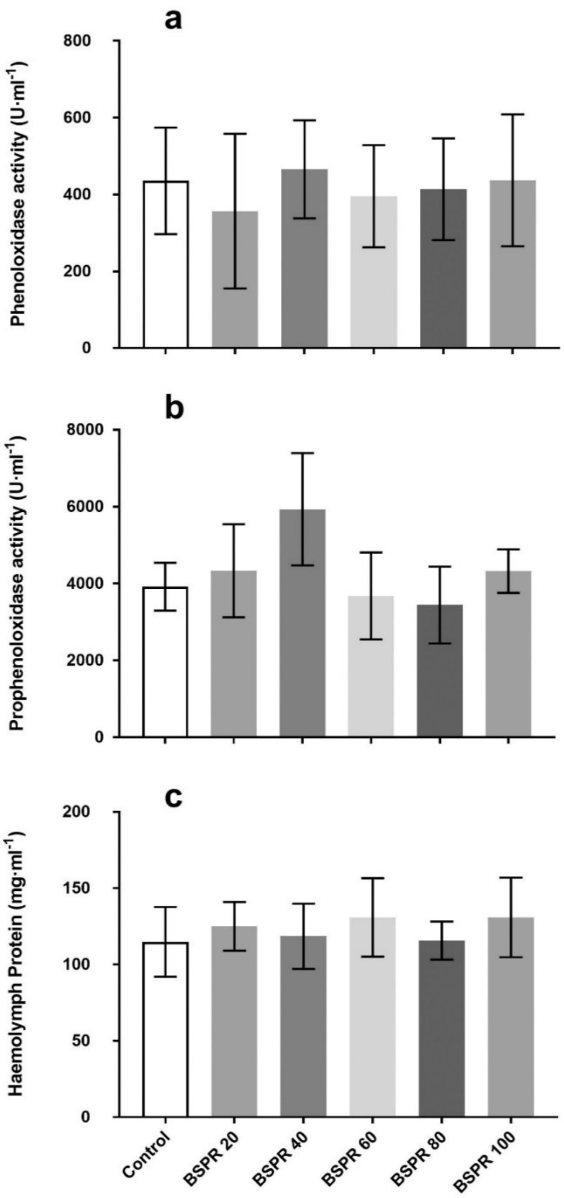

Fig. 1. Phenoloxidase, prophenoloxidase and haemolymph protein levels measured in *L. vannamei* feeding on the respective diets. Phenoloxidase and prophenoloxidase values are expressed as the mean ± sem and haemolymph protein as mean ± sd ($n = 6$). BSPR = Brown shrimp processing remains. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

content of 85%, indicating a theoretical optimal substitution level of fishmeal with BSPR.

The feed conversion ratio decreased significantly with increasing BSPR inclusion levels (Table 5). The lowest values were observed in groups receiving BSPR 80 and BSPR 100, with a FCR of 1.8 ± 0.1 and 1.9 ± 0.1, respectively. Compared to the control, containing no BSPR with an average FCR of 2.9 ± 0.4, this is an improvement of feed utilization of approximately 37%.

No diet-dependent effects were observed in haemolymph protein levels, phenoloxidase, and prophenoloxidase activities (Fig. 2). Haemolymph protein levels ranged from 115 ± 23 mg·mL^{-1} to 131 ± 26 mg·mL^{-1}. Phenoloxidase activities showed high variability and ranged from 356 ± 201 U·mL^{-1} to 466 ± 128 U·mL^{-1} (mean ± sem). Mean prophenoloxidase activities were approximately 5 times higher than phenoloxidase levels, also with high inner group variabilities and no statistically significant differences between the treatments.

4. Discussion

Alternative feed ingredient sources from marine sidestreams have great potential to be sustainable and economically valuable, driving the blue circular economy. In this study, repeated feeding experiments showed high levels of survival and excellent growth of *L.vannamei* when fishmeal was replaced with the by-product BSPR. The gross composition of the shrimp muscle tissue did not change in moisture, protein and lipid contents. These results indicate that BSPR provide well balanced and readily receptible nutrients and that increased growth does not compromise muscle tissue composition.

The palatability of feeds and feed ingredients plays a central role in feed formulation. It can positively or negatively affect feed consumption and ultimately growth performances of the cultured species (Glencross, 2020). The feeding behaviour of shrimp (searching movements, handling of feed items, ingestion/rejection of feed) is mainly driven by chemoreception (Bardera et al., 2019; Steiner and Harpaz, 1987). Therefore, attractiveness and scent of feeds are important assets. Different crustacean meals were shown to be effective feeding stimulants in shrimp feeds (Holland and Borski, 1993; Smith et al., 2005). We observed that shrimp approached and started to ingest BSPR containing feeds equally well as the fishmeal-based diets, regardless of the inclusion level. Accordingly, dietary inclusion of BSPR does not appear to influence feed acceptability in tank-based culture conditions.

The daily feed intake in both feeding experiments decreased with increasing BSPR contents. Despite reduced feed intakes, the weight gain of shrimp increased significantly, leading to an improved feed efficiency of up to 37% in the performance trial. It is probable that calculated FCR in the preliminary trial was high due to poor water stability resulting in feed loss due to leaching. On the contrary, estimation of water stability does not account for disintegrated feed material unable to be netted from the tanks, but being available for the shrimp. Due to the uncertainty in the estimation of the actual feed intake, the FCR of the preliminary-trial should be considered with caution. These factors may influence but do not negate the improved feeding efficiency observed since both feeds, control and BSPR 50, showed no significant differences in water stability.

Growth enhancing effects of crustacean meals in penaeid shrimp feeds has been reported in several studies (Amar et al., 2006; Córdova-Murueta and García-Carreño, 2002; Fox et al., 1994; Nunes et al., 2019; Shan et al., 2019; Villarreal et al., 2006). Positive effects on shrimp growth were observed at inclusion rates ranging from 3 to up to 30% of the diet, depending on the specific crustacean meal investigated. Already low krill meal (*Euphausia superba)* inclusion rates of 3 to 6% improved growth performance in *Litopenaeus stylirostris* (Suresh et al., 2011), *L. vannamei* (Ambasankar et al., 2022; Sabry-Neto et al., 2017), and *Penaeus monodon* (Smith et al., 2005). Fox et al. (1994) included differently processed shrimp head meals made from *P. monodon* at 31% in diets for juvenile *P. monodon.* At these comparatively high inclusion levels, growth of shrimp was enhanced depending on the production method of the shrimp raw materials. Specifically, raw shrimp heads that were passed through a commercial meat/bone separator and dried at low temperatures gave better growth than untreated heads, dried in solar conditions. Hydrolysed krill meal and fermented shrimp offal also showed growth-enhancing effects when added to diets for *L. vannamei* (Córdova-Murueta and García-Carreño, 2002) and *F. indicus* (Amar et al., 2006) at inclusion levels of 3 to 15% and 25%, respectively. The

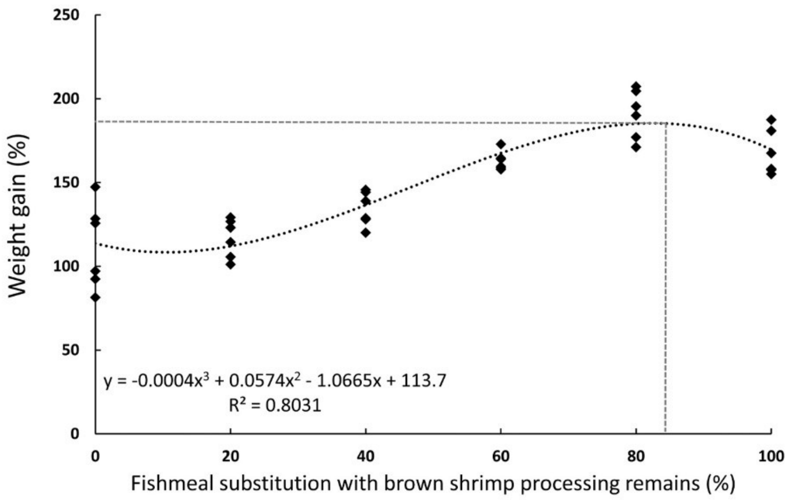

Fig. 2. Polynomial regression relating the fishmeal substitution with brown shrimp processing remains (%) and weight gain. All six data points per treatment were used for model fitting. Dashed lines indicate the local maximum ($x = 85.2$; $y = 192.1$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

performance trial revealed optimal growth at 85% replacement of fishmeal by BSPR, representing an overall dietary inclusion level of 30.6%. This value matches well with results of Villarreal et al. (2006) who determined an optimum of fishmeal replacement of 80% with red crab meal (*Pleuroncodes planipes*) in diets for juvenile *L. vannamei*. Like the BSPR used in our study, the red crab meal was boiled and oven dried at similar temperature. Compared to other crustacean meals, BSPR investigated in the current study is less effective in stimulating growth than e.g. krill. This is potentially due to the various processing steps of BSPR (cooking, freezing, drying) which may have deteriorated certain beneficial nutrients or feed components.

The underlying physiological mechanisms of the growth stimulating effect of crustacean based ingredients are not clear. Increased growth performance has mainly been linked to superior palatability, feed intake, and better nutritional profiles (Amar et al., 2006; Fox et al., 1994; Nunes et al., 2019; Shan et al., 2019; Suresh et al., 2011). Williams et al. (2005) even suggested a so far unknown growth factor present in the insoluble protein fraction of crustacean-based feed ingredients. There are also reports on insulin-like peptides found in crustacean based meals, which stimulate metabolism and growth in shrimp (Pang et al., 2021; Vega-Villasante et al., 2002 and references therein).

In line with Smith et al. (2005) using krill and crustacean meal in *P. monodon* feeds, we did not observe increased feed intake with higher weight gains. This suggests that the increased growth in our study is primarily based on a certain nutritive and/or functional benefit of BSPR. The experimental diets used in our study were formulated to contain similar digestible protein and energy levels and meet the dietary requirements of *L. vannamei*. The calculated essential amino acid (EAA) profiles of the diets used in the performance trial (Table 3) do not show major differences between each other and all EAA contents exceed dietary requirements. Only leucine shows elevated levels in diets containing high amounts of BSPR compared to the fishmeal-based feed (29.4 vs 24.8 g kg^{-1}). The dietary leucine requirement for penaeid shrimp is satisfied with 13–15 g kg^{-1} (NRC, 2011). Therefore, it is unlikely that the differences in growth are caused by protein or amino acid limitations. Brown shrimp processing remains contain a variety of key nutrients (Fricke et al., 2022). Essential nutrients such as long chained polyunsaturated fatty acids, phospholipids, cholesterol, and micronutrients that might be limited in the fishmeal-based diet were accounted for by fish oil and individual supplementations (i.e. lecithin, cholesterol, vitamin and mineral premix). Exceptions could be carotenoids and chitinous compounds inherent to BSPR but lacking in the FM based diet. Williams et al. (2005) investigated different fractions of krill and wild caught shrimp head meal and identified an unknown growth factor present in the insoluble protein fraction, probably associated with chitin-complexes. This indicates that the nutritional benefit of BSPR could be related to the chitinous parts of the cephalothorax and abdominal cuticle that are plentiful in the remains (Fricke et al., 2022). Alternatively, the observed increased growth in *L vannamei* could have been caused by hormonal stimulation related to insulin-like peptides, as mentioned previously.

Table 3
Calculated essential amino acid composition of the experimental diets prepared for the performance trial with stepwise replacement of fishmeal with brown shrimp processing remains (BSPR). Values expressed as $g\cdot kg^{-1}$, unless otherwise indicated.

Essential amino acids [$g\cdot kg^{-1}$]	Control	BSPR 20	BSPR 40	BSPR 60	BSPR 80	BSPR 100
Arginine	22.1	22.5	22.8	23.3	23.5	23.8
Histidine	8.0	8.2	8.3	8.5	8.5	8.7
Isoleucine	14.4	14.5	14.6	14.9	15.0	15.2
Leucine	24.8	25.6	26.4	27.6	28.3	29.4
Lysine	21.8	21.4	20.9	20.6	20.1	19.6
Phenylalanine	15.6	15.8	15.9	16.2	16.2	16.4
Methionine	11.6	11.6	11.7	11.8	11.8	11.8
Threonine	14.0	13.8	13.7	13.6	13.4	13.3
Valine	17.3	17.1	17.0	17.0	16.8	16.7
Tryptophan	8.2	7.4	6.6	5.9	5.1	4.3

*calculated with values provided by suppliers of the raw materials. If no information was available, values were used from the International Aquaculture Feed Formulation Database (IAFFD).

There are ambiguous findings on the nutritive value and effects of chitin and its derivatives on the growth performance in shrimp. For instance, Akiyama et al. (1989) found no digestive uptake of chitin in *L. vannamei,* while Clark et al. (1993) measured apparent chitin digestibility coefficients ranging from 33 to 52% in different shrimp species. Positive effects of chitin on growth were reported for *P. monodon* and the freshwater prawn, *Macrobrachium tenellum* (Niu et al., 2013; Santos-Romero et al., 2017). On the contrary, Fox (1993) found no significant effect of chitin on growth performance indices in juvenile

Table 4
Acceptance of brown shrimp processing remains (BSPR) containing diets, performance data, muscle composition and digestive enzyme activities of *L.vannamei* in the preliminary-trial. Values expressed as mean ± sd.

Parameter	Control	BSPR 50	BSPR 100
Acceptance (s)	20 ± 4	18 ± 4	25 ± 5
Survival (%)	92.2 ± 6.0	92.2 ± 7.9	85.9 ± 10.7
Final weight (g)	10.9 ± 0.5 $^{(a)}$	12.6 ± 0.7 $^{(b)}$	11.0 ± 1.6
Weight gain (%)	66 ± 7 $^{(a)}$	94 ± 9 $^{(b)}$	68 ± 24
Daily feed intake (% BW·day^{-1})	4.3 ± 0.4 ^{b}	3.9 ± 0.1 ^{ab}	3.7 ± 0.3 ^{a}
Feed conversion ratio	3.4 ± 0.4 ^{b}	2.3 ± 0.2 ^{a}	2.9 ± 0.7 ^{ab}
Daily molting ratio (DMR)	5.1 ± 0.8 ^{a}	7.1 ± 2 ^{ab}	8.6 ± 1.1 ^{b}
Days DMR > 10%	9 ± 1	13 ± 4	15 ± 3
Hepatosomatic index	3.6 ± 0.7	3.3 ± 0.6	3.2 ± 0.6
Haemocyanin (mmol·L^{-1})	1.3 ± 0.3	1.1 ± 0.2	1.2 ± 0.2
Muscle tissue composition (g·kg^{-1} dry basis)			
Moisture	769 ± 7	749 ± 14	773 ± 17
Protein	821 ± 20	821 ± 34	812 ± 28
Lipid	58 ± 9	50 ± 8	45 ± 6
Enzyme activities (U·mg$_{protein}^{-1}$)			
Total protease	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
Chitinase	44.2 ± 19.2	57.5 ± 13.5	29.1 ± 13.0
Esterase	110 ± 43	109 ± 42	103 ± 25
Lipase	0.04 ± 0.01	0.07 ± 0.03	0.04 ± 0.02

Values in the same row with a different superscript are significantly different from another (one-way ANOVA, $p < 0.05$). Superscript letters in parentheses of weight gain and final weight data indicate significant differences using a pairwise t-test comparison with the control.

P. monodon. Different chitin sources were used in the diets of these studies, ranging from pulverized shrimp carcasses to purified analytical grade substances. Chitin is a polymer of amino-polysaccharides consisting of β-linked *N*-acetylglucosamines. In crustaceans, chitin is an essential structural component of the exoskeleton, forming sclerotized complexes with structural proteins and calcium salts (O'Brien et al., 1991). As pointed out by Fox (1993), native chitin as that found in shrimp head meals might be of greater nutritional value than purified, chemical grade substances. In this study, all shrimp exhibited chitinolytic enzyme activities in the midgut gland, which implies certain biological relevance.

L. vannamei feeding on diets containing BSPR showed higher molting synchronism and significantly higher molting frequencies. A major share of BSPR is comprised of exoskeleton. Chitin or other substances present in the calcified protein-chitin matrix of the cuticle could facilitate the synthesis of the new exoskeleton and thus aid molting in *L. vannamei*. Ecdysis is a critical phase in the molting cycle of crustaceans (Wang et al., 2016). In intensive cultures, cannibalistic predation on freshly molted shrimp occurs frequently. Synchronisation of the molting cycle can reduce mortalities due to the inability of freshly molted shrimp to attack their conspecifics (Shan et al., 2019; Wang et al., 2016). This could also explain the higher survival rates in the performance trial, though the effect was not statistically significant. Some reports indicate that molting behaviour in crustaceans can be influenced by nutrition and micronutrient supplementation (Rao and Anjaneyulu, 2008; Suprayudi et al., 2012). Shan et al. (2019) fed diets containing freeze dried krill, *E. superba*, to *L. vannamei* and observed an increase in molting synchronism. The authors argued that phospholipids and copper present in krill meal might have caused this effect. Up to 40% of the total body copper content is bound in the respiratory pigment haemocyanin (Depledge, 1989). Haemocyanin levels can vary, depending on the nutritional status, feed ingredients, and dietary copper sources (Ambasankar et al., 2022; Pascual et al., 2003). Despite good copper bioavailability in BSPR (Fricke et al., 2022), we did not observe any dietary effects on the haemocyanin levels. The haemocyanin concentrations of 1.1–1.3 mmol·L^{-1} measured in this study are in the range typical for *L. vannamei* raised in indoor tanks (Pascual et al., 2003).

Additional response variables beyond growth performance parameters can help to understand functional effects of novel aquafeed ingredients by giving more sophisticated insights into the health status of the cultured species. Diet nutrient composition and certain feedstuffs can influence and stimulate the digestive system in shrimp, which can be accompanied by increased nutrient usage and growth performances (Brito et al., 2001; Gamboa-Delgado et al., 2003; Omont et al., 2019). Haemolymph protein levels can be used as a reference for evaluation of the physiological and nutritional status of *L. vannamei* (Pascual et al., 2003; Weiss et al., 2020). A variety of feed additives from marine and terrestrial origin were shown to improve immune responses in *L. vannamei*, such as phenoloxidase (PO) and prophenoloxidase (proPO) activities and gene expressions (Ambasankar et al., 2022; Lee et al., 2020; Weiss et al., 2020).

The efficient utilization of nutrients depends on the balanced action of extra- and intracellular digestive enzymes (Saborowski, 2015). *L. vannamei* possesses a wide array of different digestive enzymes and isoenzymes, which enable the digestion of a vast suite of dietary ingredients and compensate nutrient imbalances and enzyme inhibition (Gamboa-Delgado et al., 2003; Lemos et al., 2000; Rojo-Arreola et al., 2019). The activities of proteolytic enzymes, esterase, lipase, and

Table 5
Growth performance parameters of *L. vannamei* in the performance trial. Values expressed as g kg^{-1}, unless otherwise indicated.

Parameter	Control	BSPR 20	BSPR 40	BSPR 60	BSPR 80	BSPR 100
Survival [%]	71.7 ± 14.4	80.0 ± 4.5	82.5 ± 6.9	84.2 ± 4.9	86.7 ± 5.2	89.2 ± 7.4
Final weight [g]	9.2 ± 1.1 ^{a}	9.4 ± 0.4 ^{a}	10.2 ± 0.5 ^{ab}	11.2 ± 0.3 ^{bd}	12.4 ± 0.7 ^{c}	11.5 ± 0.6 ^{dc}
Weight gain [%]	112 ± 25 ^{a}	117 ± 12 ^{a}	134 ± 10 ^{a}	163 ± 6 ^{b}	189 ± 17 ^{c}	168 ± 14 ^{bc}
Daily feed intake (% BW·day^{-1})	4.9 ± 0.1 ^{b}	5.1 ± 0.4 ^{b}	4.8 ± 0.5 ^{ab}	4.5 ± 0.4 ^{ab}	4.2 ± 0.2 ^{a}	4.2 ± 0.2 ^{a}
Feed conversion ratio	2.9 ± 0.4 ^{b}	2.9 ± 0.3 ^{b}	2.5 ± 0.3 ^{b}	2.1 ± 0.2 ^{a}	1.8 ± 0.1 ^{a}	1.9 ± 0.1 ^{a}

Values in the same row with a different superscript letters are significantly different from another (one-way ANOVA, $p < 0.05$).

chitinase measured in this study were not affected by BSPR levels. The substrates used to determine enzyme activities in this study can be hydrolysed by a set of different enzymes. The enzyme activities reported are thus a measure of all the specific enzymes able to hydrolyse the respective substrate. Brito et al. (2001) showed the pattern of general protease was clearly distinct from the measured trypsin and chymotrypsin activities in early post larvae *L. vannamei* fed different experimental diets. Intricate enzymatic adjustments to the corresponding feed in the preliminary-trial are thus possible, but remain undetected. Our results suggest that dietary BSPR inclusion does not greatly influence or stimulate main digestive enzyme activities in the midgut gland of *L. vannamei*. Accordingly, increased growth of *L. vannamei* at higher BSPR levels cannot be attributed to elevated digestive enzyme activity.

In crustaceans, haemolymph protein levels can be affected in stressful and inadequate conditions due to higher energy usage or suboptimal nutrition (Mercier et al., 2006; Weiss et al., 2020). Haemolymph protein levels of shrimp in the preliminary trial were similar to the values reported for *L. vannamei* of comparable size, molting stage and rearing method (Cheng et al., 2002; Pascual et al., 2003). The phenoloxidase (PO) activities measured in the plasma of *L. vannamei* were slightly lower than the values reported by Huang et al. (2010) but within the same range as reported by Weiss et al. (2020). The high variability of this parameter can explain the differences to the other studies. Therefore, the PO activities measured in this study are within realistic dimensions and are not considered abnormal. Treatment of haemolymph samples with trypsin resulted in a 10-fold increase of measured proPO activity. Serine proteinases, such as trypsin, are known to trigger the prophenoloxidase activating system and induce PO activities in the otherwise inactive zymogen form, proPO, present in plasma and haemocytes (Huang et al., 2010; Ji et al., 2009). Additionally, the respiratory protein haemocyanin is capable of oxidizing monophenols and show latent PO activities as well (García-Carreño et al., 2008). Hence, the measured prophenoloxidase activity is the sum of enzymes present in haemolymph capable of oxidizing L-Dopa and can be considered a measure of the full immune response potential of the complex PO-system. Values are slightly higher than reported by Huang et al. (2010) which is probably related to PO activity originating from the haemocytes in our samples. These results indicate a good health and physiological status of shrimp feeding on all experimental diets. However, the tested physiological parameters were not influenced by dietary treatments and do not further elucidate the growth promoting effect of BSPR.

5. Conclusion

Brown shrimp processing remains (BSPR) are an excellent by-product based feed ingredient for *L.vannamei* reared in recirculating aquaculture systems. BSPR are of greater nutritional value to *L.vannamei* than the fishmeal used in this study made of multi-species trimmings and by-products. The optimum fishmeal replacement level is 85% or 306 $g \cdot kg^{-1}$ BSPR in total feed. Improved growth, increased feed utilization, and high survival rates underpin the quality of this underutilized fisheries by-product. The definite reason for the recorded improved growth performance remains to be elucidated. However, highly available and well-balanced key nutrients, along with compounds associated with the exoskeleton of brown shrimp, and perhaps insulin like peptides may have promoted growth in *L. vannamei*.

CRediT authorship contribution statement

Enno Fricke: Methodology, Investigation, Formal analysis, Data curation, Writing – original draft. **Matthew James Slater:** Validation, Resources, Writing – review & editing, Supervision. **Reinhard Saborowski:** Validation, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no conflict of interest related to the work presented in this paper.

Data availability

Data will be made available on request.

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Insect Farming Industry

The following two chapters (2.4 and 2.5) describe underutilized by-products from black soldier fly larvae (*Hermetia illucens*) production and their use as feed ingredient for *Litopenaeus vannamei*

Remains of black soldier fly larvae production: sheddings of the larvae (exuviae), cocoons, and adult flies (imagines). The black line indicates 10 mm

2.4 Publication III

Utility of by-products of black soldier fly larvae (*Hermetia illucens*) production as feed ingredients for Pacific Whiteleg shrimp (*Litopenaeus vannamei*)

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Utility of by-products of black soldier fly larvae (*Hermetia illucens*) production as feed ingredients for Pacific Whiteleg shrimp (*Litopenaeus vannamei*)

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Abstract

Projected growth in insect production as alternative feedstuffs will yield novel by-products that are potentially valuable for aquafeed applications. We analyzed the nutrient composition of three by-products occurring from black soldier fly larvae production (exuvia, cocoon, imago) and the bioavailability of key nutrients for Pacific Whiteleg shrimp (*Litopenaeus vannamei*). Protein accounted for 317 g kg^{-1} in exuviae, 433 g kg^{-1} in cocoons, and up to 521 g kg^{-1} in adult flies (imagines). Considerable amounts of essential amino acids were detected in imago meal, which significantly matched the ideal dietary amino acid composition for penaeid shrimp ($r^2 = 0.66$, $p = 0.0076$). Exuviae and cocoons contained moderate amounts of lipids (64–140 g kg^{-1}), while imagines comprised 356 g kg^{-1} total lipid. Saturated fatty acids predominated in all insect materials (47%–83% of total fatty acids). Chitin concentration was highest in cocoons and exuviae (154 and 139 g kg^{-1}) and low in imagines (51 g kg^{-1}). A feeding trial with shrimp, *L vannamei*, revealed apparent digestibility coefficients of 20%–59% for protein, 24%–54% for energy, 25%–49% for carbon, and 27%–68% for copper. Defatting of imago meal increased the digestibility of protein, energy, and carbon by

77%, 64%, and 61%, respectively. Defatted imago meal can serve as a protein supplement for shrimp diets.

KEYWORDS
alternative feedstuffs, apparent digestibility coefficients, circular economy, insect by-products, nutrients

1 | INTRODUCTION

The global human population is predicted to reach around 9.7 billion by 2050 (United Nations, 2022). One of the major humanitarian challenges is to secure food availability without exceeding planetary boundaries (Campbell et al., 2017). So far, it is estimated that the global food production sector is responsible for 25% of greenhouse gas emissions (Mrówczyńska-Kamińska et al., 2021), a situation requiring drastic change. Reduction of food waste and circular production patterns are required and frame central goals in policy (European Commission, 2020; United Nations, 2021). Within this context, insects may provide a suitable source material for converting organic waste streams into high value protein and lipid sources. However, as a result of previous disease outbreaks (e.g., transmissible spongiform encephalopathies), hygienic and health concerns exist that have to be addressed regarding the use of animal waste streams as feed ingredients (European Commission, 2001). Ruminant proteins, meat-and-bone meal, catering waste, and manure are prohibited as substrates for insects that are to be used as food/feed in the European Union (European commission, 2009a, 2009b). Yet, recent changes in EU legislation have authorized insects for use in aquafeed (European Commission, 2008), poultry, and pig feed (European Commission, 2021), unlocking further applications for this novel live-stock.

The larvae of the black soldier fly (BSF) (*Hermetia illucens*, Diptera: Stratiomyidae) thrives on numerous organic waste streams (Gold et al., 2018; Jucker et al., 2020; Spranghers et al., 2017) and is an ideal candidate for establishing a circular bio-economy (Liu et al., 2022). In Europe, the technology for industrial insect production is rapidly advancing (Derrien & Boccuni, 2018). In 2018, total insect production was estimated to be 2000 metric tons and is expected to reach 1.2 million metric tons by 2025 (Wunsch, 2020). The total annual monetary turnover of insect feed operators is projected to exceed 2 billion euros by 2023 (IPIFF, 2021), demonstrating the growing market of this industry. Most insect producers in Europe are farming BSFs (Derrien & Boccuni, 2018). Late larvae stages are the main product, and the nutritious larva meal is used as feed for poultry, livestock, and aquaculture (Henry et al., 2015; Makkar et al., 2014; Spranghers et al., 2017). For the production of larvae, all life stages of this insect, from egg to the adult fly (imago), are cultivated (Tomberlin et al., 2002), which, in turn, generates certain by-products.

After hatching, *H. illucens* passes through five larval stages before developing into the pupae (Soetemans et al., 2020). Between each stage, larvae shed their exuviae in order to grow (Hahn et al., 2022). In the pupa stage, the insect metamorphizes into the adult fly (imago) (Tomberlin & Sheppard, 2002). The empty cocoons of the pupae provide another residue of BSF larvae production. Sexual reproduction of *H. illucens* takes place in the imago stage and after a few days of laying eggs, the adult fly dies (Tomberlin & Sheppard, 2002). These three BSF larvae production residues (exuviae, cocoons, and imagines) have been investigated as chitin sources (Hahn et al., 2022; Soetemans et al., 2020), but there is a dearth of research on their potential as sources of biomolecules and nutrients for aquaculture, in particular shrimp species.

The current study presents a detailed characterization of the chemical composition of these insect residues and discusses their relevance for shrimp nutrition. A controlled digestibility trial with Pacific Whiteleg shrimp (*Litopenaeus vannamei*) demonstrates the bioavailability of key nutrients and provides baseline information to investigate the potential of these materials as ingredients for aquafeed production.

2 | MATERIALS AND METHODS

2.1 | By-products of BSF larvae production

Exuviae, cocoons, and imago of BSFs (Figure 1) were obtained from a local insect farm (Farmcycle GmbH, Bremen, Germany). After collection at the production site, the raw materials were transported to the facilities of the Alfred Wegener Institute in Bremerhaven, Germany, and stored at −20°C. The insect materials were oven dried at 60°C for 48 h in glass dishes. After drying, exuviae, cocoons, and imagines were ground to a fine powder with a knife mill (GM 200, Retsch, Germany) in intervals of 30 s with 30 s pauses in between to avoid excess heat development. This process was repeated until all particles of the ground material passed through a 500-μm sieve. The final homogenous meals of the insect by-products were stored at 4°C in air tight containers until further use.

Defatting of the imago meal was done with 99.8% ethanol puriss (Sigma-Aldrich, Germany) as described by L'hocine et al. (2006) and Zhao et al. (2016). Fat extraction was performed at room temperature in 1-L glass bottles at a solvent to meal ratio of 3:1 (v/w) and under continuous rotation of the horizontally placed bottles on a tilt/roller mixer (RS-TR05, Phoenix Instrument, Germany). After 60 min of extraction and another settling period of 30 min in the upright bottles, the lipid-containing supernatant was carefully removed using a glass pipette and discarded. The remaining meal was spread onto glass dishes, left to vaporize under a fume hood overnight, and oven dried the following day for 48 h at 60°C.

2.2 | Biochemical analyses

The moisture and ash contents of the meals were determined following the standard methods 934.01 and 942.05 of the Official Analytical Chemists (AOAC, 2010). An elemental analyzer model Eurovector EA3100 (Eurovector, Pavia,

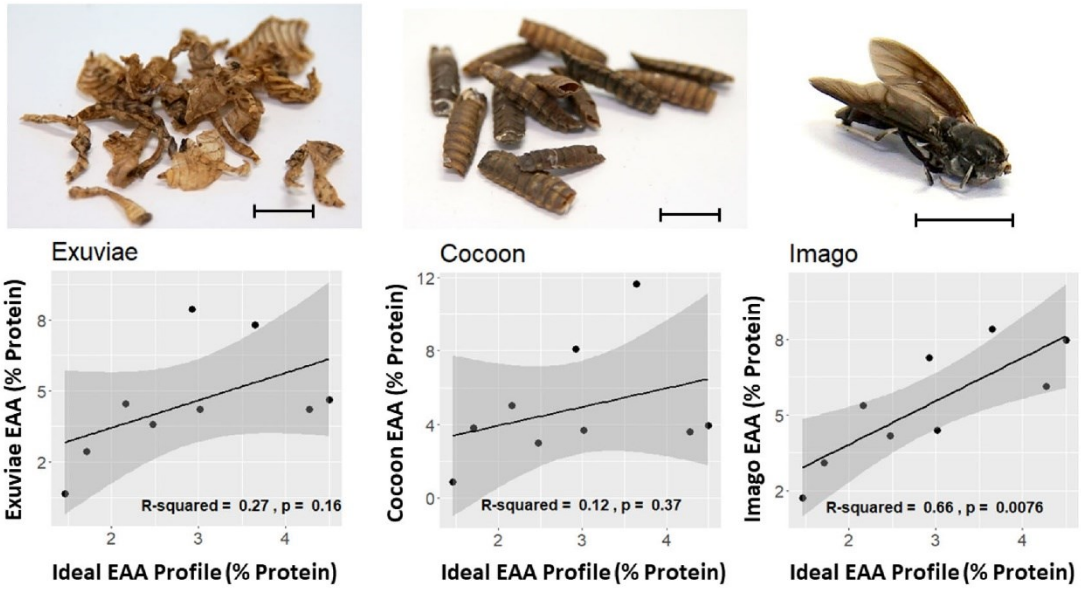

FIGURE 1 Images of the different by-products of industrial black soldier fly (*Hermetia illucens*) larvae production: exuviae of larvae, cocoons, and imagines (from the left to the right). The scale indicates a length of 1 cm. Graphics below each image show the linear correlation of the ideal essential amino acid (EAA) profile of dietary protein for shrimp (National Research Council, 2011) and the EAA contents in the respective raw material, expressed as % of protein.

Italy) was used to determine the carbon and nitrogen contents. Chitin was extracted after Percot et al. (2003) and determined gravimetrically. In brief, samples were demineralized with HCl (0.25 mol·L^{-1}) for 15 min at a solid to liquid ratio of 1:40 (w/v). Consecutive deproteinization was carried out with NaOH (1 mol·L^{-1}) at a ratio of 1:30 (w/v) for 24 h at 70°C. Subsamples of the extracted chitin were then combusted in a muffle furnace for 2 h at 600°C (method 942.05, AOAC (2010)) to correct the chitin contents for residual ash. The chitin bound nitrogen was subtracted from the total nitrogen content of the insect meal. The protein content was determined by multiplying the corrected nitrogen content by 6.25 (Dumas, 1831). Total lipid was measured gravimetrically after Folch et al. (1957) using dichloromethane/methanol (2:1, v/v) as solvent, as described by Postel et al. (2000) and Koch et al. (2023). Energy content was measured using a bomb calorimeter (Parr 6100, Parr Instrument Company, USA). A commercially available test kit (Boehringer, Germany) was used to determine the cholesterol content. Fatty acid (FA) and amino acid (AA) profiles were measured by a certified laboratory (LUFA Nord-West, Germany; methods: ASU L 13.00-26;2008-06, ASU L 13:00-27/2;2012-01 and VO (EG) 152/2009). Yttrium and minerals (P, K, Ca, Mg, Cu) were analyzed by an external commercial laboratory (Institute Dr. Nowak, Ottersberg, Germany) and measured via inductively coupled plasma-optical emission spectrometry (ICP-OES).

2.3 | Digestibility trial

The digestibility trial was performed with test diets containing the insect meals and a reference diet. The reference diet was formulated with fishmeal and soy protein concentrate meal as primary protein sources (Table 1). Yttrium

TABLE 1 Ingredient composition of the reference diet.

Ingredient	Reference diet (g kg^{-1})
Fishmeal[a]	260
Soy protein concentrate[b]	220
Wheatmeal[c]	409
Fish oil[a]	20
Lecithin (soy)[d]	20
Gluten (wheat)[e]	25
Sodium alginate[f]	25
Vitamin and mineral premix[g]	10
Met-Met[h]	5
Yttrium oxide[i]	5
Cholesterol[j]	1

[a] Fishmeal: protein 64%, lipid 9%, and ash 21%; Bioceval GmbH & Co. KG (Cuxhaven, Germany).

[b] Protein 60%, lipid 1%, and ash 6%; Köster Marine Proteins GmbH (Hamburg, Germany).

[c] Protein 11%, lipid 2%, and ash 1%; Mühle Schlingemann e.K. (Waltrop, Germany).

[d] Louis Francois SAS (Croissy-Beaubourg, France).

[e] Kröner-Stärke GmbH (Ibbenbühren, Germany).

[f] BOS FOOD GmbH (Meerbusch, Germany).

[g] Vitamin and mineral premix (g kg^{-1} diet): retinyl acetate (3000 IU), cholecalciferol (3000 IU), dl-a-tocopherol 0.3, menadione 0.04, thiamine 0.03, riboflavin 0.03, pyridoxin-HCL 0.06, cyanocobalamin 0.15 (mg kg^{-1}), nicotinic acid 0.07, D-pantothenic acid 0.07, choline chloride 1, folic acid 6 (mg kg^{-1}), biotin 0.5 (mg kg^{-1}), vitamin C 0.125, inositol 0.3, iron 0.05, copper 0.04, manganese 0.02, zinc 0.075, iodine 2 (mg kg^{-1}), selenium 0.3 (mg kg^{-1}), cobalt 0.06 (mg kg^{-1}), and magnesium 0.3; Spezialfutter Neuruppin GmbH & Co KG (Neuruppin, Germany).

[h] Evonik Industries AG (Essen, Germany).

[i] Fisher Scientific GmbH (Schwerte, Germany).

[j] Merck KGaA (Darmstadt, Germany).

was added as indigestible marker at a concentration of 5 g kg^{-1}. All ingredients of the experimental diets were homogenously ground and sieved (<500 μm). The test diets contained the respective insect meals and the reference diet mash at a ratio of 3:7 (w/w). Water was added to the feed mixtures to achieve a water content of approximately 15%. The resulting dough was mixed thoroughly and pelleted using a PP120 pellet machine (Cissonius, Zehdenick, Germany) with a die hole diameter of 2.5 mm.

A batch of 600 shrimp (*L. vannamei*), weighing approximately 10 g each, were purchased from a nearby indoor shrimp farm (Neue Meere, Gronau (Leine), Germany) and transported to the facilities of the Center of Aquaculture Research of the Alfred-Wegener Institute, Helmholtz Centre for Polar and Marine Research in Bremerhaven, Germany. The shrimp were held in two 750-L tanks (tank dimensions: 1.2 m · 1.2 m · 0.6 m). The tanks were connected to a recirculation aquaculture system (RAS), which consisted of a mechanical drum filter, a biofilter, a protein skimmer, and an UV treatment device. Shrimp acclimatized to the facility conditions for 7 days. During this acclimation time, shrimp received commercial shrimp feed (38% crude protein, 11% fat) two times daily ad libitum.

After 1 week, 180 shrimp were randomly taken from the stock tank (mean weight 10.5 ± 2.0 g) and distributed equally into 15 separate 50-L tanks (tank dimensions: 0.50 m · 0.25 m · 0.45 m), all connected to a RAS as described above. Each tank was stocked with 12 individual shrimp with an average biomass of 125.7 ± 2.1 g per tank. The experimental diets (one reference diet, four test diets) were randomly allocated to the 15 tanks, resulting in three replicates per feed. Shrimp were fed three times daily at 08:00, 11:30, and 15:00 with an equivalent of 4.5% of the shrimp biomass per day. The amount of feed was adjusted weekly, assuming a shrimp growth of 2 g per week. Feces collection commenced 1 week after starting to feed the experimental diets. Therefore, 1 hour after the feed was applied, all tanks were cleaned of uneaten feed remains, feces, and shrimp exuviae. Two hours later, freshly produced feces were carefully collected from each tank using a fine meshed net and stored at −20°C. Feces collection was carried out at 11:00, 14:30, and 18:00. After 4 weeks, approximately 25 g of fecal material (wet weight) was collected per tank and the digestibility trial was terminated. Fecal material was freeze dried, manually homogenized using a mortar and pestle, and stored in a desiccator until further measurements.

The apparent nutrient digestibility coefficients (ADC) of protein, energy, carbon, and copper in the reference and test diets were calculated as proposed by Cho and Slinger (1979):

$$\mathrm{ADC}\,(\%) = 100 - \left[100\left(\frac{Y_{\mathrm{diet}}}{Y_{\mathrm{feces}}}\right)\cdot\left(\frac{N_{\mathrm{feces}}}{N_{\mathrm{diet}}}\right)\right],$$

with N being the considered nutrient concentrations, and Y the yttrium contents in the diet and feces samples, based on dry matter.

The apparent nutrient digestibility of the insect raw materials was determined after Bureau and Hua (2006):

$$\mathrm{ADC}_{(\text{test ingredient})} = \mathrm{ADC}_{\text{test diet}} + \left[\left(\mathrm{ADC}_{\text{test diet}} - \mathrm{ADC}_{\text{ref diet}}\right)\cdot\left(\frac{0.7\cdot N_{\mathrm{ref}}}{0.3\cdot N_{\text{test ingredient}}}\right)\right],$$

where N_{ref} is the nutrient concentrations of the reference diet mash and $N_{\text{test ingredient}}$ being the nutrient contents of the tested insect-based ingredients (as is).

2.4 | Statistics

Datasets were tested for homogeneity of variance and normal distribution using the Bartlett's and Shapiro–Wilk tests, respectively. When parametric assumptions were met, group comparisons were made using one-way ANOVA. A Tukey post-hoc test was performed when statistically significant differences were detected ($p < 0.05$). If homoscedasticity and normal distribution were not given, a Kruskal–Wallis test followed by a Nemenyi post-hoc test was made. Data and regression analysis were conducted with the software R (R Core Team, 2022).

3 | RESULTS

3.1 | Chemical composition

The initial moisture contents of exuviae, cocoons, and imagines were 35%, 30%, and 33%, respectively. Oven drying reduced the moisture content to approximately 5% in all materials, resulting in dry matter contents from 949 to 958 g kg^{-1} (Table 2). The crude protein content was lowest in exuviae with mean values of 317.1 ± 10.1 g kg^{-1} (mean ± SD). Cocoons showed approximately 10% higher crude protein levels, while the imago material had crude protein levels of 521.0 ± 8.7 g kg^{-1}. Defatting the imago meal significantly increased the crude protein content to almost 60% (596.7 ± 10.1 g kg^{-1}) and lowered the lipid content by 34%. Compared to imagines, exuviae and cocoons showed markedly lower total lipid levels. The energy content of the insect materials followed the same tendency as the lipid values: highest energy levels in the imago meal (26.9 ± 0.1 MJ kg^{-1}) and lowest in the cocoon meal (18.8 ± 0.7 MJ kg^{-1}). Chitin was found at similar concentrations in exuviae and cocoons, ranging from 139.2 ± 3.7 g kg^{-1} to 153.9 ± 6.1 g kg^{-1}, respectively. Imago meal comprised approximately one third of this amount. Defatting of the imago meal increased the chitin content by 19% to 60.4 ± 3.9 g kg^{-1}. All materials showed low amounts of cholesterol ranging from 1.2 ± 0.3 g kg^{-1}, in defatted, to 2.8 ± 0.3 g kg^{-1} in untreated imago meal. The ash content was similar in exuviae, imagines, and defatted imago meal. In contrast, the ash content in the cocoon meal reached values almost three times as high with 177.2 ± 1.0 g kg^{-1}. Cocoons also showed highest calcium concentrations of 41.8 g kg^{-1}, while exuviae and imagines contained only 6.2 and 0.6 g kg^{-1}, respectively (Table 3). The other tested macrominerals (phosphorus, potassium, and magnesium) were present at similar levels in the insect by-products, not exceeding 2% of the raw material. The copper content was highest in exuviae with 32 mg kg^{-1}, while the cocoons and imagines showed lower values of 22 and 16 mg kg^{-1}, respectively.

The insect materials contained high relative levels of amino acids that are essential for shrimp (EAA; Table 4). The essential amino acid index (EAAI) ranged from 1.5 to 1.9. Exuviae and cocoons were largely similar in the amino acid profiles, with some variations in leucine and glycine. Cocoons contained more than twice as much leucine, and approximately 60% more glycine than exuviae. Despite high levels of EAA, both materials showed a low correlation with the ideal shrimp dietary amino acid profile reported by the National Research Council (2011). The coefficient of

TABLE 2 Gross nutrient composition of exuviae, cocoons, imago, and defatted imago meal of *Hermetia illucens* expressed in g kg^{-1} "as is," unless otherwise indicated (values are presented as the mean ± standard deviation, $n = 3$–5).

Nutrient (g kg^{-1})	Exuvia	Cocoon	Imago	Imago-defat
Dry matter	949.3 ± 0.6^{a}	958.0 ± 1.2^{b}	951.6 ± 2.6^{ab}	950.7 ± 5.7^{ab}
Crude protein	317.1 ± 10.1^{a}	432.8 ± 25.1^{b}	521.0 ± 8.7^{c}	596.7 ± 10.1^{d}
Gross energy (MJ kg^{-1})	21.0 ± 0.6^{ab}	18.8 ± 0.7^{a}	26.9 ± 0.1^{b}	24.7 ± 0.1^{ab}
Total lipid	139.4 ± 2.6^{b}	63.8 ± 2.9^{a}	355.8 ± 1.7^{d}	234.4 ± 2.2^{c}
Chitin	139.2 ± 3.7^{c}	153.9 ± 6.1^{d}	50.6 ± 2.3^{a}	60.4 ± 3.9^{b}
Cholesterol	1.6 ± 0.3^{a}	1.5 ± 0.2^{a}	2.8 ± 0.3^{b}	1.2 ± 0.3^{a}
Ash	74.0 ± 0.2^{c}	177.2 ± 1.0^{d}	42.6 ± 0.5^{a}	46.5 ± 0.5^{b}

Note: Different superscript letters in the same row indicate significant differences ($p < 0.05$).

TABLE 3 Mineral content of exuviae, cocoons, and imago meal of *Hermetia illucens* as is expressed in g kg^{-1} unless otherwise indicated.

Mineral	Exuvia	Cocoon	Imago
Calcium	6.2	41.8	0.6
Phosphorus	8.2	10.6	9.0
Potassium	17.7	19.7	7.9
Magnesium	3.0	4.6	3.1
Copper (mg kg^{-1})	32.0	22.0	16.0

TABLE 4 Amino acid content (g kg^{-1}) of by-products derived from BSF larvae production.

Amino acid	Exuvia	Cocoon	Imago	Imago-defat
Arginine	10	11	24	29
Histidine	7	12	14	16
Isoleucine	11	16	22	25
Leucine	17	36	32	37
Lysine	11	12	30	35
Phenylalanine	9	9	18	20
Methionine	3	3	9	12
Threonine	10	12	18	22
Valine	18	25	28	32
Aspartic acid	19	23	42	50
Glutamic acid	30	31	50	59
Alanine	20	27	32	37
Cysteine	3	2	4	5
Glycine	20	33	22	25
Serine	12	16	17	20
Proline	19	23	25	28
Tyrosine	12	19	19	22
EAAI	1.5	1.5	1.8	1.9

Note: Essential amino acid index $(\mathrm{EAAI}) = \sqrt[n]{\frac{\mathrm{Arg}_p}{\mathrm{Arg}_i} \times \frac{\mathrm{His}_p}{\mathrm{His}_i} \times \frac{\mathrm{Iso}_p}{\mathrm{Iso}_i} \times \ldots \times \frac{\mathrm{Thr}_p}{\mathrm{Thr}_i}}$ with the subscript *p* referring to insect protein (exuvia, cocoon, imago, and imago-defat) and *I* referring to the ideal protein for penaeid shrimp (National Research Council, 2011).

determinations reached 0.27 and 0.12 for exuviae and cocoons, respectively (Figure 1). The imago meal contained higher concentrations of all amino acids (except leucine in cocoons) and reached an EAAI of 1.8. The correlation with the ideal dietary EAA was statistically significant ($r^2 = 0.66$, $p = 0.0076$).

All insect materials contained a high share of saturated fatty acids (SFAs; Figure 2), accounting from 47% of the total FAs in exuviae, to up to 83% in imagines. Lauric acid (C12:0) was the prevalent FA, accounting for 26% in exuviae, to more than 60% of the total FA contents in imagines. Other SFA, such as myristic acid (C14:0), palmitic acid (C16:0), and stearic acid (C18:0), made up a small share of 2%–13% of the total FA. The absolute contents of mono- and polyunsaturated fatty acids (MUFA, PUFA) were highest in exuviae and imagines, while defatted imago meal and cocoons showed lower levels (Table 5). Oleic acid (C18:1(n-9)) was the predominant MUFA, while the PUFAs linoleic- (C18:2(n-6)) and linolenic acid (C18:3(n-3)) were found in varying levels throughout the tested materials. The

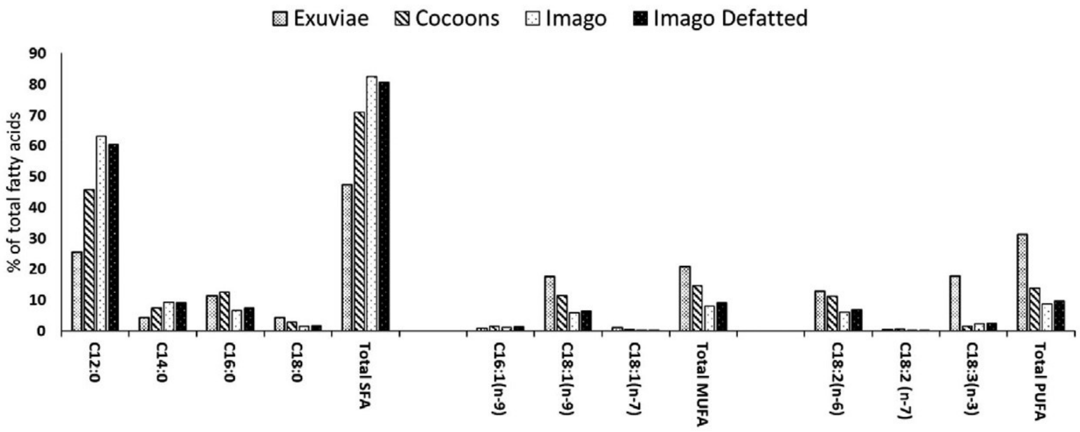

FIGURE 2 Fatty acid profile the different by-products of industrial black soldier fly (*Hermetia illucens*) larvae production: exuviae of larvae, cocoons, and imagines, expressed as % of total fatty acids.

TABLE 5 Fatty acid content (g kg^{-1}) of by-products derived from BSF larvae production.

Fatty acids	Exuvia	Cocoon	Imago	Imago-defat
Saturated fatty acids (SFA)				
C10:0	0.7	0.4	2.8	1.7
C12:0	30.7	21.1	169.6	117.6
C14:0	5.1	3.4	24.9	18.0
C16:0	13.7	5.8	18.1	14.5
C17:0	0.3	0.1	0.3	0.3
C18:0	5.2	1.4	4.1	3.5
C20:0	0.3	0.1	0.4	0.4
C22:0	0.3	0.1	0.4	0.3
Total SFA	56.6	32.6	221.5	157.1
Monounsaturated fatty acids (MUFA)				
C16:1(n-9)	1.2	0.7	3.4	2.7
C18:1(n-9)	21.1	5.2	15.7	12.9
C18:1(n-7)	1.4	0.2	0.6	0.6
C20:1(n-9)	0.4	0.2	1.0	0.7
Total MUFA	25.1	6.8	21.7	17.7
Polyunsaturated fatty acids (PUFA)				
C18:2(n-6)	15.5	5.2	16.5	13.4
C18:3(n-3)	21.4	0.7	6.6	5.1
Total PUFA	37.4	6.4	23.7	19.0

relative FA profile (Figure 2) showed that the contribution of MUFA and PUFA was highest in exuviae with 21% and 31% of the total FAs and decreased in cocoons to 15% and 14%. Imagines and defatted imagines contained the lowest percentage of MUFA and PUFA with 8%–10%.

3.2 | Digestibility

The apparent protein digestibility was lowest in exuviae with 19.6 ± 10.7% (Table 6). Both cocoons and imagines had a similar apparent protein digestibility of approximately 35% and 38%. The apparent energy and carbon digestibility of exuviae, cocoons, and imagines followed the same trend: higher values in the exuvia, lower apparent digestibility in imago, and lowest in cocoons. This pattern was also seen in the apparent copper digestibility, reaching values of 68.2 ± 12.9% in exuviae and 26.6 ± 14.6% in cocoons. Defatting of the imago meal resulted in an increase of all ADC. Apparently, 59.1 ± 4.4% of the protein of defatted imago meal was bioavailable for *L. vannamei*, leading to a digestible protein content of 370.7 g kg^{-1}. This was an increase of approximately 77%, compared to the digestible protein level of untreated imago meal with 209.6 g kg^{-1}. The digestible carbon and energy contents increased by 61% and 64%, when imago meal was defatted, reaching absolute digestible values of 227.9 g kg^{-1} and 13.8 MJ kg^{-1}.

4 | DISCUSSION

Exploring suitable nutrient sources from by-products for aquaculture feeds is essential for the development of sustainable aquaculture. BSF larvae are a valuable animal feed ingredient suitable for terrestrial and aquatic animal nutrition. There are numerous biological by-products remaining during BSF larvae production and this study reports initially the detailed chemical composition of these by-products and the bioavailability of key nutrients for *L. vannamei*. These insect remains contain considerable amounts of crude protein (30%–60%) and low (<10%) to moderate (18%) amounts of ash. A high share of EAA and the presence of biomolecules such as cholesterol and chitin, as well as micro-minerals like copper, make these raw materials especially interesting as ingredients for shrimp feed.

The crude protein content of exuviae and cocoons is comparable to that of plant-based ingredients such as pea, canola, lupin, and peanut meal, which have been successfully tested as alternative protein ingredients in shrimp feeds (Cruz-Suarez et al., 2001; Liu et al., 2012; Smith et al., 2007; Weiss et al., 2020). The imago life stage of the BSF showed a crude protein content similar to that of the BSF larvae, which varies between 400 and 600 g kg^{-1} (Cummins Jr et al., 2017; Richardson et al., 2021; Spranghers et al., 2017). The amino acid profile of imago largely resembles that of the BSF larvae with high levels of EAA such as leucine, isoleucine, and lysine (Cummins Jr

TABLE 6 Apparent digestibility coefficients (ADC) for energy, carbon, protein, and copper of black soldier fly larvae production residues and the calculated amount of the respective digestible nutrient (on dry matter basis, values are expressed as mean ± standard deviation).

Nutrient ADC (%)	Exuvia	Cocoon	Imago	Imago-defat
Protein	19.6 ± 10.7^{a}	35.4 ± 16.6^{ab}	38.3 ± 8.3^{ab}	59.1 ± 4.4^{b}
Energy	44.4 ± 9.2^{bc}	24.4 ± 8.3^{a}	29.9 ± 1.4^{ab}	53.1 ± 3.0^{c}
Carbon	45.7 ± 13.2	25.1 ± 10.2	27.7 ± 14.1	49.2 ± 5.0
Copper	68.2 ± 12.9^{b}	26.6 ± 14.6^{a}	36.7 ± 15.9^{ab}	N/A
Digestible nutrient				
Protein (g kg^{-1})	65.6	160.0	209.6	370.7
Energy (MJ kg^{-1})	9.8	4.7	8.4	13.8
Carbon (g kg^{-1})	227.9	113.2	141.7	227.9
Copper (mg kg^{-1})	23.0	6.1	6.2	N/A

Note: Values with different superscript letters are statistically different ($p < 0.05$).

et al., 2017; Oteri et al., 2021; Spranghers et al., 2017). All insect by-products potentially provide sufficient EAA needed for shrimp nutrition as indicated by the high EAAI. However, the low correlation between EAA in exuviae and cocoons versus the ideal EAA of dietary protein implies some amino acid (AA) imbalances, if these resources were used as single AA sources in shrimp diets. In contrast, the significant correlation of EAA between imago meal and the ideal protein requirements suggests that imago meal is a more appropriate protein source for *L. vannamei*.

The different AA composition of exuvia and cocoon meals compared to imago meal could be explained by the predominantly structural and chitin-bound proteins in these exoskeleton-based materials. Exoskeletons of insects primarily consist of sclerotized chitin and protein that act as a protective barrier against the external environment (Willis, 1987). Proteins associated with exoskeletons show specific AA compositions, which, in turn, are linked to the physio-chemical property of the cuticle in different life stages of insects (Andersen et al., 1995; Kumari et al., 1995). Chitin is another major biomolecule in insect exoskeletons and accounts for 20% and 40% in *H. illucens* exuviae of different life stages (Hahn et al., 2018). The values for exuviae and cocoons obtained in the current study are lower (14%–15%). This discrepancy might be because of natural variation in the biological material, impurities of the raw materials, and/or differences in the chitin determination methods. The nutritional relevance of chitin and its derivatives for penaeid shrimp remains under debate (Akiyama et al., 1989; Brol et al., 2021; Clark et al., 1993; Fox, 1993; De los Santos-Romero et al., 2017). Nevertheless, excellent aquafeed ingredients such as shrimp head meals contain chitin in the range of 90–178 g kg^{-1} (Fox et al., 1994; Fricke et al., 2022; Synowiecki & Al-Khateeb, 2000) and *L. vannamei* possess chitinases that indicate their capability to digest chitin (Sotelo-Mundo et al., 2009).

Liu et al. (2017) found changes in the crude lipid content between 5% and 32% of the dry mass throughout the different life stages of *H. illucens*. The lipid content increased steadily up to 28% during the feeding phase of the larvae, but decreased again drastically to 7% in late pupal stage. After metamorphosis to imago, the fat content reached highest values of more than 30%, presumably accumulating energy reserves for reproduction (Liu et al., 2017). These variations in lipid content are consistent with our findings, showing intermediate lipid values in exuviae, low contents in cocoons, and highest in the imago.

The changes in FA composition throughout the lifespan of *H. illucens* are also coherent with the FA profile of BSF production residues that originate from the respective life stage. While SFAs are predominant over the whole lifespan, unsaturated FAs, such as oleic acid (C18:1(n-9)), linoleic acid (C18:2(n-6)), and linolenic acid (C18:3(n-3)), make up a larger share in early larval stages (Liu et al., 2017). Exuviae from larvae stages also showed highest shares of unsaturated FAs.

Shrimp do not tolerate high levels of dietary lipids (National Research Council, 2011). Therefore, high fat insect meals are often defatted to achieve a more suitable nutrient profile (Cummins Jr et al., 2017; Motte et al., 2019; Oteri et al., 2021). Defatting of imago meal in our study reduced the total lipid content without altering the FA profile. While the defatting also resulted in a decrease in cholesterol and the overall energy content, non-fat nutrients such as protein, chitin, and ash increased accordingly.

The apparent digestibility of protein, energy, and carbon increased in defatted imago meal. It seems that a high lipid content negatively affects nutrient bioavailability. This is in line with Glencross et al. (2002), who found significantly reduced lipid digestibility in *Penaeus monodon* when fed diets containing 135 g kg^{-1} of total lipid.

The apparent nutrient digestibility of exuviae, cocoons, and imagines did not exceed 50% (except copper in exuviae). In comparison, the apparent protein digestibility of BSF larvae meals in *L. vannamei* range between 72% and 85% (Li et al., 2022; Shin & Lee, 2021). The protein of other insect species such as the mealworm (*Tenebrio molitor*) is even less efficiently digested by *L. vannamei*, with values ranging from 42% to 57% (Li et al., 2022; Panini et al., 2017). This is in contrast to the findings of Shin and Lee (2021), who reported ADC protein values of 84% for mealworm meal. The mealworm used in the study of Shin and Lee (2021) differed in proximate composition, containing less protein and less lipid compared to the mealworm used in the other studies (Li et al., 2022; Panini et al., 2017). It appears that the bioavailability of the nutrients in insect meals is strongly affected by insect taxa, life-stage of the insect, and the gross nutrient composition of the material. For instance, chitin has been assumed to negatively influence nutrient digestibility and is sometimes classified as anti-nutritional and indigestible fiber (Barroso et al., 2014; Kroeckel et al., 2012; Shiau & Yu, 1998, 1999). Despite the presence of chitinolytic enzymes in the digestive tract of *L. vannamei* (Huang et al., 2010), chitin seems to be poorly digested with ADCs of around 30% (Clark et al., 1993; Shin & Lee, 2021). Higher concentrations of undigested chitin might therefore interfere with the absorption of other nutrients, similar to the effects of dietary non-starch-polysaccharides (Glencross et al., 2012). Furthermore, proteins present in insect exoskeletons that are sclerotized or bound to chitin could be less accessible for digestion as pointed out by Panini et al. (2017). This might explain the low ADC protein measured in chitin-rich exuviae and cocoons, which are primarily composed of exoskeletal material.

Glencross et al. (2002) observed that the digestibility of FA in *P. monodon* increased with the share of unsaturated FAs and with shorter FA chain lengths. The low energy digestibility observed in all tested materials of the current study is probably caused by the low digestibility of the SFAs that are predominant in the insect materials. The slightly higher apparent energy and carbon digestibility in exuviae could therefore be explained by the higher share of well-digestible MUFA and PUFA.

Insects comprise a large variety of antimicrobial compounds such as peptides, FAs, and chitin/chitosan (Lagat et al., 2021; Saadoun et al., 2022; Saviane et al., 2021). Improved pathogen resistance and immunological parameters were reported for *L. vannamei* when fed with *H. illucens* larvae and *T. molitor* (Choi et al., 2018; Motte et al., 2019; Richardson et al., 2021). Insect-based immunostimulants could provide a viable and safe alternative to antibiotics (Saadoun et al., 2022), which have been banned in livestock feed in the European Union (European Commission, 2003). Exuviae, cocoons, and imagines contain biologically relevant compounds (e.g., chitin, lauric acid, cholesterol, copper), which may promote growth, health, and disease resistance. Dietary supplementation of chitin and its derivatives was shown to improve performance in penaeid shrimp (Brol et al., 2021; Niu et al., 2013; Shiau & Yu, 1998). Copper is an essential micronutrient for crustaceans, needed for many metabolic processes and essential in hemocyanin synthesis (Culotta et al., 2006; Depledge, 1989; National Research Council, 2011). Cholesterol is considered indispensable in shrimp diets, and supplementation of purified cholesterol is one of the key drivers in feed production cost (Kanazawa et al., 1984; Smith et al., 2001; Zhang et al., 2019). Especially in diets increasingly replacing marine ingredients with plant-based alternatives, by-products of BSF larvae production could be included as dietary cholesterol source.

The ADC of copper was highest in exuviae, resulting in 23 mg kg^{-1} of bioavailable copper. This value seems high in comparison with the other insect materials investigated in this study, but is only about half the amount of digestible copper found in brown shrimp processing remains (Fricke et al., 2022). Phosphorus was present in similar levels across the tested insect materials and were slightly higher than in early BSF prepupa (Liu et al., 2017). The high calcium content found in cocoons indicates a high degree of calcification, thus explaining the rigid structure of this material. Information on the dietary requirements of minerals in shrimp is scarce and hard to determine because marine crustaceans can absorb certain minerals directly from the ambient seawater (National Research Council, 2011). Yet, information on mineral contents in feed ingredients is relevant to formulate high precision feeds and account for mineral interactions, for example, appropriate calcium/phosphorous ratios, to ensure sufficient phosphorous availability (Davis et al., 1993).

5 | CONCLUSIONS

Despite promising chemical compositions of BSF larvae production by-products, the bioavailability of key nutrients is limited for *L. vannamei*. Defatting of imago meal resulted in a significantly increased nutrient digestibility. These findings, along with an improved nutrient profile, encourage further investigation of defatted imago meal as an alternative protein source in shrimp feeds. Exuviae and cocoons, with high chitin, copper, and cholesterol contents are as well interesting candidates as functional ingredients, rather than replacing macronutrients in aquafeeds. Along with growing industrial production of insects, the availability of these novel by-products will increase accordingly, ensuring a year-round steady supply. These novel resources can represent sustainable and efficient feed ingredients in the future.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no personal nor financial interests that could have appeared to influence the work of this study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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2.5 Manuscript I

Defatted black soldier fly (*Hermetia illucens*) imago meal as replacement for fish meal in diets for Pacific Whiteleg shrimp (*Litopenaeus vannamei*)

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Defatted black soldier fly (*Hermetia illucens*) imago meal as replacement for fish meal in diets for Pacific Whiteleg shrimp *Litopenaeus vannamei*

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Keywords

- Alternative protein
- Insect
- Circularity
- By-products

ABSTRACT

Imagines, the dead adult stage of the black soldier fly, *Hermetia illucens*, are a by-product of large-scale larvae production. The suitability of this novel side stream as protein source in diets for juvenile Whiteleg Shrimp, *Litopenaeus vannamei* (initial weight 5 – 6 g), was investigated in controlled feeding experiments in recirculating aquaculture systems. Three experimental diets replacing 50% (I50), 75% (I75) and 100% (I100) of the fish meal content of the diets with defatted imago meal (IM) were tested in an acceptability trial and a growth trial. The acceptability of all IM-containing diets was reduced, leading to significantly lower apparent feed intake of the I75 and I100 diets during the growth experiment ($p = 0.001$). Shrimp growth was reduced in all IM-containing diets ($p = 0.0005$). Inclusion of IM led to lower dry matter contents in shrimp muscle ($p = 0.0136$) and lower lipid contents in midgut glands ($p = 0.0169$). Survival, digestive enzyme activities, and hemolymph parameters were not affected by dietary treatment, implying no adverse effects of IM on general shrimp health. Based on these findings, defatted imago meal cannot replace dietary fish meal at the levels tested in this study. Lower IM inclusion rates ($< 100\ g \cdot kg^{-1}$), further defatting of IM, and addition of attractants are suggested to improve the nutritional quality of imago meal.

1. INTRODUCTION

Cost effective, high-quality protein ingredients are required to meet increasing demand for sustainable aquaculture feeds (Kim et al., 2019; Tacon & Metian, 2015; Tacon, 2019). Conventional protein sources from fisheries or agriculture contribute to the overexploitation of natural resources and may compete directly with use as human food (Fry et al., 2016; Naylor et al., 2021). A resilient and efficient food producing sector can be achieved through a circular bioeconomy addressing environmental impacts and improving resource utilization (Dahiya et al., 2018; Yang et al., 2023). Insects are capable of converting organic waste streams into high-value nutrients (Jucker et al., 2020; Liu et al., 2022). Nutrient contents of insect meals are high, commonly ranging from 40 to 60% in protein and 20 to 50% in lipids (Liu et al., 2017; Panini et al., 2017; Peh et al., 2021; Perez-Santaescolastica et al., 2023). Different insect species such as crickets, mealworm, silkworm, housefly larvae, and black soldier fly larvae have been investigated as fish meal replacement in diets for fish and shrimp (Panini et al., 2017; Peh et al., 2021; Rahimnejad et al., 2019; Wang et al., 2017).

The black soldier fly (BSF), *Hermetia illucens*, is one of the most studied insects for aquaculture feeds. Larvae meals made from the late pre-pupae stages of this insect are generally used for feed applications (Soetemans et al., 2020). Successful dietary fish meal replacement with BSF larvae meal has been reported for commonly cultured species such as *Salmo salar, Oncorhynchus mykiss*, *Oreochromis niloticus*, *Dicentrarchus labrax* and *Litopenaeus vannamei* (Abdel-Tawwab et al., 2020; Biasato et al., 2022; Bruni et al., 2020; Nunes et al., 2019; Tippayadara et al., 2021).

The industrial production of insects is advancing rapidly and is expected to reach 1.2 million metric tons globally by 2025 (Derrien & Boccuni, 2018; Wunsch, 2020). As production increases, novel by-products that contain considerable amounts of nutrients and biomolecules, suitable for further applications are becoming available (Soetemans et al., 2020; Yildirim-Aksoy et al., 2022). By-products of BSF larvae production include hulls of the larvae (exuviae), empty cocoons, the adult life stage (imago), and the remaining frass (Fricke et al. 2024, Soetemans et al. 2020, Yildirim-Aksoy et al. 2022). Utilization of these by-products can reduce waste, create value, and pose cost-effective feed ingredients.

The adult stage of the black soldier fly has a life span of only few days. It is cultured for reproduction and egg production (Soetemans et al., 2020; Tomberlin et al., 2002). Imagines, the dead adults, contain high amounts of protein (521 $g \cdot kg^{-1}$), lipid (356 $g \cdot kg^{-1}$), essential amino acids, and minerals. They are potentially suitable as feed ingredient for the Pacific Whiteleg shrimp, *Litopenaeus vannamei* (Fricke et al., 2024). Defatting of imago meal enhanced the overall nutrient profile and improved the apparent digestibility of key nutrients in *L. vannamei* (Fricke et al., 2024). However, further information whether defatted imago meal can be included in diets and how this novel by-product affects overall performance and health in *L. vannamei* is not available.

The aim of the present study was to investigate the suitability of defatted imago meal as protein source and fish meal substitute in feeds for *L. vannamei*. To assess the effects of the ingredient on feed palatability an acceptability trial was conducted. Growth performance, feed utilization, tissue composition, hemolymph parameters, and digestive enzyme activities were then investigated in a 40-day feeding trial with juvenile *L. vannamei*.

2. MATERIALS AND METHODS

2.1 Defatted black soldier fly imago meal

Approximately 3 kg of dead adult *Hermetia illucens* flies (imagines) were provided by the insect farm Farmcycle GmbH (Bremen, Germany) and stored in airtight plastic containers at -20 °C at the Alfred Wegener Institute (Bremerhaven, Germany). The imagines were oven dried at 60 °C for 48 h on glass trays. After drying, the insects were ground to a fine powder with a knife mill (GM 200, Retsch, Germany). To avoid heating of the material, grinding was done in intervals of 30 s, followed by 30 s pauses. The imago meal was defatted with ethanol (99.8%, Sigma-Aldrich, Germany) after L'hocine et al. (2006) and Zhao et al. (2016), as adapted for imago meal (*H. illucens*) by Fricke et al. (2024). After defatting and drying, the resulting defatted imago meal (IM) was sieved (< 500 µm) and stored at -4 °C in airtight containers until production of the experimental feeds within two weeks.

2.2 Experimental feeds

Four experimental diets were formulated to meet the nutrient requirements of *L. vannamei* (NRC, 2011): one control diet (Control containing 26% fish meal) and three diets replacing 50% (I50), 75% (I75) and 100% (I100) of the fish meal content (Table 1). The fish meal (FM) used for diet formulations was made of sardine and mackerel processing remains (Köster Marine Proteins GmbH, Hamburg, Germany). All diets were formulated to contain similar digestible energy (15 MJ·kg^{-1}) and digestible protein levels (350 g·kg^{-1}). For IM, the bioavailability of energy (13.8 MJ·kg^{-1}) and protein (370.7 g·kg^{-1}) were based on the findings of Fricke et al. (2024). Digestible nutrient values for FM and other macro ingredients were obtained from the International Aquaculture Feed Formulation Database (IAFFD). Nutrient profiles of diets were balanced using different amounts of soy protein concentrate, wheat meal, gluten, coconut oil, sodium alginate, methionine and lysine. Coconut oil was used to balance the high lipid content of IM.

Table 1. Ingredient composition of experimental diets prepared for acceptance and controlled feeding trials. Control = control diet containing 26% fish meal, I50 = diet with 50% replacement of fish meal with imago meal, I75 = diet with 75% replacement of fish meal with defatted imago meal and I100 = diet with 100% replacement of fish meal.

Ingredient ($g \cdot kg^{-1}$)	Control	I50	I75	I100
Fish meal [1]	260	130	65	-
Defatted imago meal [2]	-	130	195	260
Soy protein concentrate [1]	300	328	356	384
Wheatmeal [3]	356	319	294	269
Cod liver oil [4]	15	15	15	15
Coconut oil [5]	24	16	8	0
Lecithin (soy) [6]	20	20	20	20
Gluten (wheat) [7]	-	25	30	35
Sodium alginate [8]	10	-	-	-
Vitamin and mineral premix [9]	10	10	10	10
Met-Met [10]	2	3	3	3
L-Lysin [11]	1	2	2	2
Cholesterol [12]	2	2	2	2

[1] Köster Marine Proteins GmbH (Hamburg, Germany)

[2] Entosus GmbH (Bremen, Germany)

[3] Mühle Schlingemann e.K. (Waltrop, Germany)

[4] Bioceval GmbH & Co. KG (Cuxhaven, Germany)

[5] Rapunzel Naturkost GmbH (Legau, Germany)

[6] My Lecithin e.K., (Neu Wulmstorf, Germany)

[7] Kröner-Stärke GmbH (Ibbenbühren, Germany)

[8] Gioia Group s.r.l., (Turin, Italy)

[9] vitamin and mineral premix ($g \cdot kg^{-1}$ diet): retinyl acetate (3000 IU), cholecalciferol (3000 IU), dl-a-tocopherol 0.3, menadione 0.04, thiamine 0.03, riboflavin 0.03, pyridoxin-HCL 0.06, cyanocobalamin 0.15 ($mg \cdot kg^{-1}$), nicotinic acid 0.07, D-pantothenic acid 0.07, choline chloride 1, folic acid 6 ($mg \cdot kg^{-1}$), biotin 0.5 ($mg \cdot kg^{-1}$), vitamin C 0.125, inositol 0.3, iron 0.05, copper 0.04, manganese 0.02, zinc 0.075, iodine 2 ($mg \cdot kg^{-1}$), selenium 0.3 ($mg \cdot kg^{-1}$), cobalt 0.06 ($mg \cdot kg^{-1}$), magnesium 0.3; Spezialfutter Neuruppin GmbH & Co KG (Neuruppin, Germany)

[10] Evonik Industries AG (Essen, Germany)

[11] Borgstädt Solutions UG (Langenfeld, Germany)

[12] Merck KGaA (Darmstadt, Germany)

For feed production, micro-ingredients (quantities $< 50\ \mathrm{g \cdot kg^{-1}}$) were first mixed thoroughly by hand before the macro-ingredients were added. Fish oil, lecithin and coconut oil were blended and then slowly poured onto the diet mash under constant stirring. Once all ingredients were added, water ($300\ \mathrm{mL \cdot kg^{-1}}$) was sprinkled onto the ingredient mixture. The final dough was continuously mixed for one hour in a planetary mixer (GGM Gastro International GmbH, Ochtrup, Germany). Thereafter, the diet mash was pelleted into 2-mm pellets using a pellet mill (PP120, Cissonius, Zehdenick, Germany). Freshly produced pellets were spread out on large trays and air dried at room temperature for 72 h. After drying, feeds were stored in sealable plastic bags until the start of the experiments one week later.

Experimental feeds were analyzed for dry matter and ash content following the standard methods of the Official Analytical Chemists (AOAC, 2010). The nitrogen content was determined with an elemental analyzer (Euro Elemental Analyzer, Eurovector SPA, Italy) via combustion at high temperatures and separation of resultant gaseous species. Crude protein was calculated by multiplying the nitrogen content by factor 6.25 (Dumas, 1831). Total lipids were determined gravimetrically after lipid extraction of samples using dichloromethane / methanol (2:1, v/v) (Koch et al., 2023). The energy content was measured using an oxygen bomb calorimeter (Parr 6100, Parr Instrument Company, USA). Crude fiber content was measured by a certified laboratory for feed analysis (LUFA Nord-West, Germany) according to the standard method recommended by the European Union (European Commission Regulation No 152/2009).

2.3 Shrimp and experimental system

Feeding experiments were registered at the responsible authorities of the State of Bremen (Altfeed, TA21-04) and conformed with the appropriate regulations and guidelines of the German animal protection laws. Approximately 1000 live juvenile shrimp, *L. vannamei*, were purchased from the indoor shrimp farm Aquapurna GmbH (Wunstorf, Germany) and transported to the Center for Aquaculture Research (ZAF) of the Alfred-Wegener Institute in Bremerhaven, Germany. To recover from the transport and acclimatize to the culture conditions of the facility, shrimp were held in two aerated tanks ($0.75\ \mathrm{m^3}$ each) connected to a recirculating aquaculture system (RAS) for seven days. The RAS consisted of a moving-bed biofiltration unit, mechanical filter, protein skimmer, ozone generator, and UV-sterilizer. The illumination of the facility followed a 10:14 h light:dark cycle. During the acclimation period, shrimp were fed ad libitum every day in the morning and afternoon with grow out feed for shrimp (38% protein, 7% fat, $18.3\ \mathrm{MJ \cdot kg^{-1}}$ gross energy, ø = 1.7mm, Le Gouessant, Lamballe-Armor, France). Water parameters (dissolved oxygen, temperature, pH, and conductivity) were measured continuously via sensors and a digital monitoring system (Senect GmbH & Co. KG, Landau Germany). Nitrogenous compounds (NH_3, NO_2-, NO_3-) in the water were determined twice per week with a QuAAtro39 Continuous Segmented Flow Analyzer (SEAL, Analytical GmbH, Norderstedt, Germany).

Water parameters were maintained within the following average values (± standard deviation): dissolved oxygen: 91.2 ± 3.8%; temperature: 28.6 ± 0.4 °C; pH: 7.9 ± 0.2, conductivity: 34.4 ± 0.6 mS·mL^{-1}, NH_3: 0.1 ± 0.1 mg·mL^{-1}; NO_2-: 0.2 ± 0.1 mg·mL^{-1}; NO_3-: 116.1 ± 32.7 mg·mL^{-1}.

Table 2. Nutrient profile and selected amino acid composition of the fish meal and defatted imago meal and the relative difference of each nutrient, expressed in percent points.

Nutrient (g·kg^{-1}, unless otherwise indicated)	Fish meal	Imago meal	% difference
Dry matter	924	950	+3
Crude protein	682	597	-12
Total lipid	96	234	+144
Gross energy (MJ·kg^{-1})	21	25	+19
Ash	143	47	-67
Amino acids			
Arginine	45.2	29.0	-36
Histidine	16.8	16.1	-4
Isoleucine	32.1	24.9	-22
Leucine	51.5	36.8	-29
Lysine	59.2	35.3	-40
Phenylalanine	27.3	20.3	-26
Methionine	21.2	11.5	-46
Threonine	28.0	21.6	-23
Valine	41.5	32.2	-22

Table 3. Chemical composition of experimental diets (as determined) and estimated amino acid profile. Control = control diet containing 26% fish meal, I50 = diet with 50% replacement of fish meal with defatted imago meal, I75 = diet with 75% replacement of fish meal with defatted imago meal and I100 = diet with 100% replacement of fish meal with defatted imago meal.

Parameter ($g \cdot kg^{-1}$, unless otherwise indicated)	Control	I50	I75	I100
Chemical composition [a]				
Dry matter	938	925	923	921
Crude protein	427	437	443	435
Total lipid	100	104	109	108
Crude fiber	23	38	46	55
Ash	73.6	57.4	56.7	46.4
Gross energy ($MJ \cdot kg^{-1}$)	19.6	20.5	20.2	20.5
Amino acids [b]				
Arginine	27.3	26.7	26.9	27.0
Histidine	10.4	11.0	11.3	11.7
Isoleucine	18.5	18.7	19.1	19.4
Leucine	30.6	32.1	32.8	33.4
Lysine	29.1	28.1	27.6	27.1
Phenylalanine	19.1	19.7	20.2	20.6
Methionine	10.4	10.6	10.2	9.0
Threonine	15.8	15.9	16.2	16.5
Valine	22.0	22.0	22.2	22.4

[a] values are mean values of 2 to 4 replicates, depending on the parameter.

[b] based on calculated values

2.4 Acceptability trial

A total of 24 shrimp with an average weight of 9.3 ± 1.1 g were distributed evenly into 4 separate glass aquaria (48×38×49 cm, length×width×height) - 6 shrimp per tank. The aquaria were connected to a RAS with the same components as outlined in section 2.3. Shrimp were left to acclimatize to the new environment for one week. During this time, shrimp were fed with grow-out feed as above, at 4.5% of the biomass per day, provided in two rations at 09:00 and 14:00. After the acclimation period, shrimp feeding behavior was recorded with a video camera setup (Sony A7sII, 60 fps, ISO4800; Sigma 12-24@18 mm, f2,8) in front of the aquaria. At each recorded feeding event one experimental diet was randomly assigned to one experimental tank each. A total of 18 feed pellets of the assigned feed were placed into the assigned aquarium. Shrimp behavior was then recorded for 20 min. Pre-trials with standard grow out feed revealed that shrimp ingested 100% of the provided pellets within a 20 min period. Analysis of the video material allowed the time until start of feeding (first contact), rejections of feed pellets once approached (feed rejection), and feed pellet consumption over time (% of pellet consumption after 10 min and 20 min) to be recorded exactly. Feed acceptability observations were made once per day at afternoon feeding (14:00). In the morning, shrimp received standard grow out feed. Each day, experimental feeds were randomly allocated to the shrimp tanks anew. Observations with each experimental feed were made in quadruplicate, resulting in four experimental days in total.

2.5 Growth Trial

A 40-day growth trial was conducted in 16 separate 50-L tanks (50×25×45 cm, length×width×height), connected to the RAS as described in section 2.3. Water inflow was adjusted to 100 $L \cdot h^{-1}$ in each tank and constant aeration was provided by submerged diffuser air stones. A total of 224 juvenile shrimp with an average weight of 5.4 ± 1.0 g were distributed evenly across all tanks, resulting in a stocking density of 14 individuals per tank. Care was taken that the size distributions of shrimp remained similar across all tanks with a coefficient of variance from 17.2 to 20.1%. Experimental diets (Control, I50, I75, I100) were randomly allocated to the tanks, resulting in four replicates per treatment. Feed was provided according to the feeding rates proposed by Tacon et al. (2013). Shrimp that died during the first 3 days of the trial were replaced by shrimp of similar weight. If mortalities occurred thereafter, the amount of feed for the respective tank was adjusted accordingly. Each week, the feeding rate was adjusted assuming a shrimp growth rate of 2 $g \cdot week^{-1}$. To account for the feed intake, uneaten feed remains were collected after 1 h if present, using a fine-meshed net. Feed remains were dried in a microwave oven (SAM 225, CEM Microwave Technology, USA) and weighed the following day. The feeding rate was not increased when the uneaten feed exceeded more than 15% of the provided feed. At the end of the growth trial, all shrimp were netted from the tanks, killed in an ice slurry, counted, and individually weighed.

2.6 Hemolymph parameters

At the end of the growth trial, hemolymph of three shrimp in the intermolt stage was sampled for total hemocyte counts (THC), plasma total protein, and glucose levels. The molt-stage of shrimp was determined after Robertson et al. (1987). Before withdrawing hemolymph from the base of the fifth pereiopod, the puncture site was cleaned with 75% ethanol. Hemolymph was sampled with a sterile 1-mL plastic syringe filled with 100 µL cold anticoagulant (27 $mmol \cdot L^{-1}$ trisodium citrate, 310 $mmol \cdot L^{-1}$ sodium chloride, osmolality 640 $mmol \cdot kg^{-1}$). Depending on the amount of withdrawn hemolymph, anticoagulant was added to reach a final ratio of 1:1 (w/w). Care was taken, that the duration of shrimp capture, killing, molt-staging, and subsequent hemolymph collection did not exceed 10 min. Subsamples (30 µL) of vortexed hemolymph samples were directly analyzed for total hemocyte counts using a Cellometer Spectrum (Nexelcom Bioscience, Lawrence, MA, USA). The residual hemolymph was centrifuged for 10 min at 800 g and 4 C° (Biofuge fresco, Heraeus Instruments, Hanau, Germany). The supernatant (plasma) was aliquoted, shock frozen in liquid nitrogen, and stored at -80 °C until further analysis. Plasma total protein and glucose concentrations were then analyzed using an automated blood analyzer (Fuji DRI-CHEM NX500, Tokyo, Japan).

2.7 Digestive enzyme assays

Midgut glands of three shrimp per replicate were carefully dissected and weighed to the nearest 0.001 g. Samples were shock frozen in liquid nitrogen and stored at -80°C. Crude enzyme extracts were prepared by homogenizing midgut gland tissue in ultrapure water (Mili-Q, Merck, Germany) at a ratio of 1:5 (weight to volume) using a Precellys® lysing kit (Bertin Instruments, France). Homogenized samples were centrifuged at 16,800 g for 30 min and 4 °C (5430R Eppendorf, Germany). The supernatant was carefully removed beneath the thin lipid layer at the surface using a micropipette, aliquoted, and stored at -80 °C. Trypsin, chymotrypsin, and leucine aminopeptidase activities were measured using substrates releasing p-nitroaniline. Free p-nitroaniline was quantified spectrophotometrically at 405 nm applying an extinction coefficient of $\varepsilon_{405} = 10.2\ \mathrm{L \cdot mol^{-1} \cdot cm^{-1}}$. Enzyme assays were run in microplates using 0.1 $\mathrm{mol \cdot L^{-1}}$ Tris/HCl at pH 8, with substrate concentrations of 1 $\mathrm{mmol \cdot L^{-1}}$. The change of absorption was determined using a Muliskan FC plate reader (Thermo Fisher Scientific, Schwerte, Germany) with reads taken every 30 s for 10 min at room temperature. Substrates used for trypsin, chymotrypsin, and leucine aminopeptidase were N-benzoyl-L-arginine-p-nitroanilide, N-succinyl-ala-ala-pro-phe-p-nitroanilide, and L-leucine-p-nitroanilide (Merck, Darmstadt, Germany).

Enzyme assays for esterase, lipase and chitinase activities were conducted with fluorogenic substrates based on 4-methylumbelliferyl (MUF). Assays were done with 0.1 $\mathrm{mol \cdot L^{-1}}$ Tris/HCl at pH 7 and substrate concentrations of 0.1 $\mathrm{mmol \cdot L^{-1}}$ for 10 min at room temperature. The fluorescence at λ_{ex}= 355 nm and λ_{em}= 460 nm was measured with a plate fluorometer (Fluoroskan Ascent FL, Thermo Fischer, Germany). Esterase, lipase, and chitinase activities were measured with 4-methylumbelliferyl-butyrate, 4-methylumbelliferyl-oleate, and 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide (Merck, Darmstadt, Germany) as substrates. Protein contents of the crude enzyme extracts were measured using the protein dye-binding method (Bradford, 1976) with a commercial protein assay (Bio-Rad Laboratories GmbH, Feldkirchen, Germany). Specific enzyme activity (U) was expressed as one µmol of substrate released in one minute per mg protein ($\mathrm{U \cdot mg_{prt}^{-1}}$).

2.8 Tissue analysis

A further three shrimp per tank were sampled for muscle and midgut gland tissue to analyze dry matter and lipid contents. First, the midgut gland was carefully removed and weighed. Then the abdomen of the shrimp was dissected and the exoskeleton removed. Midgut gland and muscle tissues were stored in individual sample vials, shock frozen in liquid nitrogen and stored at -80 °C. Samples were lyophilized and homogenized manually using a mortar. Dry matter and lipid contents were measured following the methods described in section 2.2.

2.9 Calculations and data analysis

Shrimp performance data, hepatosomatic indices (HSI), and feed utilization parameters were calculated as follows:

$$Survival(\%) = \frac{final\ number\ of\ shrimp}{initial\ \ number\ of\ shrimp} \times 100$$

$$Weight\ gain\ (\%\ initial\ weight) = \frac{(final\ body\ weight(g) - initial\ body\ weight(g))}{initl\ body\ weight\ (g)} \times 100$$

$$HSI = \frac{weight\ midgut\ gland\ (g)}{body\ weight\ (g)} \times 100$$

$$Apparent\ feed\ intake\ (g) = feed\ input\ (g) - recovered\ feed\ (g) \times CF$$

The correction factor (CF) accounts for the amount of feed lost due to leaching (Smith et al., 2005):

$$CF = \frac{dry\ weight\ feed\ input\ (g)}{dry\ weight\ feed\ recovered\ \ (g)}$$

For which 2 g of feed were placed in the tanks (= *dry weight feed input*), without the presence of shrimp and recovered after 1 h (=*dry weight feed recovered*) following the protocol of the growth trial.

$$FCR = \frac{Apparent\ feed\ intake\ (g)}{corrected\ biomass\ gain\ (g)}$$

The corrected biomass gain was calculated as described by Kitabayashi et al. (1971) accounting for dead shrimp occurring during the trial:

$$Corrected\ biomass\ gain\ (g)\ = BM_{final} + \left[\frac{(BW_{initial} + BW_{final})}{2} \times\ deaths\right] - BM_{initial}$$

with

BM_{final} = final total shrimp biomass (g)

$BW_{initial}$ = average initial body weight (g)

BW_{final} = average final body weight (g)

$BM_{initial}$ = initial total shrimp biomass (g)

All data are expressed as mean values ± standard deviation (SD) unless otherwise stated. All data sets were checked for homogeneity of variance and normal distribution using the Bartletts and the Shapiro-Wilks test. When homoscedasticity and normal distribution was given, group comparisons were done using one-way analysis of variance (ANOVA). If significant differences were present, a Tukey´s post-hoc test was applied to detect differences between treatments. If parametric assumptions failed, a Kruskal-Wallis test followed by a Nemenyi post-hoc test were applied. Percentage data (survival, $\text{Consumption}_{10\text{min}}$, $\text{Consumption}_{20\text{min}}$) were arcsine transformed prior to analysis. Feed rejection counts were analyzed using a generalized linear model with Poisson error distribution. Differences between groups were tested using estimated marginal means. The significance level for all tests was set at α = 0.05. Data analysis and graphics were done with the open access software R (R Core Team, 2019).

3. RESULTS

The fish meal (FM) made of sardine and mackerel processing remains was substantially lower in lipid and contained 12% more crude protein than the imago meal (IM, Table 2). Fish meal was also richer in all compared amino acids than IM. Lysine and methionine exhibited the highest deviation between FM and IM (> 40%). The chemical composition of the experimental diets (Table 3) was similar in dry matter, crude protein, total lipid, energy, and ash contents. However, a steady increase in crude fiber levels from 23 $g \cdot kg^{-1}$ in the Control diet to 55 $g \cdot kg^{-1}$ in the I100 diet was present. Based on feed formulations, the calculated amino acid contents were similar between the experimental diets. Slightly increasing levels of histidine, isoleucine, leucine, and phenylalanine were estimated for higher levels of IM inclusion. Conversely, lysine content in the Imago 100 diets was moderately lower than in the Control diet, with estimated values of 27.1 $g \cdot kg^{-1}$ and 29.1 $g \cdot kg^{-1}$, respectively (Table 3).

Observations of the acceptability trial revealed, that on average, shrimp approached pellets faster, rejected less, and consumed more of the control feed compared to the IM containing diets (Table 4). The variability of the observed behavior parameters was high and statistically significant differences were only detected in the amount of feed consumed after 10 min. After 10 min, shrimp consumed twice the amount of the control diet, compared to the I100 diet (one-way ANOVA, $p = 0.0179$). The responses of shrimp to pellets containing smaller amounts of imago meal (e.g. I50) did not differ from the I100 diet, in which fish meal was completely replaced with imago meal.

Table 4. Feeding behavior of shrimp receiving the experimental feeds during the acceptability trial. Values expressed as mean ± standard deviation, n = 4. Control = control diet containing 26% fish meal, I50 = diet with 50% replacement of fish meal with defatted imago meal, I75 = diet with 75% replacement of fish meal with defatted imago meal and I100 = diet with 100% replacement of fish meal with defatted imago meal.

Parameter	Control	I50	I75	I100
First contact (s)	8 ± 2	23 ± 18	12 ± 8	25 ± 18
Feed rejections	4 ± 2	8 ± 4	7 ± 5	6 ± 2
Consumption $_{10\ min}$ (%)	83 ± 14 ^{b}	57 ± 14 ^{ab}	56 ± 16 ^{ab}	43 ± 16 ^{a}
Consumption $_{20\ min}$ (%)	100 ± 0	72 ± 28	69 ± 27	71 ± 17

Weight gain of shrimp feeding on imago diets was approximately half that of shrimp in the control treatment, that increased mean body weight by 121 ± 17% (one-way ANOVA, $p = 0.0005$) (Table 5). Mean growth (weight gain) of shrimp feeding on insect containing diets (IM) was similar across all treatments and ranged from 57 to 63%. Throughout the feeding trial, the apparent feed intake was highest in the Control and I50 treatments with mean values of 206 ± 20 g and 205 ± 12 g, respectively. Shrimp in the I50 and I75 treatments consumed significantly less feed during the feeding experiment with values ranging from 144 ± 27 g and 167 ± 12 g (one-way ANOVA, $p = 0.001$). The feed conversion ratio (FCR) was lowest in the control diet (2.7 ± 0.4) and high in the imago treatments (4.1 ± 1.5 to 5.2 ± 1.5). Although the effect of diet treatment on hepatosomatic indices was not statistically significant (one-way ANOVA, $p = 0.06$), the HSI was lowest in the control treatment (3.3 ± 0.2) but higher across all imago diets with mean values from 3.8 ± 0.3 to 3.9 ± 0.3. The dry matter content of the midgut glands (Figure 2) indicates higher moisture content in midgut glands of shrimp feeding on the imago diets although the differences were not statistically significant (Kruskal-Wallis, $p = 0.168$). Diet treatments significantly affected the lipid content of midgut glands (one-way ANOVA, $p = 0.0169$). Highest lipid levels were observed in the control diet. As in the midgut gland, the dry matter of shrimp muscle decreased significantly in shrimp of the I50, I75, and I100 treatments (one-way ANOVA, $p = 0.0136$). Muscle lipid content remained similar across all treatments with values ranging from 48 ± 3 $g \cdot kg^{-1}$ in I50 to 54 ± 8 $g \cdot kg^{-1}$ in the control treatment.

Digestive enzyme activities measured in crude extracts of the midgut glands were not affected by dietary treatment and were similar in all groups (Table 6). Hemolymph parameters (THC, plasma glucose, plasma protein) of shrimp did not differ between dietary treatment groups as well (Table 7).

Table 5. Performance data of *L. vannamei* after a 40-day feeding trial with four experimental diets. Values expressed as mean ± standard deviation, n = 4. Control = control diet containing 26% fish meal, I50 = diet with 50% replacement of fish meal with defatted imago meal, I75 = diet with 75% replacement of fish meal with defatted imago meal and I100 = diet with 100% replacement of fish meal.

Parameter	Control	I50	I75	I100
Initial weight (g)	5.2 ± 0.4	5.5 ± 0.1	5.4 ± 0.2	5.2 ± 0.2
Final weight (g)	11.5 ± 0.7	9.0 ± 0.8	8.7 ± 1.1	8.2 ± 1.0
Weight gain (%)	121.4 ± 16.8 ^{b}	63.3 ± 18.2 ^{a}	61.5 ± 19.3 ^{a}	57.1 ± 14.3 ^{a}
Survival (%)	73.2 ± 12.2	76.8 ± 6.8	62.5 ± 12.2	64.3 ± 19.3
Apparent feed intake (g)	206.1 ± 19.5 ^{c}	205.0 ± 11.5 ^{bc}	144.1 ± 26.9 ^{a}	167.0 ± 11.9 ^{ab}
FCR	2.7 ± 0.4	5.0 ± 1.4	4.1 ± 1.5	5.2 ± 1.5
HSI	3.3 ± 0.2	3.9 ± 0.3	3.8 ± 0.3	3.9 ± 0.1

Table 6. Digestive enzyme activities measured in *L. vannamei* after controlled 40-day feeding trial fed four experimental diets. Values expressed as mean ± standard deviation, n = 4. Control = control diet containing 26% fish meal, I50 = diet with 50% replacement of fish meal with defatted imago meal, I75 = diet with 75% replacement of fish meal with defatted imago meal and I100 = diet with 100% replacement of fish meal with defatted imago meal.

Enzyme	Control	I50	I75	I100
Chymotrypsin ($U \cdot mg_{protein}$)	1.16 ± 0.10	1.09 ± 0.22	1.04 ± 0.20	1.29 ± 0.28
Trypsin ($U \cdot mg_{protein}$)	0.19 ± 0.06	0.13 ± 0.02	0.13 ± 0.02	0.17 ± 0.05
Leu-AP [a] ($mU \cdot mg_{protein}$)	15.46 ± 5.02	13.24 ± 2.02	12.78 ± 2.83	13.50 ± 3.21
Esterase ($U \cdot mg_{protein}$)	108.41 ± 12.63	91.83 ± 5.58	83.07 ± 16.17	93.21 ± 12.61
Lipase ($mU \cdot mg_{protein}$)	31.66 ± 8.72	35.04 ± 17.80	42.79 ± 27.90	30.09 ± 11.87
Chitinase ($U \cdot mg_{protein}$)	66.50 ± 19.46	65.01 ± 5.75	53.71 ± 21.78	58.47 ± 12.54

[a] Leucine aminopeptidase

4. DISCUSSION

Viable sustainable diet sources for high-value aquaculture shrimp are imperative to future industry growth. To date there is no information available whether meal made from the adult life stage (imago) of *Hermetia illucens* can replace fish meal in diets for Pacific Whiteleg shrimp. In the current study, the replacement of fish meal by defatted imago meal resulted in stagnated shrimp growth, regardless of the replacement level. Shrimp performance in the control treatment was similar to previous feeding trials in the same systems, reaching a 120% increase of the initial body mass on average over a period of approximately 6 to 7 weeks (Fricke et al., 2023; Weiss et al., 2020). In contrast, shrimp feeding on IM diets grew only half as much in the current study.

Chen et al. (2021) used full-fat larvae meal from the black soldier fly (BSFLM) with 326 $g \cdot kg^{-1}$ crude lipid. Shrimp growth was at the same level as the control up to dietary inclusion of 95 $g \cdot kg^{-1}$. However, growth and feed conversion were significantly impaired at an inclusion rate of 142.5 $g \cdot kg^{-1}$, which corresponded to a FM replacement level of 30%. These results are consistent with findings of Cummins Jr et al. (2017) who used partially defatted BSFLM (151 $g \cdot kg^{-1}$ crude lipid) and observed decreasing shrimp growth at inclusion levels above 91 $g \cdot kg^{-1}$. These reports of stunted growth at high BSFLM inclusion levels suggest that dietary concentrations of IM used in the current trial (> 130 $g \cdot kg^{-1}$) were above the tolerable level for shrimp.

In contrast, Wang et al. (2021) successfully replaced 60% of the fish meal content in diets for *L. vannamei* (dietary inclusion 235 $g \cdot kg^{-1}$) with defatted BSFLM, containing 91 $g \cdot kg^{-1}$ crude lipid. Recently, Nunes et al. (2023) demonstrated, that defatted BSFLM (76 $g \cdot kg^{-1}$ crude lipid) can completely replace the FM content in diets for juvenile shrimp and maintain high shrimp performance. According to the authors, appropriate supplementation with crystalline amino acids, adequate supply of n-3 fatty acids, and incorporation of feed attractants were key in BSFLM diet formulations.

Experimental diets in the current study were supplemented with lysine and methionine to adjust for differences in the calculated amino acid composition between diets. The considered amino acid concentrations were above, or in the upper margin of recommended values for lysine (16.3 - 20.5 g·kg^{-1}), arginine (19.6 – 23.2 g·kg^{-1}), methionine (6.6 – 9.1 g·kg^{-1}), and threonine (11.8-15.1 g·kg^{-1}) (Lin et al. 2015; Xie et al., 2012; Zhou et al., 2012; Zhou et al., 2013). However, these values merely represent estimated values and the actual amino acid compositions may vary. Apparent digestibility coefficients of protein and energy were taken into account for IM feed formulations. Yet, detailed information on the bioavailability of essential amino acids is lacking. Hence, limited bioavailability of essential amino acid and/or fluctuations in raw material amino acid contents could explain the reduced growth in IM treatments.

Table 7. Hemolymph parameters measured in *L. vannamei* fed four experimental diets. Values expressed as mean ± standard deviation, n = 4. Control = control diet containing 26% fish meal, I50 = diet with 50% replacement of fish meal with defatted imago meal, I75 = diet with 75% replacement of fish meal with defatted imago meal and I100 = diet with 100% replacement of fish meal with defatted imago meal.

Parameter	Control	I50	I75	I100
THC (×10^6 cells·ml^{-1})	5.9 ± 2.5	6.7 ± 0.5	7.4 ± 2.3	7.8 ± 3.4
Total protein (g·dl^{-1})	7.3 ± 0.9	7.1 ± 0.9	7.6 ± 1.4	7.3 ± 0.8
Glucose (mg·dl^{-1})	32.5 ± 5.6	31.0 ± 3.9	29.0 ± 7.5	30.0 ± 2.7

As pointed out by Chen et al. (2022), the varying results in BSFLM nutritional studies with *L. vannamei* could be related to the different pre-treatment methods applied. According to the authors, higher lipid contents and varying fatty acid composition of the diets might be the key factors limiting dietary inclusion of BSFLM in shrimp feeds. In the current feeding trial, imago meal was defatted to reduce the excessive lipid content (from 356 g·kg^{-1} to 234 g·kg^{-1} total lipid) and to improve the overall nutrient profile. Nevertheless, the lipid content of defatted IM was more than twice as high as in the BSFLM used by Wang et al. (2021) and Nunes et al. (2023). Approximately 80% of the total fatty acids in IM are saturated FAs, while the remaining 20% are mono-, and polyunsaturated FAs with equal shares (Fricke et al. 2024). Saturated fatty acids (SFA) are not well digested by penaeid shrimp and are probably responsible for reported low energy digestibility of IM (Glencross et al., 2002; Fricke et al. 2024). Elevated levels of saturated fatty acids have been suggested to reduce growth performance in shrimp (Lim et al., 1997; Chen et al., 2022;). However, to balance out the high lipid contents of the I100 diet, coconut oil was supplemented in those diets which had lower IM contents (ref. Table 1). The fatty acid profile of coconut oil is similar to that of IM, with high levels of short chain saturated acids such as lauric acid (Lim et al., 1997; Marina et al., 2009). The gross levels of saturated fatty acids should therefore be similar in all experimental diets used in the current trial. By keeping amounts of cod liver oil constant in all diets, sufficient supply of n-3 fatty acids was provided in all experimental diets. It is therefore unlikely that inappropriate FA supply is the main reason for the reduced growth in IM treatments.

Along with decreasing growth performance, fish meal replacement by IM caused changes in midgut gland and muscle tissue composition. Increased water content and lowered lipid levels in crustaceans are often linked to starvation or poor feed quality (Barclay et al., 1983; Ghosh, 2018; Jones & Obst, 2000; Wu & Dong, 2002). The midgut gland (syn. hepatopancreas), is considered the primary storage organ in decapod crustaceans, thereby reflecting the nutritional status of the animal (Armitage et al., 1972; Jones & Obst, 2000). Jones and Obst (2000) observed an increase in moisture, while lipid, protein, and ash decreased in the hepatopancreas of starved freshwater crayfish, *Cherax destructor*. Similarly, Barclay et al. (1983) found a decreasing trend in moisture and lipid in both, abdomen and midgut gland of *Penaeus esculentus* during feed deprivation. The lower dry matter and lipid contents observed in the tissues of *L. vannamei* in the current trial can thus result from inadequate nutrient supply to shrimp feeding on IM diets. However, it remains unclear how the inclusion of IM causes these changes in shrimp nutritional status.

Lipid reduction in whole body and hepatopancreatic tissue has been reported in shrimp and fish feeding on BSFLM (Chen et al., 2022; Cummins Jr et al., 2017; Wang et al., 2019). Chen et al. (2022) found that full-fat BSFLM induced changes in the energy metabolism in *L. vannamei*. While the expression of genes involved in glycolysis and the Krebs Cycle was decreased, genes and metabolites involved in lipolytic pathways were partially elevated with dietary BSFLM inclusion. Thus, increased lipid oxidation could explain the lower lipid levels measured in midgut glands of shrimp feeding on IM in the current study. Another reason for the lowered lipid levels in shrimp could be related to the availability of cholesterol in the IM-supplemented diets. In vertebrates, cholesterol is needed for bile acids synthesis which play a crucial part in lipid emulsification and absorption (Kortner et al., 2013; Molinero et al., 2019). The role of cholesterol in bile acid metabolism in crustaceans is not well described. However, Su et al. (2022) found interactive effects of these two compounds on lipid metabolism and growth of *L. vannamei*. Replacement of FM by BSFLM resulted in lowered cholesterol concentrations in the hemolymph of *L. vannamei* (Chen et al., 2022; Wang et al., 2021). It is argued that chitin, abundant in insect feed meals, might interfere with cholesterol absorption in the digestive tract and cause hypocholesterolemia (Wang et al., 2019; Wang et al., 2021). Despite cholesterol supplementation to the experimental diets, cholesterol availability could have been reduced in IM diets, influencing overall lipid uptake.

Levels of digestive enzyme activities were similar as in previous feeding trials with *L. vannamei* (Fricke et al., 2023). Regardless of the changes in the chemical composition of the midgut glands, the digestive function was not impaired. This is in line with the study of Wang et al. (2021) who found no adverse effects of BSFLM on digestive enzyme activities in *L. vannamei*. Hemolymph parameters, as well, were in the same range as reported previously for juvenile *L. vannamei* raised under indoor culture conditions (Mercier et al., 2006; Pascual et al., 2003; Weiss et al., 2020). However, values of total hemolymph protein, glucose, and total hemocyte counts can differ largely in the literature, probably depending on the different laboratory methods applied. Nevertheless, the current findings indicate that overall shrimp health and digestive physiology does not appear to be profoundly affected by IM-supplementation. This is also reflected in the survival of shrimp, which did not differ between dietary treatments. However, overall mortality was high throughout the growth trial for unknown reasons. Mortalities of about 40% or higher are not unusual in feeding trials made in clear water systems of similar duration (Amar et al., 2006; He et al., 2022; Shan et al., 2019; Weiss et al., 2020). In this trial, the visual appearance of shrimp was normal and the water parameters were consistently in the range suitable for shrimp rearing. The high mortalities may be linked to poor recovery from the live-transport.

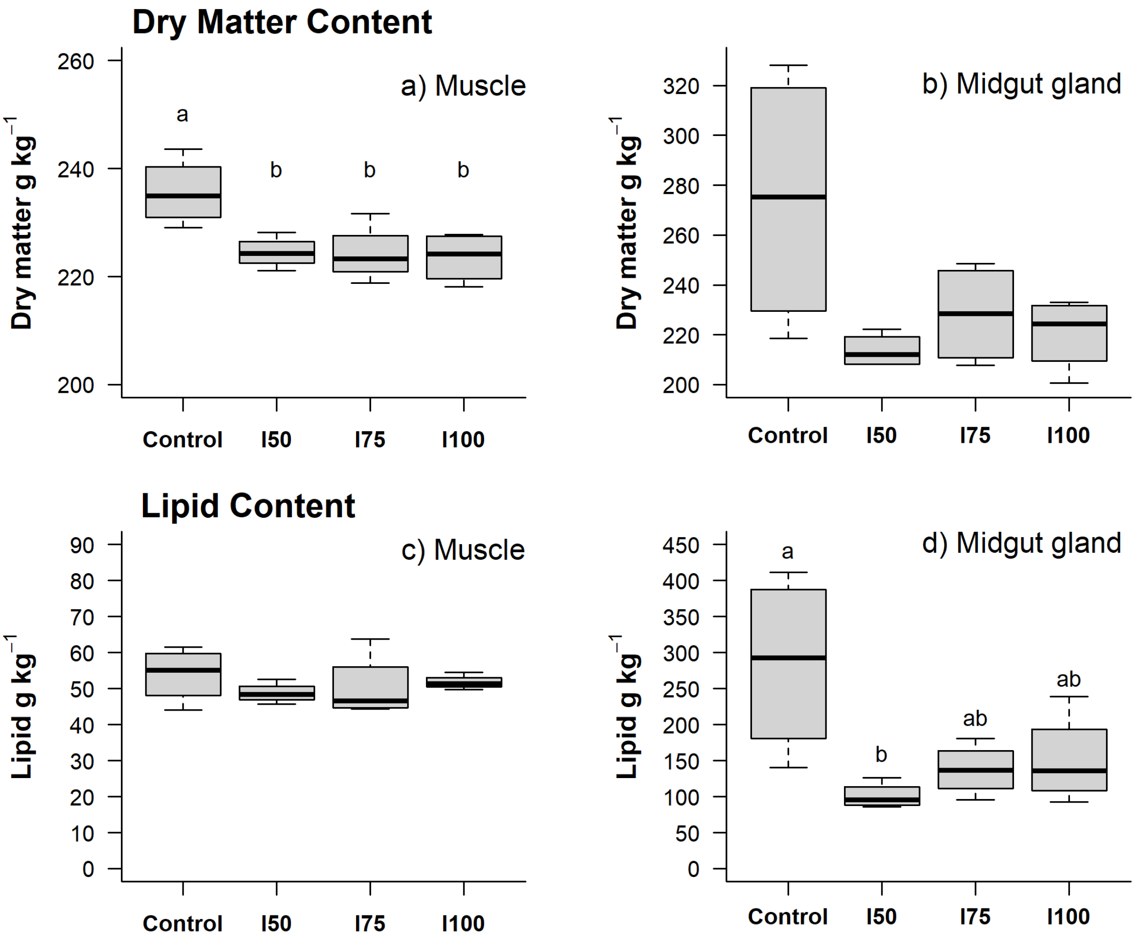

Figure 1. Dry matter and lipid content measured in the muscle (a, c) and midgut gland (b, d) of *L. vannamei* fed four experimental diets. Control = control diet containing 26% fish meal, I50 = diet with 50% replacement of fish meal with defatted imago meal, I75 = diet with 75% replacement of fish meal with defatted imago meal and I100 = diet with 100% replacement of fish meal with defatted imago meal.

A pivotal aspect of feed ingredient evaluation is the resulting feed acceptability and feed intake, which are the first factors influencing growth (Glencross, 2020). The acceptability trial offers preliminary observations on palatability of what appear to be suboptimal IM feeds. The low attractivity of IM containing diets is further evident in the apparent feed intake which was significantly lower in I75 and I100 diets. The lowered palatability of the insect-containing diets is likely to have adversely affected shrimp performance. As a cause of the reduced feed intake, energy supply to maintain the basal metabolism and growth was limited. This might have induced a physiological situation similar to feed deprivation or starvation, causing the reductions in dry matter and lipid contents similar to the observations made by Jones and Obst (2000) and Barclay et al. (1983). Still, this does not explain the observations in the Imago 50 treatment, in which average feed consumption was almost identical to the control group. The high FCR in this treatment indicates that the diet was of low nutritional quality. Hence, the reduced shrimp performance seen in the IM treatments are likely a result of the combined effect of reduced feeding, improper EAA supply, and changed energy metabolism.

5. CONCLUSION

Defatted imago meal cannot replace fish meal in diets for *L. vannamei* at the levels tested in this trial. Dietary IM inclusion resulted in low acceptability of feed pellets and reduced feed intakes during the course of the experiment. However, shrimp survival, hemolymph parameters and digestive enzyme activities of shrimp were not negatively affected by IM inclusion. Therefore, lower inclusion rates, not exceeding 100 $g \cdot kg^{-1}$ might be more suitable. More efficient defatting of IM could be beneficial and allow higher inclusion rates. Inclusion of feed attractants such as squid, or krill meal may be applicable to mask the negative effect of IM on feed palatability.

The projected global increase of BSF larvae production will concurrently generate substantial biomass of the adult life stage of this insect. Utilization of this novel by-product would increase value, reduce waste and enhance bioeconomic circularity. This study is an initial investigation on the suitability of this novel raw material as proteinaceous feed ingredient for the aquaculture sector. Further investigations are required to improve the utilization of imago meal.

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Cosmetic and Insect Farming By-Products

The following chapter describes the aqueous extraction residue of *Saccharina latissima* (algae pomace) and cocoons from *Hermetia illucens* as potential feed additive for *Litopenaeus vannamei*

Dried algae pomace of *Saccharina latissima* (left) and cocoons of *Hermetia illucens (right)*. The black line indicates 10 mm.

2.6 Manuscript II

Evaluating diets for Pacific Whiteleg shrimp, *Litopenaeus vannamei* supplemented with algae pomace (*Saccharina latissima*) and cocoons (*Hermetia illucens*) in clear water recirculating-, and biofloc systems

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KEYWORDS

- Feed additive
- By-products
- Insects
- Seaweed

ABSTRACT

Two underutilized by-products, cocoons of the black soldier fly and pomace from macroalgae, were evaluated as feed additives for Pacific Whiteleg shrimp, *Litopenaeus vannamei*, in two different farming systems. In a first experiment (clear water), diets were formulated to contain 0%, 1%, 2.5% and 5% of the test ingredients with equivalent digestible protein and energy levels. Diets were fed to juvenile shrimp (4 to 7 g) reared in a clear water recirculating aquaculture system in 100 L tanks. After 42 days, growth, survival, feed conversion, and hematological parameters (phenoloxidase, prophenoloxidase, plasma glucose, plasma protein) were evaluated. In the second experiment (biofloc system), juvenile shrimp (6 to 7g) were raised in 600 L biofloc systems and 3 experimental diets (control, 5% algae pomace, 5% cocoons) were tested. After 36 days, growth, survival, feed efficiency, gut microbial, and total hemocyte counts of shrimp were examined. On day 37, shrimp were subjected to an abrupt cold thermal shock for 1 h and the cumulative mortality was monitored for 12 h after returning to baseline temperature. In the first experiment, weight gain of shrimp was reduced in the algae pomace treatments ($p = 0.0008$), but not in any cocoon treatment. Survival significantly increased at 5% dietary inclusion of both algae pomace and cocoons compared to the control ($p = 0.046$ and $p = 0.015$, respectively). No differences were detected in any other parameters. In the second experiment, shrimp growth was reduced in both cocoon and algae pomace treatments ($p = 0.002$). No differences were observed in survival, resistance to cold thermal shock, gut bacteria, and total hemocyte counts among treatments. Despite positive effects seen in recirculating clear water systems, the tested ingredients have limited potential as feed additives and can negatively affect shrimp growth.

1. INTRODUCTION

The growing demand for fish and seafood in human nutrition is expected to be covered by increasing aquaculture production (OECD/FAO, 2023). Boosting aquaculture production is associated with intensive culture conditions and increased use of formulated feeds (Asche et al., 2013; N'Souvi et al., 2024; Tacon & Metian, 2015). High stocking densities and intensive rearing conditions can facilitate disease outbreaks, with dramatic impacts on fish and shrimp productions (Asche et al., 2013; Lafferty et al., 2015; Shinn et al., 2018). In formulated feeds, the share of plant-based feed ingredients is continuously growing as a consequence of stagnating fisheries and increasing prices (Naylor et al., 2021; Tacon et al., 2011). Pushing the tolerance limit of carnivorous species to plant-based ingredients raises concerns about animal health and could lead to increased disease susceptibility (Bu et al., 2018; Kokou et al., 2012; Monteiro et al., 2021). Additionally, extreme weather situations are expected to occur more frequently in the upcoming decades (IPCC, 2022). Climate variability and abrupt changes in abiotic factors can cause stress in fish and shrimp and facilitate the spread of pathogens (Kautsky et al., 2000; McCoy et al., 2017).

Antibiotics have long been used in aquaculture to control diseases and enhance performance of cultivated fish and shrimp (Hernández Serrano, 2005). However, excessive usage of antibiotics led to accumulation of antibiotic residues in the environment and increased antimicrobial resistances, which poses risks to public health (Cabello, 2006; Chen et al., 2020). The issues related to antibiotics in animal feeds resulted in a ban of the sub-therapeutic use of antibiotics in the European Union (European Commission, 2003). As an alternative to antibiotics, feed additives can be used to increase resistance to disease, resilience to abiotic stressors, and improve the overall performance of aquatic animals (Dawood et al., 2018; Fuchs et al., 2015). Feed additives can be defined as non-nutritive ingredients, or non-nutritive compounds of ingredients that either influence the chemical or physical characteristic of the feed, or affect the cultured animal's performance or quality of resulting products (Barrows, 2000). They are a diverse group of materials, ranging from pure chemicals, e.g., organic acids or nucleotides, to raw materials containing a vast suite of bioactive compounds, such as medicinal plants (Dawood et al., 2018; NRC, 2011).

Marine crustaceans belong to the most valuable aquaculture commodities worldwide, with a global annual production exceeding 12 million tons in 2022 (FAO, 2024). The growth of the shrimp aquaculture sector is marked by episodes of disease and declines in production volumes (Shinn et al., 2018). Although some form of immune memory has been recorded in crustaceans, a vertebrate-like adaptive immune system is lacking and vaccinations have mostly been ineffective (Barreto et al., 2023; Kulkarni et al., 2021). Feed additives that improve performance and health are therefore an important and promising strategy to tackle the above listed challenges in shrimp aquaculture.

The growing seaweed and insect industry generate biological materials with potentially bioactive properties (Fricke et al., 2024; Lourenço-Lopes et al., 2020). Side-streams of these novel industries are therefore worth to be explored as suitable feed additives for shrimp.

Macroalgae contain a wide range of bioactive substances including peptides, carotenoids, phenolics, fatty acids, and carbohydrates (Dethier et al., 2005; Harnedy & FitzGerald, 2011; Karsten et al., 1996; Wang et al., 2009). Because of this great diversity of compounds, seaweed biomass can be processed into a multitude of products, bearing huge potential for emerging industries (Buschmann et al., 2017; Shibasaki & Ueda, 2023). In the cosmetic industry, macroalgae are being used for their properties as hydrocolloids, thickening agents, emulsifiers and anti-aging ingredients (Lourenço-Lopes et al., 2020). Aqueous extracts obtained from the brown algae *Saccharina latissima* are used for cosmetical skin-care products. Algae pomace is hereby the residue remaining after the extraction procedure and is an underutilized by-product. In aquaculture, macroalgae are gaining attention as potent feed additive for a range of species. Dietary inclusion of macroalgae was shown to stimulate feeding, increase growth, enhance immunological functions, promote anti-inflammatory processes, and result in better animal product quality (i.e. pigmentation, organoleptic properties) (Choi et al., 2015; Cruz Suárez et al., 2008; Monteiro et al., 2021; Valente et al., 2016). Utilization of algae pomace as feed additive would promote a circular bioeconomy and create further value to this marine resource.

The industrial production of insects for feed and food is a growing business with global productions, expected to reach 1.2 million metric tons by 2025 (Derrien & Boccuni, 2018; Wunsch et al., 2020). Insect meals have primarily been investigated as a protein source to replace fish meal in diets for aquaculture, due the high amounts of protein (40 - 60%) and favorable amino acid profiles (Henry et al., 2015). Apart from the nutritive value, a growing number of publications have reported immunomodulatory effects of insect meals in fish and shrimp (Chen et al., 2021; Motte et al., 2019; Mousavi et al., 2020; Richardson et al., 2021). Insects contain a range of compounds such as peptides, fatty acids, or chitin/chitosan with potential antimicrobial effects (Lagat et al., 2021; Saadoun et al., 2022; Saviane et al., 2021). Yet, there is little information about insects as functional additive in aquafeeds for shrimp. Cocoons of the black soldier fly, *Hermetia illucens*, are a by-product of larvae production (Soetemans et al., 2020). The insects go through metamorphosis from larvae into the adult imago stage, leaving behind the cocoons. Cocoons contain about 40% protein, 6% lipids, high amounts of chitin (15%), and a range of minerals (Fricke et al., 2024). The digestibility of protein (35%) and energy (24%) in Pacific Whiteleg shrimp, *Litopenaeus. vannamei*, was low, presumably due to the high chitin content. However, chitin and chitosan were shown to improve growth, survival, and the antioxidant status of penaeid shrimp (Brol et al., 2021; Niu et al., 2013; Shiau & Yu, 1998). Moreover, cocoons contain biologically relevant compounds such as lauric acid, cholesterol, and copper, needed in metabolic and immune related processes in shrimp (Fricke et al., 2024).

The aim of this study was to investigate two novel by-products, algae pomace and cocoons of the black soldier fly, as feed additives in diets for Pacific Whiteleg shrimp. First, the chemical composition and apparent protein and energy digestibility of algae pomace was investigated. Information on the chemical composition and nutrient digestibility of insect cocoons are available from previous work (Fricke et al., 2024). Then, a controlled feeding trial in a clear water recirculating aquaculture system was conducted to define appropriate dietary inclusion levels for both materials, ranging from 1 to 5%. Hematological parameters (plasma glucose, plasma protein, phenoloxidase-, and prophenoloxidase activities) were monitored to examine the health status of shrimp. Based on these findings, a second feeding trial of 36 days was conducted in biofloc systems. After the rearing period, gut microbiology, total hemocyte counts, and resistance of shrimp to a cold thermal shock was investigated.

2. MATERIALS AND METHODS

2.1 Raw materials

Frozen algae pomace (*Saccharina latissima)* was collected from oceanBASIS GmbH (Kiel, Germany). Cocoons of the Black Soldier Fly, *Hermetia illucens* were provided by Farmcycle GmbH (Bremen, Germany). Both raw materials were transported to the facilities of the Alfred-Wegener-Institute, Helmholtz Centre for Polar and Marine Research (Bremerhaven, Germany), and stored at -20 °C. Algae pomace and cocoons were dried at 60 °C in glass trays until a moisture content of approximately 5% was reached, which took up to 48h for algae pomace. Dried materials were ground with a knife mill (GM 200, Retsch, Germany). The resulting powder was passed through a 500-µm sieve and stored in airtight containers in a temperature-controlled room at -4 °C.

2.2 Digestibility assay for algae pomace (*Saccharina latissima*)

2.2.1 Diet preparation and experimental setup

A reference diet containing fish meal and soy protein concentrate as the bulk protein sources was formulated to meet the dietary requirements of *Litopenaeus vannamei* (NRC, 2011). Yttrium oxide was added to the diet as an inert marker at 5 $g \cdot kg^{-1}$ (Table 1). All ingredients were sieved (< 500 µm). To avoid fractioning of ingredients, coarse particles (> 500 µm) were ground repeatedly with the knife mill until all particles passed through the sieve. Micro ingredients (quantities < 100 $g \cdot kg^{-1}$) were first mixed manually before the macro ingredients were subsequently added. Oil and lecithin were combined and then added to the mixture. Afterwards, water was added (~ 300 $mL \cdot kg^{-1}$) and the dough was continuously mixed for one hour in a planetary mixer (GGM Gastro International GmbH, Ochtrup, Germany). The test diet was prepared by mixing 1400 g of the reference diet mash with 600 g dried algae pomace meal (ratio 7:3 on weight basis), as it is commonly done in aquaculture nutritional studies (Glencross et al., 2007). A pelleting machine (PP120, Cissonius, Zehdenick, Germany) was used to produce pellets with a diameter of 2 mm. Freshly produced pellets dried at room temperature for 72 h before they were stored in air tight, sealable plastic bag until the start of the feeding experiment, two weeks later.

Table 1. Ingredient composition of the reference diet and test diet containing algae pomace (*S. latissima*) used for a digestibility trial with *L. vannamei*.

Ingredient ($g\cdot kg^{-1}$)	Reference	Algae pomace
Fish meal [1]	260	182
Soy protein concentrate [2]	220	154
Wheat meal [3]	409	286.3
Cod liver oil [4]	20	14
Lecithin (soy) [5]	20	14
Gluten (wheat) [6]	25	17.5
Sodium alginate [7]	25	17.5
Vitamin and mineral premix [8]	10	7
Met-Met [9]	5	3.5
Yttrium oxide [10]	5	3.5
Cholesterol [11]	1	0.7
Algae pomace [12]	-	300

[1] Protein 68.2%, lipid 9.6%, ash 14.3%; J. Müller AG (Brake, Germany)

[2] Protein 60%, lipid 1%, ash 6%; Köster Marine Proteins GmbH (Hamburg, Germany)

[3] Protein 11.4%, lipid 2.4%, ash 1.4%; Schapfen Mühle GmbH & Co. KG (Ulm-Jungingen, Germany)

[4] Bioceval GmbH & Co. KG (Cuxhaven, Germany)

[5] Louis Francois SAS (Croissy-Beaubourg, France)

[6] Kröner-Stärke GmbH (Ibbenbühren, Germany)

[7] Gioia Group s.r.l., (Turin, Italy)

[8] vitamin and mineral premix ($g\cdot kg^{-1}$ diet): retinyl acetate (3000 IU), cholecalciferol (3000 IU), dl-a-tocopherol 0.3, menadione 0.04, thiamine 0.03, riboflavin 0.03, pyridoxin-HCL 0.06, cyanocobalamin 0.15 ($mg\cdot kg^{-1}$), nicotinic acid 0.07, D-pantothenic acid 0.07, choline chloride 1, folic acid 6 ($mg\cdot kg^{-1}$), biotin 0.5 ($mg\cdot kg^{-1}$), vitamin C 0.125, inositol 0.3, iron 0.05, copper 0.04, manganese 0.02, zinc 0.075, iodine 2 ($mg\cdot kg^{-1}$), selenium 0.3 ($mg\cdot kg^{-1}$), cobalt 0.06 ($mg\cdot kg^{-1}$), magnesium 0.3; Spezialfutter Neuruppin GmbH & Co KG (Neuruppin, Germany)

[9] Evonik Industries AG (Essen, Germany)

[10] Fisher Scientific GmbH (Schwerte, Germany)

[11] Merck KGaA (Darmstadt, Germany)

[12] oceanBASIS GmbH (Kiel, Germany)

Live shrimp (weighing approximately 5 – 10 g) were purchased from an indoor shrimp farm (Aquapurna GmbH, Wunstorf, Germany) and transported to the Centre of Aquaculture Research of the Alfred Wegener Institute (Bremerhaven, Germany). Shrimp were held in clear water recirculating aquaculture systems consisting of a moving bed biofiltration unit, a protein skimmer, an ozone supply, UV treatment, and a temperature-control unit. The facility was illuminated from 08:00 to 18:00. During an acclimation period of one week, shrimp were fed a commercial shrimp grow out feed (38% protein, 7% fat, 18.3 $MJ \cdot kg^{-1}$ gross energy, Ø = 1.7 mm, Le Gouessant, Lamballe-Armor, France) twice per day at libitum. Water parameters were monitored in the morning and afternoon each day (Table 7). Ammonia, nitrite, and nitrate were measured two times per week using a QuAAtro39 Continuous Segmented Flow Analyzer (SEAL Analytical GmbH, Norderstedt, Germany).

Twelve aquaria (dimensions: 0.50 × 0.25 × 0.45 m, length×width×height) equipped with air diffuser stones were connected to the RAS described above. A total of 156 shrimp (10.3 ± 2.3 g) were stocked, resulting in 13 individuals per tank. The reference and test diet were randomly allocated to 6 aquaria each. Feed was provided at 4.5% of the shrimp biomass per day, fed in three portions at 08:00, 11:30, and 15:00 hrs. Shrimp acclimatized to the experimental diets for one week, before fecal samples were collected. Therefore, one hour after feeding, all aquaria were cleaned from uneaten feed remains, feces, and molts. Two hours later, freshly produced and intact feces were carefully collected using a fine meshed net, drained from excess water, and stored at -20°C. Fecal samples of two aquaria were pooled, resulting in triplicate samples per feed treatment. The experiment was terminated after 3 weeks and fecal samples were lyophilized, manually homogenized with a mortar, and stored in a desiccator until chemical analysis.

Apparent digestibility coefficients (ADC) for protein and energy were calculated using the equation proposed by Cho and Slinger (1979):

$$\mathrm{ADC}\ (\%) = 100 - \left[100\left(\frac{\mathrm{Y_{diet}}}{\mathrm{Y_{faeces}}}\right) \cdot \left(\frac{\mathrm{N_{faeces}}}{\mathrm{N_{diet}}}\right)\right]$$

where Y is yttrium oxide and N the considered nutrient content in the diet or in the feces (on dry matter basis). The apparent digestibility coefficients for protein and energy of the algae pomace were calculated as described by Bureau and Hua (2006):

$$\mathrm{ADC_{(AP)}} = \mathrm{ADC_{test\ diet}} + \left[(\mathrm{ADC_{test\ diet}} - \mathrm{ADC_{reference\ diet}}) \cdot \left(\frac{0.7\ \cdot \mathrm{N_{ref\ mash}}}{0.3\ \cdot \mathrm{N_{AP}}}\right)\right]$$

where $\mathrm{ADC_{test\ diet}}$ and $\mathrm{ADC_{reference\ diet}}$ are the respective nutrient ADCs calculated for the reference and test diet, and N_{ref} and N_{AP} are the nutrient concentrations of the reference diet mash and algae pomace meal (as is).

2.2.2 Chemical analysis

Moisture and ash contents were determined according to the methods of the Association of Official Analytical Chemists (AOAC, 2010). An elemental analyzer (Euro Elemental Analyzer, Eurovector SPA, Italy) was used to determine the nitrogen content. The crude protein content was then calculated by multiplying the nitrogen content by 6.25 (Dumas, 1831). Total lipids were determined as described by Koch et al. (2023), using dicholmethane:methanol (2:1, v/v) as solvent. The energy content was measured with a bomb calorimeter (Parr 6100, Parr Instrument Company, USA). Samples were sent to external laboratories for Yttrium (Institute Dr. Nowak, Ottersberg, Germany) and crude fiber (LUFA Nord-West, Oldenburg, Germany) analysis. Yttrium was measured using inductively coupled plasma optical emission spectrometry (ICP-OES). Crude fiber was analyzed according to the standard methods defined by the European Commission Regulation No 152/2009.

2.3 Clear water experiment

2.3.1 Feed production and experimental setup

Seven experimental diets were formulated to meet the nutritional requirements of *L. vannamei* (NRC, 2011), each containing 150 g·kg^{-1} fish meal (Table 2). Cocoons and algae pomace were tested at inclusion rates of 1%, 2.5%, and 5% (AP 1, AP 2.5, AP 5, Cocoon 1, Cocoon 2.5, Cocoon 5). Diets were formulated to contain similar digestible protein (348 g·kg^{-1}) and digestible energy levels (15.4 MJ·kg^{-1}). Nutrient profiles of the diets with different contents of cocoon and algae pomace were balanced out with soy protein concentrate and wheat meal. Increasing lipid levels caused by higher cocoon inclusion (2.5% and 5%) were balanced with reduced amounts of sunflower oil. Feed pellets were produced and analyzed as described in section 2.2 (Table 3).

Live juvenile shrimp, *L. vannamei*, weighing 4 to 7 g (Aquapurna GmbH, Wunstorf, Germany) were transported to the research facilities of the Alfred Wegener Institute (Bremerhaven, Germany). Rearing conditions were the same as described for the digestibility trial in section 2.2. After an acclimation period of seven days, 28 aquaria (0.5×0.45×0.45 m, width×length×height) were stocked with 16 shrimp each. To weigh the shrimp, excess water was carefully removed from the body using a moist paper tissue and each shrimp was individually weighed to the nearest 0.1 g. Mean shrimp body weight was 5.6 ± 1.3 g, across all tanks, with a coefficient of variance from 21.2 to 25.6%. Feed treatments were randomly assigned to the aquaria in quadruplicate. The initial feeding rate was 4.5% of the total biomass, provided in 4 portions. To account for the feed intake, 1.5 hours after feeding, uneaten feed remains were removed from the tanks using a fine meshed net. Feed remains were dried overnight in a microwave oven (SAM 225, CEM Microwave Technology, USA) and weighed the following day. The feeding rate was increased weekly, based on an overestimated weight increase of 2 g·week^{-1} of the shrimp. When the recovered feed was more than 15% of the provided amount, the feeding rate was not further increased. If mortalities occurred, dead shrimp were removed from the tanks to avoid cannibalism.

After 42 days, the growth trial was ended and all shrimp were removed from the aquaria, counted, and individually weighed. Survival and weight gain in each tank was calculated as follows:

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

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

Estimations for total feed intakes were corrected for the amount of feed dispersed in the water. Therefore, 2 g of the experimental feed was placed into the aquaria without the presence of shrimp. All conditions (flow rate, aeration, temperature) were the same as in the growth trial. After 1.5 hours, the feed was collected, dried overnight, and weighed. The weight difference was then used to calculate a correction factor (CF) as described by Smith et al. (2005):

$$\text{CF} = \frac{\text{feed input(g)}}{\text{recovered feed (g)}}$$

The total biomass gain was corrected for dead shrimp using the equation proposed by Kitabayashi et al. (1971):

$$\text{corrected BM gain (g)} = \text{BM}_{\text{final}} + \left(\frac{\text{mean BW}_{\text{initial}} + \text{mean BW}_{\text{final}}}{2} \times \text{Deaths}\right) - \text{BM}_{\text{initial}}$$

where $\text{BM}_{\text{initial}}$ /BM_{final} refer to the total shrimp biomasses (g) at the beginning and at the end of the growth trial per tank. Mean $\text{BW}_{\text{initial}}$/mean BM_{final} refer to the mean shrimp bodyweights (g) at the start and the end of the experiment, respectively. Deaths are the total number of shrimp that died during the trial.

The feed conversion factor (FCR) was then calculated as follows:

$$\text{FCR} = \frac{[\text{feed input (g)} - (\text{recovered feed(g)} \times \text{CF})]}{\text{corrected BM gain (g)}}$$

2.3.2 Hemolymph analysis

Per tank, the hemolymph of three shrimp in intermolt stage (Robertson et al., 1987) was sampled from the base of the 5th pereiopod using sterile syringes, prefilled with cold anticoagulant (27 mmol·L^{-1} trisodium citrate, 310 mmol·L^{-1} sodium chloride, osmolality 640 mmol·kg^{-1}). The puncture site was first disinfected with 75% ethanol. Depending on the volume of withdrawn hemolymph, anticoagulant was added to reach a final dilution of 1:1. Hemolymph samples were centrifuged for 10 min at 800 g and 4 °C. The supernatant was aliquoted, shock frozen in liquid nitrogen, and stored at -80 °C until laboratory analysis.

Protein and glucose concentrations in the plasma were analyzed with an automated blood analyzer (Fuji DRI-CHEM NX500, Tokyo, Japan). The method described by Huang et al. (2010) was used to determine phenol- and prophenoloxidase enzyme activities. Measurements were run in 96-well microtiter plates at 27 °C. The reaction was started by adding 1.5 mg·mL^{-1} L-Dopa in 0.1 mol·L^{-1} potassium phosphate buffer (pH 6.6) to 10 µL of plasma. Immediately after the start of the reaction, absorbance was measured every 20 s at 490 nm for a total reaction time of 200 s. To measure prophenoloxidase activity, 10 µL of diluted samples (1:10 in 0.1 mol·L^{-1} phosphate buffer, pH 6.6) were incubated for 5 min at 27 °C with 100 µL of trypsin solution (1 mg·mL^{-1} trypsin in 0.1 mol·L^{-1} phosphate buffer pH 7.6). Ten µL of the activated sample was then used for the enzyme assay described above. One unit of phenol- and prophenoloxidase activities were expressed as 0.001·of the linear increase in absorbance at 490 nm (1U = 0.001 A·min^{-1}·mL$^{-1}_{\text{haemolymph}}$).

2.4 Biofloc experiment

2.4.1 Biofloc system and experimental design

The biofloc trial was conducted at the Laboratório de Camarões Marinhos the Federal University of Santa Catarina. Two feeds containing 5% cocoons and 5% algae pomace (Cocoon, Algae pomace) and one control (Table 5) were formulated to meet nutritional requirements of *L. vannamei*. Calculated digestible protein and digestible energy levels ranged from 293 to 307 g·kg^{-1} and 12.7 to 13.1 MJ·kg^{-1}, respectively. Dietary inclusion of algae pomace and cocoons was made at the expenses of other macro ingredients (wheat meal, soybean concentrate, poultry by product meal, fish meal) and kaolin. The proportions of macro ingredients were kept constant in all diets. All ingredients were passed through a sieve to achieve a particle size of < 600µm, prior to feed production. Diets were prepared in the same way as described in section 2.2, except that mixing was done manually using large plastic trays. The resulting dough was mixed thoroughly for approximately 30 min. Feeds were produced using a pelleting machine (Mecanica Braesi, Caixas do Sul, Brazil). The resulting pellets were oven dried (Etica, Equipamentos Cientificos S.A., Sao Paolo) for 12 h at 50 °C.

The shrimp used for the study were obtained from the company Aquatec (Post-larvae with 10 days, Rio Grande do Norte state, Brazil) and kept in a 50 m^3 BFT system in a greenhouse. Shrimp were fed six times per day with a commercial feed containing 40% crude protein (Gubai, Campinas, Brazil) until reaching the desired weight for the experiment (6 g).

The biofloc trial was conducted in a greenhouse using twelve round polyethylene tanks (Ø = 120 cm, height = 150 cm, Fortlev Unidade SC, Araquari, Brazil). Each tank was equipped with air diffuser tubes, heaters with thermostat, and square structures (40×55 cm, width × height) to increase surface area for shrimp.

One day before stocking, tanks were filled with 200 L of mature chemoautotrophic biofloc water (Salinity: 35.2 ppt; TSS: 780 g·L^{-1}; pH: 7.85; NH$_4^{+}$: 0.33 mg·L^{-1}; NO$_2^{-}$: 0.15 mg·L^{-1}; NO$_3^{2-}$: 28.2 mg·L^{-}

^{1}) and 400 L of seawater (salinity: 30.1 ppt; SST: 0 g·L^{-1}; pH: 7.87; NH_4^+: 0.33 mg·L^{-1}; NO_2^- : 0.02 mg·L^{-1}; NO_3^{2-}: 0.09 mg·L^{-1}).

Each tank was stocked with 150 shrimp with an average weight of approximately 6 g, yielding a stocking density of 250 individuals·m^{-3} water. Shrimp were weighed in batches of 30-50 individuals. The mean total biomass of shrimp was 927.4 ± 7.8 g per tank. Diet treatments were randomly assigned to the tanks in quadruplicate. Shrimp were fed four times daily (09:00, 11:30, 14:00, 17:00) using the feeding table proposed by Van Wyk (1999). Daily feeding behavior was monitored using round feeding trays (Ø = 20 cm) placed on the bottom of each tank. Water quality analyses were made two times per week, according to the methods of APHA (2005). Nitrate was monitored in the beginning, in the middle, and at end of the experiment in one tank per treatment. Temperature and dissolved oxygen were measured manually with a handheld dissolved oxygen meter (YSI 550A, YSI Inc. /Xylem Inc., OH, USA) each day in the morning (08:30) and in the afternoon (16:00). A subsample of 30 shrimp per tank was weighed once a week to monitor shrimp growth. The feeding rate was adjusted accordingly. Calcium hydroxide ($Ca(OH)_2$) was added daily to maintain an alkalinity from 140 to 160 mg·L^{-1}. When ammonia values rose above 1 mg·mL^{-1}, 15.17 g of sugar (saccharose) per g of ammonia-nitrogen was added to the tanks as carbohydrate source for the microbes (Ebeling et al., 2006). Total suspended solids were maintained between 400 and 600 mg·L^{-1}. Excess suspended solids were removed from the rearing systems with a conical decanter (Ø = 56 cm, height= 76 cm, volume = 100 L) and a pump (flowrate = 160 L·h^{-1}).

After 36 days, the rearing period ended and all shrimp were counted and weighed. Shrimp survival and weight gain was calculated as described in section 2.3.1. Productivity and apparent feed conversion factor were calculated as follows:

$$\text{Productivity } (\text{kg} \cdot \text{m}^{-3}) = \frac{\text{final biomass (kg)}}{\text{tank volume } (\text{m}^3)}$$

$$\text{Apparent FCR} = \frac{\text{total amount of feed offered (g)}}{\text{corrected biomass gain (g)}}$$

2.4.2. Gut microbiological analyses

At the end of the rearing period, the gut of 5 shrimp per tank were dissected, homogenized with a mortar and pestle, and serially diluted (1:10) in 3% sterile saline (NaCl). Inoculations for heterotrophic bacteria counts were made in tryptic soy agar (Acumedia, Neogen Corporation, Lansing, USA). *Vibrionacea* bacteria counts were performed using agar plates made with thiosulphate citrate bile sucrose (Acumedia, Neogen Corporation, Lansing, USA). After an incubation time of 24 h at 30 °C, colony-forming units (CFU) were counted and expressed as CFU·g^{-1} of shrimp gut based on wet weight.

2.4.3 Thermal shock

On day 37 of the rearing period (one day after final biometric measurements), 15 shrimp were randomly caught from each tank and exposed to an abrupt, cold thermal shock. Therefore, shrimp were transferred directly from the holding tanks (water temperature 27.6 ± 0.5 °C) into 60-L aquaria (35×58×30 cm, width×length×height) filled with 25 L seawater (32 ppt, same water source used for the biofloc systems) and cooled down to 11.0 ± 0.1 °C. This temperature was previously defined in a lethal temperature 50% assay, in shrimp from the control treatment. Shrimp remained under these conditions for 1 h with constant aeration. After 1 h, shrimp were carefully netted from the 11 °C water tanks and transferred into different 60 L aquaria filled with 27.9 ± 0.6 °C seawater, equipped with submerged heaters (200 W) and constant aeration. Shrimp were kept in these conditions for 24 h and mortality was monitored.

2.4.4 Total hemocyte counts

On the last day of the rearing period (day 36), the hemolymph of three shrimp in the intermolt stage (Robertson et al., 1987) was sampled and pooled per replicate tank (pre-shock). The hemolymph of shrimp surviving the thermal shock were also sampled (1-3 shrimp, depending on survival) and pooled per tank (post-shock). Syringes (1-mL) were filled with 100 µL cold modified Alsever Solution (MAS: 27mmol·L^{-1} sodium citrate, 9m mol·L^{-1} EDTA, 115 m mol·L^{-1} glucose, 336 m mol·L^{-1} NaCl, pH 7.2). The puncture site was first cleaned with 70% ethanol and hemolymph was withdrawn from the basis of the 5th pereiopod. Depending on the amount of collected hemolymph, samples were diluted with MAS containing 4% formaldehyde, to reach a ratio of 3:1 (hemolymph: MAS). Total hemocyte counts (THC) were done with a microscope (Carl Zeiss AG, Oberkochen, Germany) and a Neubauer counting chamber at a final sample dilution of 1:30.

2.5 Statistics

Normal distribution and homoscedasticity of the data sets were analyzed with the Shapiro-Wilk and Bartletts-test, respectively. Survival data was arcsine-transformed prior to analysis. When parametric assumptions were given, one-way ANOVA followed by a Tukey post-hoc test were applied, if necessary. A Kruskal-Wallis and a Nemenyi's-test of multiple comparisons were applied when data sets were not normally distributed or if the variance was not homogenous. The significance level was set at $\alpha = 0.05$ for all tests. Shrimp performance (weight gain, survival, FCR) and inclusion rates of algae pomace and cocoons in the growth trial were modelled using quadratic regression. Data analyses were done with the open-source software R (R Core Team, 2022). Shrimp survival after the thermal shock experiment was analyzed with the Kaplan-Meier test using the survival package in R.

Table 2. Ingredient composition of the control diet and diets containing algae pomace from *S. latissima* (AP) and cocoons from *H. illucens* at 1%, 2.5%, and 5% inclusion level. Experimental diets were used for a growth trial conducted in a clear water recirculating aquaculture system with *L. vannamei*.

Ingredient	Control	AP 1	AP 2.5	AP 5	Cocoon 1	Cocoon 2.5	Cocoon 5
Fish meal [a]	150	150	150	150	150	150	150
Soy protein concentrate [b]	375	374	373	371	372	368	361
Wheat meal [c]	359	350	336	313	352	342	325
Cod liver oil [d]	20	20	20	20	20	20	20
Sunflower oil	20	20	20	20	20	19	18
Lecithin (soy) [e]	20	20	20	20	20	20	20
Gluten (wheat) [f]	30	30	30	30	30	30	30
Vitamin and mineral premix [g]	10	10	10	10	10	10	10
Alginate [h]	5	5	5	5	5	5	5
Cholesterol [i]	5	5	5	5	5	5	5
Met-Met [j]	4	4	4	4	4	4	4
L-Lysin [k]	2	2	2	2	2	2	2
Algae pomace	-	10	25	50	-	-	-
Cocoons	-	-	-	-	10	25	50

[a] J. Müller AG (Brake, Germany); [b] Köster Marine Proteins GmbH (Hamburg, Germany); [c] Schapfen Mühle GmbH & Co. KG (Ulm-Jungingen, Germany); [d] Bioceval GmbH & Co. KG (Cuxhaven, Germany); [e] Louis Francois SAS (Croissy-Beaubourg, France); [f] Kröner-Stärke GmbH (Ibbenbühren, Germany); [g] vitamin and mineral premix ($g\cdot kg^{-1}$ diet): retinyl acetate (3000 IU), cholecalciferol (3000 IU), dl-a-tocopherol 0.3, menadione 0.04, thiamine 0.03, riboflavin 0.03, pyridoxin-HCL 0.06, cyanocobalamin 0.15 ($mg\cdot kg^{-1}$), nicotinic acid 0.07, D-pantothenic acid 0.07, choline chloride 1, folic acid 6 ($mg\cdot kg^{-1}$), biotin 0.5 ($mg\cdot kg^{-1}$), vitamin C 0.125, inositol 0.3, iron 0.05, copper 0.04, manganese 0.02, zinc 0.075, iodine 2 ($mg\cdot kg^{-1}$), selenium 0.3 ($mg\cdot kg^{-1}$), cobalt 0.06 ($mg\cdot kg^{-1}$), magnesium 0.3; Spezialfutter Neuruppin GmbH & Co KG (Neuruppin, Germany); [h] Gioia Group s.r.l., (Turin, Italy);[i] Merck KGaA (Darmstadt, Germany); [j] Evonik Industries AG (Essen, Germany); [k] Borgstädt Solutions UG (Langenfeld, Germany

Table 3. Chemical composition of the control diet and experimental diets containing algae pomace from *S. latissima* (AP) and cocoons from *H. illucens* at 1%, 2.5%, and 5% inclusion level. Diets were used for a growth trial conducted in a clear water recirculating aquaculture system with *L. vannamei*. Values expressed as the mean ± standard deviation, if applicable.

Feed chemical composition*	Control	AP 1	AP 2.5	AP 5	Cocoon 1	Cocoon 2.5	Cocoon 5
Dry matter	930 ± 1	914 ± 0	902 ± 1	902 ± 0	903 ± 0	907 ± 1	908 ± 0
Crude protein	441 ± 10	449 ± 16	435 ± 19	418 ± 15	412 ± 6	414 ± 11	416 ± 9
Gross energy (MJ·kg^{-1})	19.2 ± 0.5	19.7 ± 0.1	19.2 ± 0.1	19.2 ± 0.1	19.1 ± 0.1	19.4 ± 0.1	19.3 ± 0.1
Total lipid	95 ± 2	92 ± 1	90 ± 3	86 ± 1	85 ± 1	84 ± 3	88 ± 3
Ash	60 ± 0	60 ± 0	60 ± 0	64 ± 0	63 ± 4	67 ± 0	70 ± 1
Crude fiber	23	24	31	30	28	31	37

* g·kg^{-1}, unless otherwise indicated

Table 4. Water parameters measured during the digestibility, growth and biofloc trial. Values expressed as the mean ± standard deviation.

Experiment	Temperature	Oxygen	pH	Conductivity	NH_3	NO_2-	NO_3-	TSS	Alkalinity
	°C	%		ms·cm^{-1}	mg·mL^{-1}	mg·mL^{-1}	mg·mL^{-1}	mg·mL^{-1}	
Digestibility	27.9 ± 0.7	95.8 ± 4.3	8.0 ± 0.2	32.8 ± 2.2	0.11 ± 0.03	0.34 ± 0.20	111.4 ± 6.7	-	-
Growth	28.0 ± 0.6	92.4 ± 2.5	7.7 ± 0.1	32.5 ± 0.6	0.19 ± 0.08	0.39 ± 0.13	159.8 ± 41.2	-	-
Biofloc *									
Control	27.8 ± 1.5	63.7 ± 4.1	8.0 ± 0.1	53.7 ± 2.3	0.39 ± 0.22	0.86 ± 0.30	18.5 ± 4.1	523 ± 178	152 ± 20
Cocoon	27.6 ± 2.1	64.7 ± 5.3	8.0 ± 0.1	53.4 ± 2.3	0.69 ± 1.02	0.94 ± 0.39	19.0 ± 9.5	527 ± 167	156 ± 19
Algae pomace	28.0 ± 1.4	63.9 ± 4.4	8.0 ± 0.1	53.8 ± 2.3	0.64 ± 0.70	1.04 ± 0.40	24.3 ± 9.1	525 ± 181	157 ± 21

*Average water parameter values of the feed treatments: control, cocoon, and algae pomace

Table 5. Ingredient composition of the three experimental diets used in the biofloc trial. Control = control diet, Cocoon = diet containing 5% cocoons from *H. illucens*, Algae pomace = diet containing 5% algae pomace from *S. latissima*.

Ingredient (g·kg^{-1})	Control	Cocoon	Algae pomace
Wheat meal [a]	270	255	255
Soybean concentrate [b]	235	225	228.5
Poultry meal [b]	160	150	150
Fish meal [c]	122	112	112
Kaolin [e]	70	65	61.5
Soy lecithin [e]	30	30	30
Fish oil [c]	15	15	15
Soy oil [d]	15	15	15
Magnesium sulfate [e]	15	15	15
Potassium chloride [e]	15	15	15
Vitamin mix [f]	15	15	15
Mineral mix [f]	15	15	15
Monocalcium phosphate [e]	10	10	10
Sodium chloride [e]	5	5	5
Carboxymethyl cellulose [e]	4.8	4.8	4.8
DL- Methionine [g]	2.5	2.5	2.5
Ascorbic acid [h]	0.7	0.7	0.7
Cocoons [i]	-	50	-
Algae pomace [j]	-	-	50
Estimated gross composition			
Dry matter	932	934	933
Crude protein	368	369	360
Total lipids	92	93	92
Digestible energy (MJ·kg^{-1})	13.1	12.7	12.8

[a] Moinho do Nordeste, Santana do Ipanema, Brazil

[b] BRF S. A., Itajaí, Brazil

[c] Tectron, Toledo, Brazil

[d] Louis Dreyfus Company, Rotterdam, Netherlands

[e] Roster Equipamentos Laboratoriais LTDA, Barcelona, Brazil

[f] Vitamin premix: vitamin A, 3.000.000 IU; vitamin D3, 1.000.000 IU; vitamin E, 70.000 IU; vitamin K3, 14 g, vitamin B1, 30 g; vitamin B2, 20 g, vitamin B6, 33 g; vitamin B12, 0.05 g; pantothenic acid, 40 g, biotin, 0.75 g; nicotinic acid, 70 g; folic acid, 3 g; excipient for 1 kg. Mineral premix: potassium, 6.10 g; copper, 23.33 g; zinc, 10 g; manganese, 20 g; selenium, 0.125 g; iodine 1 g; cobalt, 0.05g; excipient for 1 kg. In Vivo mix, Paulínia, Brazil

[g] Rhoster Industria e Comercio LTDA, São Paolo, Brazil

[h] L-ascorbic acid-2-monophosphate 35%, DSM Produtos Nutricionais, São Paolo, Brazil

[i] Entosus GmbH, Bremen, Germany

[j] oceanBASIS GmnH, Kiel, Germany

3. RESULTS

3.1 Chemical composition and digestibility algae pomace

Algae pomace contains a moderate amount of protein of 219 g·kg^{-1} of which 78% was apparently digested by *L. vannamei* (Table 6). The gross energy content was 15.4 MJ·kg^{-1} with an ADC$_{energy}$ of 44%. The lipid content was low, with mean values of 40 g·kg^{-1}. High amounts of crude fiber (123 g·kg^{-1}) and ash (138 g·kg^{-1}) were present in algae pomace. Analysis of the mineral content showed highest amounts for calcium (25.6 g·kg^{-1}) and potassium (19 g·kg^{-1}), while magnesium and phosphorous were present in smaller amounts of 7.1 and 1.7 g·kg^{-1}, respectively. Copper was present in minor amounts of 4 mg·kg^{-1}.

Table 6. Gross chemical composition, mineral content and digestible protein and energy values of algae pomace (*S. latissima*) and cocoons (*H. illucens*) for *L. vannamei*.

Parameter	Algae pomace	Cocoons [1]
Gross composition (g·kg^{-1})		
Dry matter	954	958
Crude protein	219	433
Total lipid	40	64
Gross energy (MJ·kg^{-1})	15.4	18.8
Ash	138	177
Crude fiber	123	-
Chitin	-	154
Minerals (g·kg^{-1})		
Calcium	25.6	41.8
Phosphorous	1.7	10.6
Potassium	19.0	19.7
Magnesium	7.1	4.6
Copper (mg·kg^{-1})	4	22
Digestibility [2]		
ADC protein (%)	77.5 ± 8.5	35.4
ADC energy (%)	44.2 ± 1.0	24.4
Digestible protein (g·kg^{-1})	170.0 ± 18.7	160.0
Digestible energy (MJ·kg^{-1})	6.8 ± 0.1	4.7

[1] data from Fricke et al. 2024.

[2] digestibility data for algae pomace are mean values ± standard deviation of 4 replicate observations.

3.2 Clear water experiment

Compared to the control, the weight gain of shrimp was significantly lower at all algae pomace inclusion levels (p = 0.0008, one-way ANOVA). Shrimp feeding on AP 1 showed lowest weight gains of 85.2 ± 4.2% (Figure 1). At higher inclusion levels of algae pomace, mean growth of shrimp increased from 87.7 ± 5.9% in AP 2.5, to 92.3 ± 6.25% in AP 5. Yet, differences in growth between algae pomace treatments were not statistically significant. Quadratic regressions showed a significant fit (p = 0.006) and a minimum for weight gain was identified at an inclusion level of 2.9% of algae pomace.

Table 7. Growth performance data of *L. vannamei* after a rearing period of 36 days in biofloc systems, feeding on the experimental diets. Control = control diet, Cocoon = diet containing 5% cocoons from *H. illucens*, Algae pomace = diet containing 5% algae pomace from *S. latissima*. Values are expressed as mean ± standard deviation, n = 4.

Parameter	Control	Cocoon	Algae pomace
Initial body weight (g)	6.20 ± 0.03	6.17 ± 0.03	6.21 ± 0.05
Final body weight (g)	11.40 ± 0.14 ^{b}	10.14 ± 0.49 ^{a}	10.49 ± 0.24 ^{ab}
Weight gain (%)	83.72 ± 2.96 ^{b}	64.34 ± 8.27 ^{a}	69.01 ± 3.43 ^{a}
Weekly growth (g·week^{-1})	1.04 ± 0.03 ^{b}	0.79 ± 0.10 ^{a}	0.86 ± 0.04 ^{a}
Survival	80.00 ± 9.03	90.33 ± 4.44	85.00 ± 14.03
Productivity (kg·m^{-3})	2.28 ± 0.23	2.29 ± 0.11	2.23 ± 0.41
Apparent FCR	3.33 ± 0.57	3.43 ± 0.41	3.04 ± 0.23

The weight gain of shrimp feeding on cocoons showed a similar trend, with the lowest weight gain at an inclusion level of 2.4%, according to the quadratic model. Weight gain was greater at higher and lower inclusion levels, reaching average weight gains of 91.1 ± 12.9% and 109.8 ± 27.6% in Cocoon 1 and Cocoon 5 treatments, respectively. Group comparisons showed no significant differences in the weight gain between all feeding treatments (p = 0.16, one-way ANOVA).

Significantly higher survival rates were observed in feed treatments with 5 % inclusion of algae pomace and cocoon (p = 0.0458 and p = 0.0153, one-way ANOVA). Based on quadratic regressions, highest survival was calculated at an algae pomace inclusion level of 3.8% and 4.7% for cocoons. The feed conversion was not affected by dietary treatment with mean values ranging from 2.2 to 3.2.

No differences were observed in plasma protein and glucose levels of shrimp feeding on the experimental diets (Figure 2). Mean protein concentrations ranged from 6.6 to 7.2 g·dL^{-1}, and glucose from 22.7 to 28.5 mg·dL^{-1}.

Activities of phenol- and prophenoloxidase in shrimp were also not affected by dietary treatment in the growth trial. Mean values in dietary treatments ranged from 156 to 391 U·mL^{-1} for phenoloxidase activities. Prophenoloxidase activities were substantially higher in all treatment groups with values of 3071 to 5913 U·mL^{-1}.

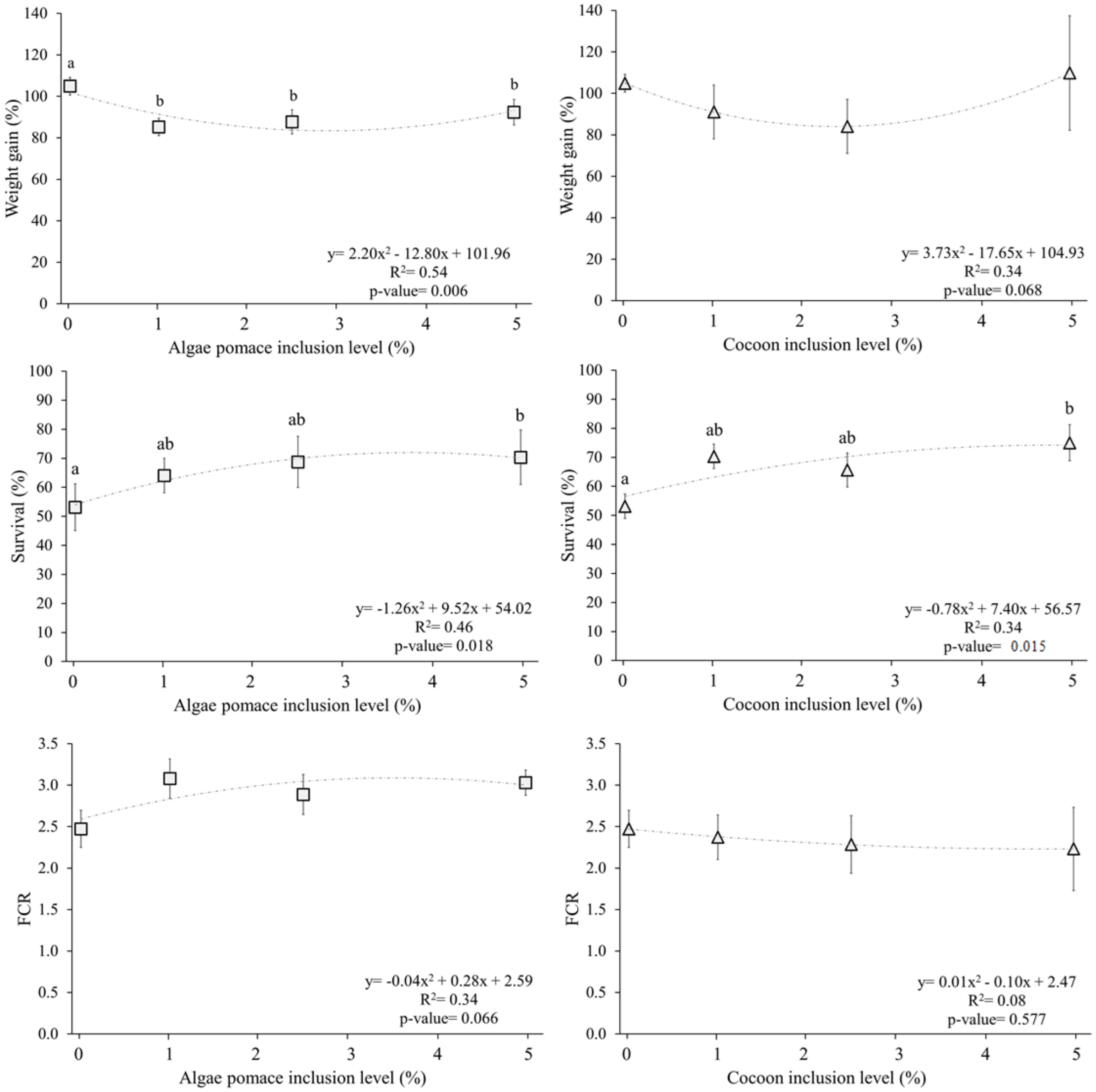

Figure 1. Mean (± standard deviation; n = 4) of weight gain, survival and feed conversion ratio (FCR) of *L. vannamei* fed diets containing different inclusion levels (0%, 1%, 2.5%, and 5%) of algae pomace and cocoons for 42 days. Different letters indicate significant differences ($p < 0.05$). Inclusion rates of the test ingredients and the respective parameters (weight gain, survival, FCR) were modeled using quadratic regression.

3.3 Biofloc experiment

After a rearing period of 36 days in biofloc systems, the mean final weight of shrimp was significantly lower in the cocoon treatment (Kruskal-Wallis, $p = 0.015$). The final body weight of shrimp feeding on algae pomace was slightly higher, but still approximately 1 g less than that of shrimp in the control treatment (Table. 7). The percentage weight gain and weekly growth was significantly higher in the control treatment compared to both, the algae pomace and cocoon treatment ($p = 0.0018$, one-way ANOVA). Survival was not affected by dietary treatments and mean values ranged 80 to 90%. Feed utilization was similar for the experimental feeds and no statistical differences were detected. Mean apparent feed conversion factors ranged from 3.0 to 3.4.

Microbiological analysis of shrimp guts revealed, that dietary inclusion of algae pomace and cocoons had no effect on the heterotrophic bacteria and *Vibrionacea* count (Figure 3).

The mortality of shrimp after a thermal shock was similar for all shrimp, regardless of the dietary treatment (Figure 4). Comparisons of the post-shock survival showed no significant differences ($p = 0.96$, Kaplan-Meier). Total hemocyte counts were not different on shrimp feeding on the experimental diets before, and after the thermal shock (Figure 5). Variability in total hemocyte counts was high and ranged from 9.3 to 17.8 × 10^7 Cells·mL^{-1}.

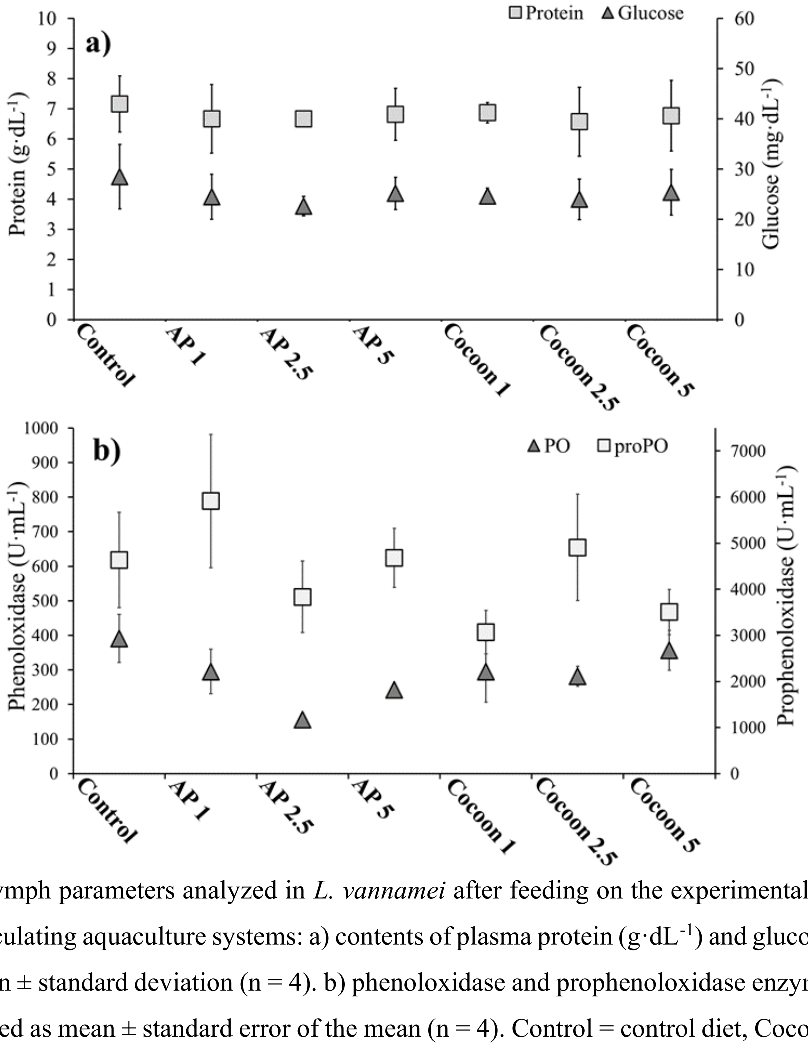

Figure 2. Hemolymph parameters analyzed in *L. vannamei* after feeding on the experimental diets for 42 days in clear water, recirculating aquaculture systems: a) contents of plasma protein (g·dL^{-1}) and glucose (mg·dL^{-1}), values expressed as mean ± standard deviation (n = 4). b) phenoloxidase and prophenoloxidase enzyme activities (U·mL^{-1}), values expressed as mean ± standard error of the mean (n = 4). Control = control diet, Cocoon = diet containing 5% cocoons from *H. illucens*, Algae pomace = diet containing 5% algae pomace from *S. latissima*.

4. DISCUSSION

4.1 Algae pomace of *Saccharina latissima*

The protein content of the algae pomace obtained from *S. latissima* was twice as high as that of *S. latissima* cultivated in commercial integrated multitrophic aquaculture (IMTA) systems (Marinho et al., 2015a). Kelp from the wild (*Laminaria digitata, L. hyperborea, Alaria esculenta,* and *S. latissima*) showed even lower protein contents with average values of about 7% of the dry matter (Schiener et al., 2015). The total lipid content of algae pomace was also higher than that of *S. latissima* harvested in Denmark, which contained highest levels in November with 3.4% of the dry matter (Marinho et al., 2015b). Large variations in the nutrient profiles of macroalgae are common and depend on the harvesting site, environmental conditions, and the season (Coaten et al., 2023; Kumar et al., 2015). The pomace used in the current study derived from the aqueous extraction of *S. latissima* for cosmetic purposes. Therefore, the structural and insoluble components such as proteins and lipids remain in the pomace and become concentrated while water soluble compounds are removed.

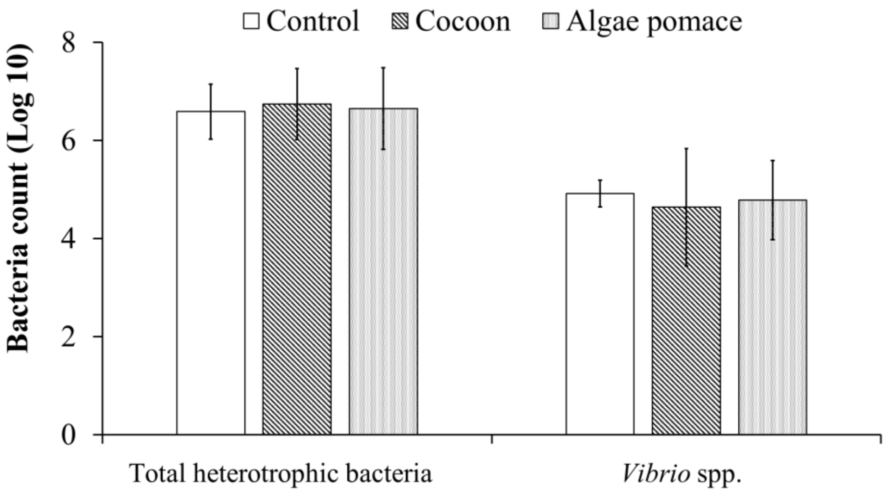

Figure 3. Total heterotrophic and *Vibrio* spp. bacteria counts from the gut of *L. vannamei* after feeding on the experimental diets in biofloc systems for 36 days. Control = control diet, Cocoon = diet containing 5% cocoons from *H. illucens*, Algae pomace = diet containing 5% algae pomace from *S. latissima*. Bar plots depict the mean and whiskers indicate the range of the standard deviation (n = 4).

Different macroalgae meals have been tested at diet inclusion rates from 0.5 to 8%, without negatively affecting the growth performance in shrimp (Cárdenas et al., 2015; Rezende et al., 2021; Rezende et al., 2022; Schleder et al., 2018). An extraction residue made from the green macroalgae *Ulva prolifera* improved shrimp growth at a dietary inclusion of about 3% (Ge et al., 2019). These results contrast the findings of the current study, where growth was reduced at all algae pomace inclusion levels. Reduced shrimp growth was also reported when the brown algae *Undaria pinnatifida* was included at levels above 5% (Niu et al., 2015). Rodríguez-González et al. (2014) tested *Ulva lactuca* and *Gracilaria parvispora* in diets for *L. vannamei* and found reduced shrimp growth in diets containing above 10% *U. lactuca*, but not in diets containing 15% *G. parvispora*. These findings indicate that the effects of macroalgae on shrimp growth depends on the level of inclusion, macroalgae species, and macronutrient content.

Despite the relatively high nutrient content of algae pomace, the reduced growth could be related to the high levels of fibers. Compared to native macroalgae meals that contain 20 to maximal 100 g·kg^{-1} fibers, the fiber content in algae pomace exceeds these values with 123 g·kg^{-1} (Cruz-Suárez et al., 2009; Rodríguez-González et al., 2014; Schleder et al., 2017b). Indigestible carbohydrates can affect digesta viscosity and enzyme substrate interactions, which ultimately reduces nutrient availability (Castillo & Gatlin III, 2015). This effect could also explain the elevated FCRs observed in in growth trial with algae pomace containing diets. The potential of macroalgae to provide macronutrients in aquafeeds is constrained, primarily by the presence of indigestible polysaccharides (Wan et al., 2019).

Certain polysaccharides such as β-glucan, lipopolysaccharides and peptidoglycan were reported to have immunomodulatory effects in fish and shrimp (Diao et al., 2013; Nya & Austin, 2010; Pan et al., 2015). Improved survival is often linked to enhanced functionalities of various parameters of the immune system. For instance, activities of phenoloxidase, a key enzyme in the innate immune system in invertebrates, were significantly increased in *L. vannamei* and *Penaeus monodon* feeding on diets supplemented with different seaweed meals and extracts (Ge et al., 2019; Niu et al., 2015; Rezende et al., 2022; Schleder et al., 2017b). Pattern recognition proteins in *L. vannamei* were shown to recognize and bind to certain seaweed polysaccharides and subsequently activate the prophenoloxidase system (Chen et al., 2016). Kitikiew et al. (2013) showed that the polysaccharide fucoidan effectively enhances phenoloxidase enzyme activities in *L. vannamei*. Wild harvested *S. latissima* contains about 6% fucoidan (based on dry matter), which is a water-soluble sulphated polysaccharide, primarily found in the cell walls of brown seaweeds (Bruhn et al., 2017; Zayed et al., 2016). In our growth trial, shrimp survival was significantly higher at an algae pomace inclusion level of 5%. However, activities of phenol- and prophenoloxidase were not affected by dietary inclusion of algae pomace. Probably, the aqueous extraction of *S. latissima* removed or substantially reduced the contents of phenoloxidase-activating polysaccharides such as e.g. fucoidan from algae pomace. Thus, the enhanced survival in the algae pomace treatment probably resulted from different underlying processes, that do not relate to the phenoloxidase system of shrimp.

The generation of reactive oxygen and nitrogen species (ROS and RNS) to oxidize and destroy pathogens and exogeneous material is a fundamental defense mechanism in shrimp (Aguirre-Guzman et al., 2009). The endogenous antioxidant system mediates the adverse effects of ROS and RNS and protects the cell from self-damage (Abele & Puntarulo, 2004). Improved antioxidative capacities of shrimp were observed in several studies testing dietary seaweed supplementation (Esquer-Miranda et al., 2016; Immanuel et al., 2012; Niu et al., 2015). Several biomolecules with antioxidative properties have been identified in macroalgae. Water-soluble antioxidants encompass phenolic compounds, phycobiliproteins and ascorbic acid, while α-tocopherol, carotenoids, and chlorophylls are present in the lipidic fraction (Plaza et al., 2008; Rodríguez-Bernaldo de Quirós et al., 2010). In algae pomace, antioxidants that might have influenced shrimp performance are probably associated with the enriched lipidic fraction.

Fatty acids present in macroalgae meals and extracts have been discussed to play a role in host defense against microbial infections (Monteiro et al., 2021). Free fatty acids have the ability to destroy bacteria by penetrating cell membranes and disrupting vital functions such as energy production and signal pathways (Dayrit, 2015; Desbois & Smith, 2010). In arthropods, changes in membrane fatty acid composition alters osmoregulatory mechanisms and membrane fluidity (Palacios et al., 2004; Teets & Denlinger, 2013). Schleder et al. (2017a) showed, that dietary inclusion of the brown algae *Sargassum filipendula* increased levels of polyunsaturated phospholipids, presumably associated with cell membranes of *L. vannamei*.

It is difficult to identify individual components of macroalgae that influence overall shrimp performance. As pointed out by Monteiro et al., 2021, multiple compounds present in macro algae can have synergistic but also antagonistic effects, likely acting together at different levels of the animals´ physiology and immune system.

4.2 Cocoons of the black soldier fly, *Hermetia illucens*

The digestible protein content of 160 $g \cdot kg^{-1}$ is similar to that of algae pomace. The low content of digestible protein in cocoons could be related to sclerotized proteins, associated with the rigid, chitin-rich outer structure of the cocoons (Fricke et al., 2024). Cocoons and algae pomace contain similar levels of total lipids, ash and fibers, given that chitin is considered as dietary fiber. However, feed supplementation with cocoons did not negatively affect shrimp growth in the clear-water RAS.

Meal made from the black soldier fly larvae is usually included in aquafeeds to replace fish meal. High levels of fish meal replacements (60 to 100%) did not negatively affect the growth performance of *L. vannamei* (Nunes et al., 2023; Wang et al., 2021). The nutrient profiles, especially lipid contents, vary considerably in the tested meals and some studies report reduced growth at dietary inclusion rates above 10% (Cummins Jr et al., 2017). The chemical composition of the black soldier fly changes drastically between the different life stages (Liu et al., 2017). Therefore, meals made from different insect life stages should be considered separately in studies as feed supplements. Cocoons are a side stream of black soldier fly larvae production with limited potential to provide macro-nutrients (Fricke et al., 2024). However, this by-product contains compounds such as cholesterol, copper, chitin, and lauric acid, which could be beneficial for penaeid shrimp. Compared to the control, the survival of shrimp was significantly enhanced in shrimp feeding on 5% dietary cocoons. Phenoloxidase activities were not affected by diet in the cocoon treatments, which indicates that increased survival is rather related to other aspects of the immune system.

There is a growing body of literature reporting on immune modulatory effects of insect meals in fish and shrimp. The health promoting effects have mainly been attributed to antimicrobial peptides, fatty acids, chitin, and chitin related derivates (Henry et al., 2018; Saadoun et al., 2022). However, the pool of bioactive compounds in insects is considered to be large and novel immune modulative substances have been discovered recently (Ohta et al., 2014; Saadoun et al., 2022; Saviane et al., 2021).

In insect meals, chitin is one of the most discussed biomolecules with potential immune modulatory functions. Effects on growth, immune stimulation, disease-, and stress resistance varies with the type of chitin (chitin, chitosan, chitosan oligomer, N-acetyl-D-glucosamine), supplementation dose, and studied species (Mohan et al., 2023). Positive effects of chitin and chitosan on growth and antioxidant status have been reported in *P. monodon* and *L. vannamei* at dietary inclusion rates from 0.1 to 0.5% (Brol et al., 2021; Niu et al., 2013). Based on calculations, the inclusion of cocoons in the experimental diets of our study resulted in chitin contents of 0.15 to 0.8%. The contents of this polysaccharide are thus in the range considered beneficial for penaeid shrimp. Similar to pathogen-associated-molecular-patterns, chitin can modulate the immune system by binding to several immune receptors, as documented in mammals (Elieh Ali Komi et al., 2018). In crustaceans, the mechanism how chitin and its derivates affect the immune response is not well explored. Yet, there is growing evidence that chitin and/or its derivates can improve several immune responses, up-regulate expression of immune related genes, and lead to higher resistance towards disease and abiotic stressors (Brol et al., 2021; Chen et al., 2021; Niu et al., 2011; Niu et al., 2013).

Lauric acid is the prevalent fatty acid in the cocoons of *H. illucens*, accounting for 2% of the dry matter (Fricke et al., 2024). Lauric acid was shown to have antimicrobial properties against gram-positive bacteria, but also against some fungi and viruses (Lieberman et al., 2006). It has been hypothesized, that lauric acid and its derivates can destroy and act on bacterial cell membranes and lipid coated viruses by interfering with cellular signaling and transduction processes (Dayrit, 2015).

A large number of diverse antimicrobial peptides can be found in insects (Saadoun et al., 2022). Their mode of action is similarly manifold and has been described to act on several levels, making them more efficient to by-pass common resistance mechanisms of microorganisms (Wang et al., 2016). In *H. illucens*, bioinformatic analyses of the transcriptome revealed the presence of 57 putatively active antimicrobial peptides (Moretta et al., 2020). Therefore, besides chitin and lauric acid, antimicrobial peptides might have also contributed to the increased survival of shrimp in the growth trial.

Plasma protein and glucose levels were not affected by any dietary treatment (cocoon and algae pomace) in the growth trial. Protein and glucose levels can be affected by rearing condition (environment), nutritive-, and health status (Mercier et al., 2006; Pascual et al., 2003; Weiss et al., 2020). Along with the unaffected phenol and prophenoloxidase activities, these results suggests that up to 5% dietary inclusion of cocoons and algae pomace does not negatively affect the nutritive and health status of *L. vannamei*. The water quality parameters during the growth trial were suitable for shrimp rearing and visual inspection of shrimp did not show any abnormalities. Unfortunately, the mortality in the control group was high, which, however, is not uncommon in growth experiments with shrimp in clear water systems (Amar et al., 2006; He et al., 2022a; Shan et al., 2019; Yamada et al., 1990). Presumably the live transport of juvenile shrimp might have imposed severe stress for shrimp, affecting shrimp performance throughout the trail.

4.3 Biofloc experiment

Shrimp performances in the biofloc trial differed from that of the growth trial in the clear water recirculating water system. Mean survival across treatments ranged from 80 to 90%, which is commonly observed in nutritional studies conducted in biofloc systems (da Silva et al., 2016; Rezende et al., 2021; Rezende et al., 2022). Shrimp survival was slightly higher in the cocoon and algae pomace treatments, but not significantly different to the control group. Growth was negatively affected in both of the feed treatments with algae pomace and cocoons. The different observations in the two experiments might have resulted from variations in feed formulations and additional nutrient supply via the microbiota present in the rearing water of the biofloc systems.

The amount of animal protein meals (fish meal and poultry meal) in diets for the biofloc trial ranged from 26 to 28%, with the control comprising the highest levels. In the RAS growth trial, fish meal was the sole animal protein (excluding cocoons), which was 15% for all diets. Ambasankar et al. (2022) tested krill meal as feed additives in diets for *L. vannamei* containing high and low amounts of fish meal. The study indicated that a minimum level of marine ingredients of 15% is required, to avoid negative effects on the growth performance of shrimp. The fish meal content in feeds used in the RAS trial were therefore in the critical lower range tolerable for shrimp. Poultry meal is an excellent protein source for shrimp and can replace up to 80% of the fish meal content in diets for *L. vannamei* (Cruz-Suárez et al., 2007). The combined animal meals (poultry and fish) in diets used for the biofloc trial were probably more generous and adequate protein sources for *L. vannamei*. Fuchs et al. (2015) hypothesized that beneficial effects of feed additives are most distinct under challenging conditions (e.g. low fish meal containing diets). This might partly explain the positive effects of algae pomace and cocoons on survival in the RAS trial, that were absent in the biofloc experiment. Nevertheless, the nutritive value of cocoons and algae pomace appears to be substantially lower than that of high-quality protein meals such as fish and poultry meal. This is presumably the primary reason for the reduced growth observed in the biofloc trial.

Trials conducted in systems in which natural productivity contributes to the nutrient supply of shrimp often reveal different results than trials in clear water systems (Cummins Jr et al., 2017; Izquierdo et al., 2006). In biofloc systems, the flocculated material is a conglomerate of different microbiota comprising flagellates, ciliates, rotifers, amoebas, nematodes, annelids, microalgae, and bacteria (Cardona et al., 2016; Gallardo-Collí et al., 2019). This biomass is an additional feed source and provides essential nutrients such as protein and fatty acids that support growth of shrimp (Anand et al., 2014; Izquierdo et al., 2006). Burford et al. (2004) estimated, that in intensive biofloc systems natural biota contributes 18 to 29% of the daily nitrogen retention of *L. vannamei*. In our study, the additional nutrient supply in the biofloc system was not enough to compensate the nutritive imbalances in the treatments with algae pomace and soldier fly cocoon. However, the availability of this secondary food source is a substantial difference between these two methods of shrimp culture and might have contributed to the distinct observations of the two experiments.

Several studies have shown that macroalgae and insect meals can affect the gut microbiome in *L. vannamei*. For instance, Schleder et al. (2020) fed a mixture of *Sargassum filipendula* and *Undaria pinnatifida* and reported reduced shrimp gut microbial richness and enhanced microbial diversity with increasing levels of *U. pinnatifida*. The effects of macroalgae on the microbial community of shrimp appears to be species specific. While *U. pinnatifida* lowered numbers of *Vibrio* in *L. vannamei*, no such effect was evident in dietary supplementation of *S. filipendula* (Rezende et al., 2021; Schleder et al., 2017b). Chen et al. (2021) found that black soldier fly larvae meal improved the intestinal microbiota in *L. vannamei*. The authors found increased numbers of *Bacillus* and *Pseudoalteromonas*, while the abundance of bacteria associated with disease such as *Virbio* spp. was reduced. Feeding fresh BSF larvae to *L. vannamei* also reduced the relative abundance of disease-related *Vibrio* and *Canditatus* Bacilloplasma, while potential probiotics such as *Motilimonas, Shimia*, *Pseudoalteromonas* and *Shewanella* increased (He et al., 2022b).

In the current study, supplementation of algae pomace and cocoons did not affect total heterotrophic bacteria and *Vibrio* counts in the gut of juvenile *L. vannamei*. Apart from feed, the bacterial composition in shrimp guts is also affected by the surrounding rearing water (Cardona et al., 2016). Huang et al. (2020) showed that the bacteria in the gut of shrimp raised in biofloc systems are associated with the bacteria found in larger-sized bioflocs. The authors concluded, that shrimp preferably feed on these larger particles, which in turn is reflected in the gut microbiome. The similarities in the microbiological analysis in between treatments could therefore indicate, that the gut microbiome of shrimp in this trial is primarily influenced by the bioflocs they were feeding on.

Temperature is an important abiotic factor that has immunosuppressive effects in penaeid shrimp when reaching critical levels (Qiu et al., 2011). To explore the physiological status and resistance of shrimp to low temperatures, cold shock experiments in which shrimp are exposed to a critical temperature that is lethal to 50% of the animals are often applied (Claessens et al., 2023; Pontinha et al., 2018; Schleder et al., 2017b). For example, diet supplementation of the macroalgae *S. filipendula* and *U. ohnoi*, as well as meal made from the protist *Aurantiochytrium* sp. improved the survival of *L. vannamei* after exposure to low temperatures of 11.5 to 13 °C for 1 hour (Coelho et al., 2023; Rocha et al., 2021; Schleder et al., 2017b). The resistance of shrimp to cold thermal stress depends on multiple factors. In ectothermic organisms, physiological effects of low temperature stress can include impairment of proteostasis, collapse of ion gradients and ion homeostasis, oxidative stress, and reduced neuromuscular functions (Hayward et al., 2014). Exposure to low temperature also reduced the numbers of total hemocytes in *L. vannamei* (Le Moullac & Haffner, 2000; Qiu et al., 2011). This could explain the slightly lower mean total hemocyte counts observed in shrimp 24 hours after exposure to 11 °C. However, there was no statistically significant difference in THC counts in shrimp before and after the temperature stress. This is in accordance with Qiu et al., (2011), who observed that exposure to low temperatures initially reduced hemocyte numbers, which returned to baseline levels after 12 h.

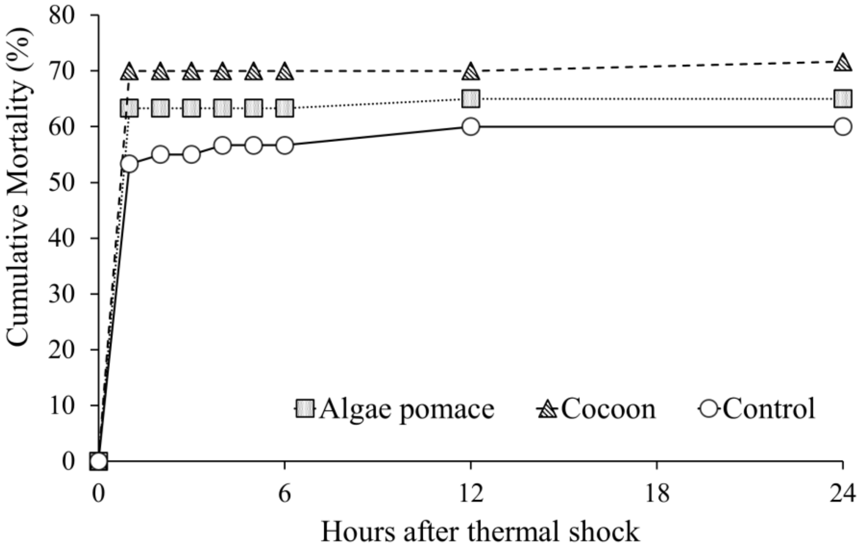

Figure 4. Mean cumulative mortality (%) of *L. vannamei* feeding on the experimental diets in biofloc systems after being exposed to a cold thermal shock (11 °C) for 1h. Control = control diet, Cocoon = diet containing 5% cocoons from *H. illucens*, Algae pomace = diet containing 5% algae pomace from *S. latissima*.

The sensitivity of *L. vannamei* hemocytes to low temperatures could be related to reduced membrane fluidity and integrity. Improved resistance to thermal stress has been linked to higher levels of phosphatidylcholines with unsaturated fatty acids, the building blocks of cell membranes (Schleder et al., 2017a). Feed additives that increase the survival of shrimp after cold exposure are often good sources of long chained polyunsaturated fatty acids such as DHA and EPA (Coelho et al., 2023). The low contents of total lipids ranging from 4 to 6% in algae pomace and cocoons might therefore explain the indifferences of thermal resistance between the dietary treatments. Yet, the detailed mode of action of how feed additives can improve the resistance to low temperature remain largely unclear.

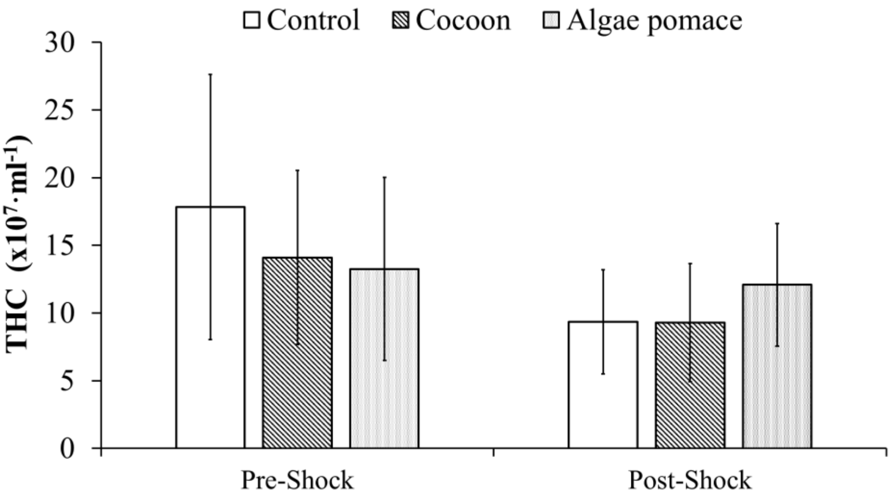

Figure 5. Total hemocyte counts (THC) in *L. vannamei* raised in biofloc systems and feeding on the respective experimental diets for 36 days. Control = control diet, Cocoon = diet containing 5% cocoons from *H. illucens*, Algae pomace = diet containing 5% algae pomace from *S. latissima*. Total hemocyte counts were made in shrimp before (Pre-Shock) and after (Post-Shock) a thermal shock test. Bar plots depict the mean and whiskers indicate the range of the standard deviation (n = 4).

5. CONCLUSIONS

Results of this study demonstrate that rearing system and feed formulation have distinct impacts on performance of shrimp when fed low amounts of algae pomace and cocoons. Both, algae pomace and cocoons increased survival when used in feed formulations containing high amounts of plant-based ingredients and raised in an environment without additional food sources. The absence of this effect in the biofloc trial is likely related to differences in feed formulation (higher share of animal protein) and a surplus of nutrients provided by the flocculated material in the rearing water of the systems. Although no significant reductions in growth were observed in the cocoon treatments in the clear water experiment, both tested feed ingredients clearly reduced the weight gain of shrimp in the biofloc trial. It can thus be concluded, that algae pomace and cocoons do not provide relevant nutrients needed for growth. Dietary inclusion of algae pomace and cocoons did not cause alterations in any hematological parameter, neither on the gross composition of gut bacteria and resistance to low temperatures. Therefore, the potential of algae pomace and cocoons to serve as feed additives is very limited, and based on this data, is not advisable.

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Chapter 3 Synoptic Discussion

3.1 Key Aspects of Feed Ingredient Evaluation

Five by-products have been identified and characterized in this work, originating from three different sectors: seafood processing, cosmetics, and animal farm (insect) industries. Four of those raw materials were tested *in-vivo* as feed or feed additives for the Pacific Whiteleg shrimp, *Litopenaeus vannamei*. The tests aimed to elucidate the bioavailability of key nutrients and to study the biological effects on this species. This fundamental characterization of these materials is essential for any further steps towards incorporation of these raw materials into feed formulations. To holistically investigate the potential of feed ingredients and permit reasonable estimation on future utilization for aquafeeds, it is crucial to consider multiple aspects of the specific ingredient. The role of feed ingredients in the broader food system reaches beyond biological assessment into social and environmental considerations (Glencross et al., 2024). Key aspects encompassing nutritional characteristics, technological implementation, economic considerations, as well as ecological and regulatory constraints should be considered. The following paragraphs introduce the key points that were taken into account to evaluate the potential of the by-products identified in this study.

3.1.1 Nutritional Value

Chemical characterization is the initial step in describing potential feed ingredients. Knowledge on the gross nutrient content, as well as more detailed resolution of the chemical composition (amino acids, fatty acids, minerals) is needed to formulate diets that match the nutritional requirements of the animal. Information on factors that influence the nutritional composition such as species, origin, season or processing methods are equally important (Glencross et al., 2007). As described in Chapter 2.1, the gross chemical composition in shrimp meals can vary considerably depending on crustacean species and processing method. For example, the protein and ash content in shrimp meals can range from 37 to 59% and 19 to 45%, respectively (Liu et al., 2013; Nwanna, 2003; Villarreal et al., 2006). The nutritional composition of macroalgae strongly depends on the season. The protein content can be twice as high in winter than in late summer (Coaten et al., 2023). Factors influencing the quality of the original material also impact the characteristics of the downstream products or by-products. The composition of raw materials, their concentration range, and their determining factors are therefore important product quality indicators by themselves that should be considered in thorough examinations (Glencross et al., 2020).

The palatability of feed or feed ingredients can be defined as the acceptance of the texture and taste for being ingested by the animal (Glencross et al., 2007). As this determines whether a feed will be eaten or not it is a critical factor in the suitability of raw materials as feed ingredients. However, low palatability of feed ingredients can be counter-balanced by the addition of feed attractants that stimulate feeding behavior, such as krill- or squid meal, hydrolysates, or certain amino acids (Nunes et al., 2019; Smith et al., 2005).

Once ingested, the nutrient digestibility of an ingredient refers to the fraction of the nutrient that is not egested with the feces but presumably is available to fuel metabolic processes of the animal (Glencross et al., 2007). The amount of digestible nutrients can vary considerably between different raw materials, even within the same class of ingredients. As for instance, in *Penaeus monodon*, nutrient digestibility varied significantly between different types of fish meal (Jack mackerel, anchoveta, tuna by-product) and also between soybean meals made from two distinct varieties (Glencross et al., 2018). Factors that influence nutrient digestibility of feedstuffs in penaeid shrimp are complex and not well understood. It has been shown, that the nutrient bioavailability can be reduced by imbalanced nutrient profiles and the presence of anti-nutritional factors such as phytate or non-starch polysaccharides (Glencross et al., 2002; Glencross, 2009a; Vandenberg et al., 2012).

The combined effects of nutrient composition, palatability, and digestibility of feed ingredients ultimately influences the feed utilization and growth responses of the cultured species (Glencross, 2020). In addition to growth, bioactive compounds present in feed ingredients can modulate the physiology and affect various aspects of the animal (Dawood et al., 2018; Dawood et al., 2022). In this study, the sum of the animal’s responses (survival, growth, digestive physiology, hematological parameters, pigmentation) to a specific feed ingredient will be regarded as the nutritional value of a raw material.

3.1.2 Technological and Economic Considerations

In order to classify the potential of a raw material within the broader context of the feed sector, the current state of production, technological feasibility, and the resulting supply of the resource is pertinent. The technology readiness level (TRL) is a measure on a scale from 1 to 9 to describe the maturation of a technology (or process) from the basic principle to the full commercial implementation (Mankins, 2009). Concerning the by-products examined in this study, the level of technological development and the scale of production will also be used to estimate the potential cost of the material. Biogenic materials that could be used as feed ingredients can also find applications in other industries. According to the Bioeconomy Strategy of the European Union, the use of biomass should be prioritized for high-value products (pharmaceutical, cosmetics) before it finds application as feed, fertilizers, or for heat generation (Figure 3.1, European Commission, 2013). Therefore, alternative uses of the raw material and competing industries need to be discussed, which could increase demands and raise prices. Feed is one of the key drivers of the total production costs in aquaculture, which in turn affects feed production principles (Kumar et al., 2017; Sánchez-Muros et al., 2020). Feed producers mostly focus on least-cost-formulations in which the cost of feed ingredients determines whether an ingredient will be included in a feed or not (Nunes et al., 2022).

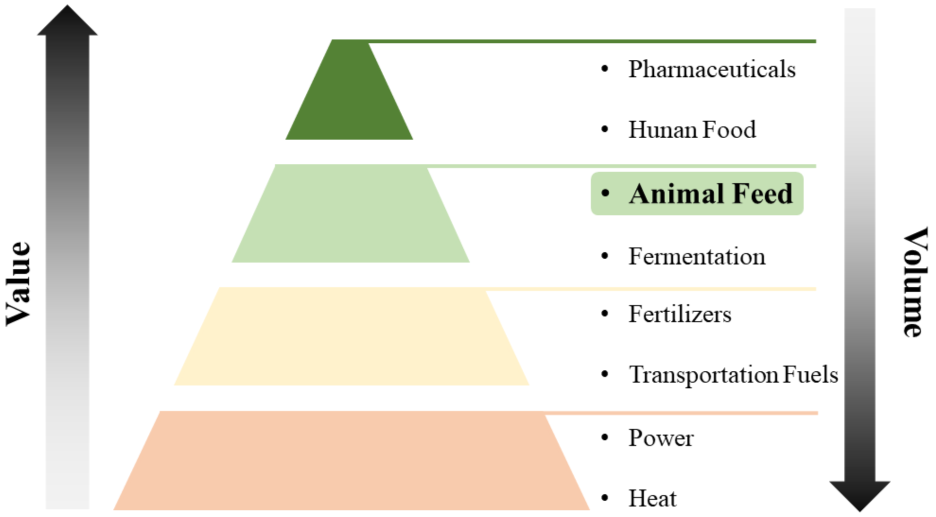

Figure 3.1. Biomass pyramid. Adopted from the European Commission (2013) and Albizzati et al. (2019).

3.1.3 Regulatory, Societal, and Environmental Constraints

In recent years, additional criteria such as feed safety, environmental footprints, and societal constraints have increasingly become important drivers in aquaculture feed developments (Glencross et al., 2024). Regulatory constraints can limit or prohibit the use of specific feed ingredients resulting from environmental, health, and/or food safety concerns. The European Union defined maximum residue levels for a range of toxins including heavy metals (Hg, As, Pb, Cd) and persistent organic pollutants (dioxin, polychlorinated biphenyls) for feed ingredients (European Commission, 2002b; European Commission, 2006). Contamination of raw material may result from anthropogenic activities or be a cause of natural accumulation. For example, use of fish oil sourced from the Baltic Sea is limited in aquafeeds because it contains high amounts of persistent organic pollutants due to the extensive industrialization of coastal areas (Cheng et al., 2016; Mikolajczyk et al., 2021). On the contrary, high concentrations of arsenic in seaweeds, mussels, and shrimp are often bound to organic compounds that accumulate naturally (Ruttens et al., 2012; Taylor et al., 2017). To improve the management of diseases such as transmissible spongiform encephalopathy, strict regulations exist in the European Union regarding the use of animal protein as feed ingredients (European Commission 2001; European Commission 2016).

Increasingly important issues are environmental considerations of raw materials intended as feed ingredients (Boyd et al., 2020). For example, utilization of fish meal as aquaculture feed has received a lot of criticism and pressure due to the environmental footprint and its societal perception (Jones et al., 2015; Naylor et al., 2000). On the contrary, feed ingredients that have low environmental impacts are highlighted to help develop eco-friendly aquafeeds, thereby fostering a positive reception of aquaculture products among consumers (Bergleiter & Meisch, 2015; Whitmarsh & Palmieri, 2011; Wilfart et al., 2023). Another example of a societal constraint is related to the use of feed ingredients sourced from genetically modified organisms (GMO). Despite not being prohibited by law, there is resistance to use GMO feed ingredients in aquafeeds mainly due to consumer perceptions and preferences in Europe (Glencross et al., 2020).

There are beneficial and negative aspects inherent to each feed ingredient and there is no "perfect aquaculture feed ingredient". A systematic examination of the opportunities and risks associated with each raw material can be holistically discussed using the structure of a Strength-Weakness-Opportunity-and-Threats (SWOT) analysis (Gasco et al., 2020). Strength and weaknesses primarily refer to the intrinsic factors of a specific feed ingredient, such as its nutritional value and technological readiness of its production. Opportunities and threats summarize external effects that influence the potential of feed ingredients such as technological innovations, consumer trends, regulatory frameworks or a changing climate.

3.2 Brown Shrimp Processing Remains

3.2.1 Strengths of Brown Shrimp Processing Remains

A fundamental strength of brown shrimp processing remains (BSPR) is the well-balanced and bioavailable nutrient content. The high protein content and excellent amino acid profile of this by-product is comparable to prime quality shrimp feed ingredients such as tuna by-product and Antarctic krill meal (Ambasankar et al., 2022; Villarreal et al., 2006). The contents of essential amino acids showed an excellent match with the ideal amino acid profile estimated for penaeid shrimp, which is highlighted in Chapter 2.1. Furthermore, a unique strength of this marine-based by-product is the presence of the essential fatty acids EPA and DHA. Additionally, BSPR contain several micronutrients that are valuable for shrimp aquaculture such as copper and cholesterol.

The most notable benefit of BSPR inclusion in shrimp diets is the growth stimulating effect observed and described in Chapter 2.2. The significantly reduced FCR revealed, that shrimp fed less but grew more, when 60 - 80% of the fish meal was substituted with BSPR. Successful substitution of fish meal by alternative feed ingredients is usually assumed when no differences to a fish meal-based control diet are observed (Cárdenas et al., 2015; Cruz-Suarez et al., 2001; Panini et al., 2017; Weiss et al., 2020). The increased performance of shrimp feeding on diets replacing the majority of fish meal with BSPR is thus an exceptional observation.

A unique feature of the brown shrimp fishery is the fact that the catch is immediately boiled on board capture vessels to prevent spoilage and facilitate the peeling process (Saborowski et al., 2022). Cooking shrimp denatures proteins and inactivates major spoilage enzymes such as proteases and phenoloxidases (Verhaeghe et al., 2016). Treatment of shrimp with chemical compounds (e.g. sodium meta-bisulfite) to prevent the enzymatically triggered melanosis is therefore not necessary. The thermal treatment also lowers the amount of bacteria and potential pathogens, which, in turn, reduces risks of disease transmission (Ježek et al., 2020). Brown shrimp feed on benthic organisms from low trophic levels and reach an age of one to three years (Campos & van der Veer, 2008; Oh et al., 2001). The short life span and the low trophic level of this organism could be a reason for the minimal amounts of contaminants that were detected in BSPR. Preventing the presence of dangerous compounds, such as persistent organic pollutants in animal feed is crucial for ensuring safety of human food (European Commission, 2006). As pointed out in Chapter 2.1, the slightly elevated arsenic levels detected in BSPR are most likely organically bound with low toxic effects. Therefore, by-products of the brown shrimp fishery can be considered a safe and low risk aquafeed ingredient.

The brown shrimp fishery has a long history in the North Sea and evolved into an intensive fishery over the last century (Saborowski et al., 2022). This fishery is a well-developed industry with established processing methods and supply chains. Annual landings regularly exceeded 30.000 tons in the last decade, resulting in approximately 20.000 tons of processing remains being generated each year (ICES, 2023). The brown shrimp fishery in Denmark, Germany, The Netherlands, and the United Kingdom (Wash) is certified by the Marine Stewardship Council. Recent audits of the industry showed acceptable results indicating a well- managed and sustainable fishery (MSC, 2023). The technological readiness of this industry can be described as mature (TRL 9) which brings certain reliability in production and supply. BSPR are not fully utilized and largely remain unused (Saborowski et al., 2022). Due to the low demand and relatively high supply, the cost for this material is assumed to be low to moderate, encouraging its utilization and increasing its potential as feed ingredient in shrimp aquaculture.

3.2.2 Weaknesses of Brown Shrimp Processing Remains

The fact that brown shrimp are cooked immediately after the catch has positive aspects, but also certain drawbacks. Levels of sensitive biomolecules can be impaired by high temperatures, due to chemical processes such as oxidation, polymerization, and cyclization (Fournier et al., 2006; Sobral et al., 2018). Boiling of *C. crangon* reduced the content of DHA and EPA by 15 and 25%, respectively (Saborowski et al., 2022). Hu et al. (2019) found that cooking of shrimp shells lowered the amount of astaxanthin by approximately 40%. Despite the positive aspects of cooking brown shrimp, the nutrient content of BSPR is also partly reduced, which is an intrinsic weakness of this material.

Total annual landings of *C. crangon* have continuously increased from 1960 until the end of the last century. However, since the beginning of the 21st century, this steady increasing trend stagnated (ICES, 2023). This indicates that the natural stocks are likely exploited at the maximum sustainable yield and further increase in total landings of this marine resource cannot be expected in future. The growth potential of this resource is therefore limited. Furthermore, landings of brown shrimp are subjected to strong fluctuations. The year 2017 was marked by exceptional low landings of 22,565 tons, while in 2018 total catches amounted to 46,015 tons (ICES, 2023). The inconsistent landing volumes and concomitant unpredictable supply are disadvantages of BSPR.

Although the brown shrimp fishery can be considered sustainable and well-managed, certain negative environmental aspects remain. Brown shrimp are caught via bottom trawling, with heavy gear that physically impacts the seabed and poses a threat to the benthic fauna (Bruns et al., 2023; de Groot, 1984). In order to catch brown shrimp, small meshed nets are required which lead to large amounts of by-catch and capture of juvenile shrimp (Neudecker & Damm, 2010). Although undersized juvenile shrimp are sieved and released, Temming et al. (2022) found that a large fraction of these discarded shrimp does not survive this process.

Another environmental constraint is CO_2 emissions by the brown shrimp industry. Compared to other fishing types (e.g. mobile gear), fishing industries using towed gear have high energy requirements (Gascoigne & Willsteed, 2009). In the North Sea, the annual fishing effort for brown shrimp is estimated to be approximately 10 million horsepower days at sea, consuming fossil fuel (ICES, 2023). Additionally, processing of shrimp (peeling, sorting, packing) is typically done in countries with low labor costs. Therefore, energy required for transportation and cooling further increase CO_2 emissions (Saborowski et al., 2022).

3.2.3 Opportunities of Brown Shrimp Processing Remains

There is a growing body of literature that reports growth stimulating effects of crustacean meals in penaeid shrimp (Amar et al., 2006; Córdova-Murueta & García-Carreño, 2002; Villarreal et al., 2006). Similar to the effects of BSPR described in Chapter 2.2, Andam et al. (2024) found that growth of *L. vannamei* post larvae was significantly enhanced when 75% of fish meal was replaced with mysid meal (*Mesopodopsis* sp.). Villarreal et al. (2006) found an optimal fish meal replacement level of 80% with red crab meal (*Pleuroncodes planipes*). These findings are surprisingly similar, which supports the hypothesis of a so far uncharacterized growth factor in crustacean meals (Williams et al., 2005). Gallardo et al. (2003) isolated and described peptides from the hepatopancreas of lobster (*Panulirus argus*) with insulin-like effects. Several peptides have been described with hormonal functions that regulate metabolic processes, molting, growth, and reproduction in shrimp (Huberman, 2000; Udomkit et al., 2004). *C. crangon* contain high shares of polar phospholipids which are highly bioavailable and known to promote growth and survival in many crustacean species (Mika et al., 2014; Zhang et al., 2019). The presence of a growth-enhancing factor in BSPR provides great opportunities for this resource. Isolation and proper characterization of such a compound could help to develop innovative and high precision feed formulations, using the growth factor as a potent feed additive. Understanding the mechanisms of a natural hormone-like compound could create opportunities for several aspects of shrimp cultivation such as an ethical appropriate reproduction (avoidance of eye stalk ablation) and overall shrimp welfare.

Already existing technological innovations could lower the environmental impact of the brown shrimp fishery and thus that of BSPR. Pulse trawling is considered a promising alternative to lower the amount of by-catch in beam trawlers (Soetaert et al., 2015). Electrical pulses induce a contraction of the shrimp muscles, causing them to jump out of the sediment into the water column, thereby facilitating their capture. The mechanical contact of the ground gear with the seabed can thus be minimized and shrimp can be caught more selectively, reducing by-catch by up to 76% (Verschueren et al., 2019). Alternative ship propulsion systems can be applied to reduce the dependence of fishing vessels on fossil fuels. Eco-friendly alternatives based on renewable fuel (e.g. hydrogen or methanol), electric energy, or hybrid systems are currently considered to reduce the environmental impact of fishing vessels (Gabrielii & Jafarzadeh, 2020; Hwang et al., 2022). Automated peeling of shrimp poses an opportunity to process shrimp locally in an economically viable manner, which would also reduce long transport routes and associated CO_2 emissions. Centralized peeling would facilitate BSPR generation in sufficient amounts to create stable and short supply chains.

3.2.4 Threats Related to Brown Shrimp Processing Remains

Shrimp waste streams are a primary source for chitin and its derivatives, that find applications in biomedical, nutraceutical, cosmetic, and wastewater industries (Amiri et al., 2022; Vieira et al., 2023). In India, the utilization of shrimp shells for chitin extraction is diverting biomass streams away from animal feed production (Muñoz et al., 2018). Pharmaceuticals, fine chemicals, and the human food sector create greater value than the animal feed sector (Figure 3.1) and thus have a competitive advantage, threatening the use of BSPR as aquafeed ingredient.

On the long term, the future of the brown shrimp fishery in the Wadden Sea, the primary fishing ground of this species, is uncertain. The Wadden Sea is one of the world's largest tidal wetlands, renowned for its unique natural features and outstanding ecological relevance (Kabat et al., 2012). At the same time, this coastal area is among the most degraded and threatened estuarine and coastal ecosystems worldwide (Lotze et al., 2006). The first protection measures date back to the beginning of the 20th century and in 2009 the Wadden Sea was acknowledged as an UNESCO World Heritage Site (Kabat et al., 2012; UNESCO, 2009). Commercial fishing is among the most damaging human activities in the Wadden Sea, affecting the ecological structure of this ecosystem (Lotze et al., 2005). In 2023, the European Commission proposed the phasing out of mobile bottom fishing in Marine Protected Areas by 2030 (European Commission, 2023). This would have drastic impacts on the brown shrimp fishery and possibly lead to a closure of these activities.

Although there is a great level of uncertainty of how climate change will impact fisheries, it is safe to acknowledge that it poses a major threat to natural brown shrimp populations (Brander, 2010). Rising temperatures and ocean acidification are predicted to change the community structure of the Wadden Sea (Pansch, 2019). A rising sea-level could lead to the permanent submersion of tidal flats and wetlands, changing sedimentation rates, and heavily altering the physical characteristics of the coastal area (FitzGerald et al., 2008; Kabat et al., 2012).

3.3 Adult Black Soldier Flies (Imagines)

3.3.1 Strengths of Adult Black Solider Flies

The nutrient dense meal made from the adult fly of *Hermetia illucens* contains high levels of protein. After defatting, the crude protein content of almost 60% is comparable to high protein aquafeed ingredients such as fish meal, krill meal, poultry-by-product meal, and soy protein concentrate (Ambasankar et al., 2022; Bauer et al., 2012; Cruz-Suárez et al., 2007). The imago protein contains all essential amino acids required by *L. vannamei* in adequate concentrations (Chapter 2.3). Similar to BSPR, imago meal contains biomolecules and minerals that are especially interesting and valuable for shrimp feeds (e.g. cholesterol, copper).

The perishability of organic material is related to high moisture contents, which facilitate the growth of microorganisms and degradation through hydrolytic reactions (Park, 2008). Unprocessed dead adult flies have a relatively low moisture content of about 33% and do not spoil rapidly (Chapter 2.3). This facilitates the short-term storage and transportation of the dead flies until they are further processed. Additionally, less energy is required to fully dry this material.

Adult black soldier flies are a by-product of insect farming. Therefore, aspects that are related to this sector also need to be considered when evaluating the potential of imago meal as aquaculture feed. Mass rearing of insects has great potential as an environmentally sustainable alternative to meet the growing global demand for protein (Berggren et al., 2019; Smetana et al., 2021; van Huis, 2020). Insects are very resource-efficient in converting organic matter into biomass with high nutritional value (Rumpold & Schlüter, 2013). Oonincx et al. (2010) estimated that the production of insects emits lesser greenhouse gases compared to conventional livestock such as pigs and cattle. Insects such as *H. illucens* prefer dry environments to pupate and are able to satisfy their water demand from the substrates they are raised on (Rumpold & Schlüter, 2013; van Huis et al., 2020). The water footprint of proteins produced by insects is thus very small, even compared to plant-based proteins (Röthig et al., 2023). Insect farming does not require large areas of land. A life cycle assessment revealed that the production of 1 kg of dried protein of BSF larvae requires 0.05 $m^2 \cdot a^{-1}$, while 8.7 $m^2 \cdot a^{-1}$ are needed to produce1 kg of soybean protein (Salomone et al., 2017).

The cultivation of *H. illucens* does not contribute to the exploitation of any natural populations as it is the case with fish meal made of species caught from the wild. Replacing fish meal with *H.illucens* adult fly meal would mitigate the immense pressure on wild fish stocks and marine ecosystems.

3.3.2 Weaknesses of Adult Black Solider Flies

Despite the promising levels of nutrients, the nutritional value of black soldier fly imagines for *L. vannamei* is restrained and is not suitable to replace high amounts (> 50%) of dietary fish meal, as it was shown in Chapter 2.4. Smaller replacement levels (< 25%) could be feasible, as reported in studies evaluating full fat black soldier fly larvae meal (Chen et al., 2021; Cummins Jr et al., 2017). Nevertheless, these findings indicate limits for the utilization of this resource for shrimp feeds. The main nutritional bottlenecks of imago meal are presumably the unappealing flavor for *L. vannamei* and an imbalanced nutrient bioavailability, considering that 40% of protein is apparently not digested (Chapter 2.3). Attempts to balance out the digestible protein and energy contents in the diets used in experiment outlined in Chapter 2.4 were not successful. Other nutrient imbalances have likely contributed to the observed impairments of shrimp performances (e.g. imbalanced fatty acid profiles, high chitin contents). However, protein and essential amino acid bioavailability are probably the most relevant factors.

The reduced attractiveness of imago meal may be masked by supplementing strong feed attractants such as krill meal or marine protein hydrolysates (Derby et al., 2016; Nunes et al., 2006). Reduced inclusion levels of imago might also negate the low palatability of imago meal for shrimp. Nonetheless, this remains a disadvantage and needs to be adequately addressed.

The high lipid content of adult *H. illucens* flies is not suitable for penaeid shrimp. The meal must be defatted before it can be used as ingredient for shrimp feed. This processing step potentially adds to the cost of the material. Furthermore, the fatty acid profile of imagines is not ideal due to the lack of the essential fatty acids EPA and DHA. Additionally, the high amount of short-chained saturated fatty acids does not provide great nutritive value for penaeid shrimp (Glencross et al., 2002; NRC, 2011).

Insects have received a lot of attention in the discussion of how to cover the growing global demand of protein. However, they are not primary producers and need suitable substratum to grow on (Glencross et al., 2024). The growth of insects, as well as their nutritional composition, is highly dependent on the source and quality of the substrates they are raised on (Bosch et al., 2019; Tschirner & Simon, 2015). Feed conversion ratios for black soldier flies can range from 1.2 to more than 10, depending on the substrate (Oonincx et al., 2015; ur Rehman et al., 2017). Perednia et al. (2017) found that on average, black soldier flies convert 41% of the carbon present in feed into insect biomass. The remaining organic matter is thus lost to the atmosphere, primarily in the form of CO_2 and methane (CH_4). Large amounts of biomass are therefore needed to fuel the expected growth required for industrial insect cultivation. Regulatory constraints in the European Union limit the use of possible substrates to raise insects. For example, animal protein, food waste, and manure are not allowed to grow insects designated for animal feed (European Commission, 2009a, 2009b).

Large scale production of insects requires energy for climate control, insect processing (biomass fractioning and drying), and other automated processes such as feeding, sensorics, and handling (Smetana et al., 2021; van Huis & Oonincx, 2017). The high energy demand for the cultivation of *H. illucens* is one of the key factors influencing cost and environmental sustainability (Salomone et al., 2017). Although the technology for industrial insect production is advancing rapidly, this sector is not fully developed with a low technological readiness and economic scale (Gold et al., 2018). In order to be considered as viable feed ingredient, large and guaranteed quantities of insect proteins are required (van Huis & Oonincx, 2017). Currently, the availability and price of insect protein cannot compete with other alternative aquafeed ingredients (Niyonsaba et al., 2021; Röthig et al., 2023).

According to EU regulation 1069, animals that died in another way than being slaughtered, including animals killed for disease control, fall into the animal by-product category 2, and cannot be used as animal feed (European Commission, 2009). Adult black soldier flies naturally die shortly after hatching and the dead flies were collected to produce defatted imago meal. The commercial use of meal from dead imagines is therefore prohibited in the European Union. However, if adult black soldier flies are intended to be used as animal feed, the imagines could be killed immediately after they laid their eggs.

3.3.3 Opportunities of Adult Black solider Flies

The unfavorable results of substituting fish meal with defatted imago meal merely represent the first attempt to evaluate this novel raw material. Further research could help to identify bottlenecks that limit the use of imago meal for shrimp. Once these are known, steps could be undertaken to overcome these nutritive impediments. Stronger defatting, fractioning, or further pre-treatment methods could help to improve the nutritional value of imago for *L. vannamei*.

The nutrient composition of insects is strongly influenced by the substrates they are feeding on (Meneguz et al., 2018). For instance, Fuso et al. (2021) found significantly differentiated amino acid profiles in black soldier fly prepupae raised on different vegetable by-products. Truzzi et al. (2020) were able to enrich prepupae with long chained polyunsaturated fatty acids by mixing microalgae into the feed substrates. This offers the opportunity to manipulate and tailor the nutrient profile of *H. illucens* to meet the dietary requirements of *L. vannamei*.

Insects can feed on materials that are metabolically unavailable for most vertebrates and re-introduce nutrients into the human food system that would otherwise be lost. For example, municipal organic solid wastes, manure, and lignin-rich substrates such as agricultural waste streams can be bio-converted by black soldier flies (Gold et al., 2018; Ramzy et al., 2022). Furthermore, using BSF for bio-waste treatment can considerably reduce GHG emissions that would otherwise be produced by composting (Perednia et al., 2017). Up to 31% of the emissions can be avoided if insects used to treat bio-waste would replace globally traded fish meal in animal feeds (Mertenat et al., 2019). Despite the current regulatory restrictions in Europe, the development of the legal framework for this industry is an ongoing process, which just started in the 21st century (European Commission, 2017; European Commission, 2021). If hygienic and disease related concerns are scientifically addressed and convincingly ruled out, changes in the regulatory framework can be expected and unlock the full potential of insect farming.

Furthermore, inclusion of insects in aquafeeds could be used as marketing tool for the aquaculture product, pointing out the environmental benefits that are associated with insects. Studies conducted in Greece and Spain revealed that there is a positive attitude towards the inclusion of insect proteins in aquaculture feeds (Llagostera et al., 2019; Rumbos et al., 2021).

Modern insect farming is commonly done in climate-controlled and enclosed facilities with stable temperatures, adequate humidity, and air exchange (Cadinu et al., 2020). The production of insects is therefore decoupled from the environment, which offers several advantages and opportunities. Year-round stable conditions enable resilient production, ensuring reliable supply, and good plannability, which is essential for the commercial viability of raw materials as feed ingredients (Glencross et al., 2020). The possibility to control all inputs (e.g. feed substrates) reduces the risks of contamination and allows for efficient management of biosecurity. As a consequence of global warming, changing weather patterns, and extreme weather events pose a severe threat to the global food system (Campbell et al., 2016). By artificially controlling the environment, insect farming systems are unaffected by heat waves, heavy rainfall and droughts. Indoor insect farming can thus help strengthen the resilience of the food and feed producing sector against climate change. Furthermore, the high energy demand of insect production may be covered by renewable energy sources (e.g., solar and wind energy), which could drastically reduce the GHG emissions of these high-tech farms.

3.3.4 Threats Related to Adult Black Solider Flies

Similar to BSPR, imagines are a source of chitin. As the commercial interest in this biopolymer is increasing, competition for insect materials might also increase, reducing the availability and rising prices for this resource. Alternative use of imagines in other sectors, e.g. medical, cosmetic, food, or agricultural, thus threatens its potential as aquafeed ingredient (Morganti et al., 2008; Park & Kim, 2010; Shahidi et al., 1999; Shamshina et al., 2020).

The scalability and success of insect farming depends on the availability and access of large amounts of biomass, which is already subjected to intense competition (Gold et al., 2018). Biomass suitable as feed substrate is also central in the development of a sustainable bioeconomy and is used for a range of processes and products including energy generation, biofuels (e.g. methane, ethanol), biochar, and bio-based chemicals (e.g. polymers, lubricants, pharmaceuticals) (Antar et al., 2021). Along with the ongoing decarbonization process, the interest and competition for biomass will presumably increase in the near future.

Feed substrates may contain a range of hazardous compounds including pathogenic microbes, mycotoxins, pesticides, heavy metals, and persistent organic chemicals (Gold et al., 2018). The bioaccumulation and transfer of these compounds into the food chain may pose a serious threat to public health, and there are still significant knowledge gaps on this subject (van der Fels-Klerx et al., 2018; Vandeweyer et al., 2021). While pharmaceuticals, PCBs, PAHs, and dioxins are not accumulated by BSF, heavy metals are taken up and can reach critical levels (Charlton et al., 2015; Lalander et al., 2016; Purschke et al., 2017). If these health concerns cannot be ruled out and certain substrates, such as bio-waste, remain a potentially hazardous feed source, the expansion of this industry will be challenging.

Overall human energy consumption is steadily increasing as the global population grows and economies are expanding (Bilgen, 2014). The widespread use of fossil fuels to produce energy further accelerates climate change and recent political crisis caused a drastic increase of energy prices in Europe (Farghali et al., 2023). Mass rearing of insects requires considerable amounts of energy, which is one of the main factors contributing to global warming of this industry (Boakye-Yiadom et al., 2022). Rising energy costs pose another threat to the development and economic viability of the insect producing sector. In the context of energy and nutrient efficiency, the question also has to be raised, whether it is desirable to raise one livestock to feed another livestock.

Guidelines for welfare and appropriate rearing conditions for insects as livestock remain to be established to avoid any ethical conflicts (Voulgari-Kokota et al., 2023). Existing animal welfare legislations for farmed animals typically do not apply for insects (European Commission, 1998). However, there is scientific evidence that insects are sentient and capable of experiencing pain (Gibbons et al., 2022). As the industrialization of insect mass-rearing is advancing rapidly, knowledge on insect health, behavior, and welfare is lacking behind (Berggren et al., 2019).

The black soldier fly is native to the Americas but is now found across tropical, subtropical, and temperate regions around the world (Kaya et al., 2015). First reports of *H. illucens* in subtropical regions of Europe (Malta) date back to 1929. Since the late 1980s, black soldier flies are reported to occur in more temperate regions (Marshall et al., 2015). The potential of black soldier flies to become a problematic invasive species is currently considered to be low (Spranghers et al., 2017). Nevertheless, with climate change the spread of non-native species will become more likely (Berggren et al., 2019). There is evidence that *H. illucens* is spreading further north (Roháček & Hora, 2013). Invasive terrestrial arthropod species can cause severe ecological and economic damage (Kenis & Branco, 2010). Precautionary approaches to ensure that cultivated *H. illucens* do not become invasive are important in the further development of this industry.

3.4 Cocoons of the Black Soldier Fly

Like adult black soldier flies, cocoons are a side stream of BSF larvae production. All aspects influencing insect farming described in Chapter 3.3 thereby also affect the applicability of cocoons. To avoid redundancies, these arguments are not explicitly mentioned again in the following section.

3.4.1 Strengths of Black Soldier Fly Cocoons

Cocoons were tested at small dietary supplementation levels aiming to improve health and performance of *L. vannamei* in this work. Cocoons are a rich source of chitin (15%) which initially led to the idea to explore them as feed additive in shrimp feeds. Chitin and its derivatives have the potential to enhance growth, promote the immune system, and increase survival of crustaceans (Mohan et al., 2023). However, scientific findings regarding this topic are controversial. Beneficial effects of chitinous substances appear to vary with dietary dose, species, feed formulation, rearing conditions, and the applied chitin derivative (Brol et al., 2021; Cheng et al., 2021; Fox, 1993; Niu et al., 2011a; Niu et al., 2013). Chitin of BSF cocoons is very similar to that of crustaceans, with an alpha crystalline structure of the chitin fibers (Figure 3.2) (Soetemans et al., 2020). Shrimp often consume their own exuviae after ecdysis and possess the digestive enzymes to break down this polysaccharide (Clark et al., 1993; Fox, 1993; Huang et al., 2010; Watanabe et al., 1998). Therefore, shrimp may be able to digest and utilize cocoon chitin as well.

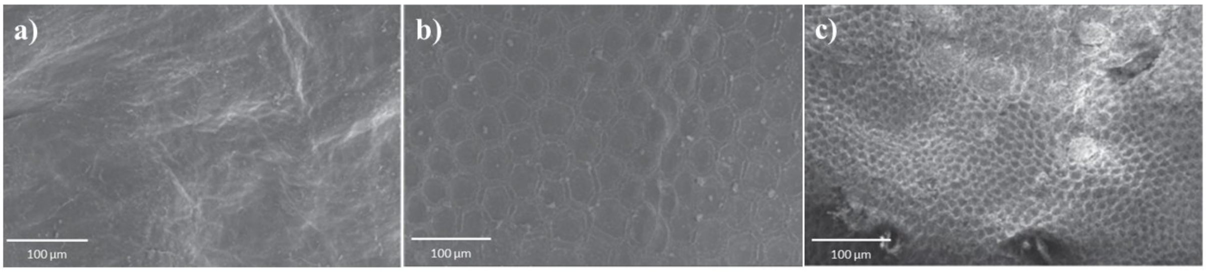

Figure 3.2. Scanning electron microscopical images (SEM) showing the surface morphologies of chitin samples extracted from a) shrimp, b) black soldier fly cocoons and c) black soldier fly larvae, magnitude ×250 (Soetemans et al., 2020). Permission to use this image is granted under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License (CC BY-NC-ND).

The exceptional high amount of minerals found in cocoons such as calcium, potassium, phosphorous, and copper are another strength of this material that may support health and growth of *L. vannamei* (Chapter 2.3). Additionally, the low moisture content in cocoons of 30% prevents the organic material of perishing rapidly, enables short-term storage, and facilitates drying.

As shown in Chapter 2.5, dietary supplementation of cocoons can result in improved survival of shrimp. Supplementing diets that contain high levels of plant-based ingredients with 5% cocoons increased average shrimp survival by more than 40% when raised in a clear water recirculating aquaculture system (RAS). This insect residue can thus be used to formulate feeds that are specifically designed for RAS culture systems, which are increasingly gaining relevance in shrimp production, especially in industrialized countries (Euroshrimp, 2021; Röthig et al., 2023). However, further research is needed to validate the observed effects.

3.4.2 Weaknesses of Black Soldier Fly Cocoons

The digestibility trial described in Chapter 2.3 showed that most of the nutrients in cocoons are not bioavailable. Apparently 35% of the protein and only 25% of the carbon is digested by *L. vannamei*. Unprocessed cocoons, as used in this study, are thus not a suitable source of macronutrients. Soetemanns et al. (2020) found that chitin from cocoons has a more crystalline structure with a higher thermal degradation temperature than chitin from other BSF life-stages. Presumably, the nutrients bound or integrated to the rigid sclerotized structure of cocoons are not easily accessible for enzymatic degradation during the digestion process in shrimp. Chitin extracted from BSF cocoons also has lower water-holding and emulsifying capacities compared to commercial chitin and chitin from other insect species (Ndiritu et al., 2023). These properties may also have contributed to the low overall nutrient availability and the reduced growth of shrimp by more than 23% observed during the biofloc trial (Chapter 2.5). The fact that cocoons can impede the overall growth performance of shrimp is a central weakness of this raw material. Furthermore, investigations of physiological parameters of shrimp could not explain the increased survival seen in the RAS-trial, nor was this effect seen in the biofloc system. Therefore, substantial uncertainties remain concerning the beneficial effects of cocoons, which have to be addressed before this raw material can be recommended as a feed additive.

3.4.3 Opportunities of Black Soldier Fly Cocoons

Chemical or physical pre-treatment methods might be useful to break down the rigid molecular structures of cocoons to increase its nutritional value. To access protein from feathers, horns, or wool, the indigestible keratin structure can be broken by acid or base treatment, thermal treatment, or enzymatic hydrolysis (Eslahi et al., 2013; Martínez-Alvarez et al., 2015; Mokrejs et al., 2011; Zhang et al., 2014). For instance, hydrolyzing feather meal greatly improves the bioavailability of protein, making it suitable for use in feeds for fish and shrimp (Bureau et al., 1999; Bureau et al., 2000; Mendoza et al., 2001). Treatment with chemical agents or harsh physical conditions disrupts organic material nonspecifically, while enzymatic hydrolysis offers a more precise and controllable molecular breakdown (Callegaro et al., 2019). Commercial enzyme products, such as Alcalase®, Corolase® and Flavourzyme®, are available on the market that are typically used for hydrolyzing rest raw materials (Lindberg et al., 2021). These products are a mixture of different endo-, and exopeptidases of microbial origin that could break up the sturdy protein structures of cocoons. Chitinases and chitin deacetylases can be added to liberate chitin-bound proteins and degrade long chitin chains into shorter and water-soluble oligomers (Kaczmarek et al., 2019). Furthermore, hydrolysates made from insects can exhibit a range of bioactive properties, including antioxidant characteristics (Nongonierma & FitzGerald, 2017).

Controlled hydrolysis thus offers opportunities to increase the nutritional value and promote immune stimulating effects of cocoons.

3.4.4 Threats Related to Black Soldier Fly Cocoons

Cocoons contain the highest amounts of chitin among all the tested by-products in this study and are thus prone to be the most competitive source for this versatile natural polysaccharide. Methods for the efficient extraction of chitin from BSF cocoons have been developed and described for its use in several pharmaceutical applications (Artilia et al., 2023; Hahn et al., 2022; Ndiritu et al., 2023; Wu & Wang, 2018). The growing volume and diversity of insect materials that become available through mass productions enabled the discovery of multiple applications, which has only just begun. Therefore, sophisticated high-value applications could potentially limit the viability of cocoons and other insect materials as aquaculture feed ingredients.

3.5 Algae Pomace

3.5.1 Strengths of Algae Pomace

The aqueous extraction residue of *Saccharina latissima* has the potential to increase shrimp survival in clear water conditions with high plant containing feed formulations as demonstrated in Chapter 2.5. Macroalgae contain a range of bioactive molecules with proven beneficial effects on shrimp (Chen et al., 2016; Esquer-Miranda et al., 2016; Immanuel et al., 2012; Rezende et al., 2021). Water-soluble compounds were presumably removed during the aqueous extraction of *S. latissima*. However, a fraction of bioactive compounds, likely associated with the lipid fraction, apparently remained in the pomace and promoted shrimp survival during the growth experiment in the recirculating clear water system.

Aside this promising nutritional aspect, dominant strengths of algae pomace are the vast environmental benefits that characterize this resource as a sustainable feed ingredient. Kelp species are among the most efficient primary producers, showing even higher productivity than terrestrial crops (Gao & McKinley, 1994). The contribution of macroalgae to global CO_2 capture is significant (Pessarrodona et al., 2023). Compared to terrestrial crops, seaweed production does not require arable land, freshwater, and expensive fertilizers (Gao & Beardall, 2022; Wan et al., 2019). By taking up inorganic nutrients, macroalgae provide crucial ecosystem services and help to ameliorate eutrophic coastal areas (Neveux et al., 2018). Macroalgae farms can mitigate ocean acidification on a local scale by interfering with the dissolved inorganic carbon system and raising the pH (Xiao et al., 2021). Natural seaweed beds, and to some extent seaweed farms, provide habitat for many marine organisms and support marine biodiversity (Almanza & Buschmann, 2013; Forbes et al., 2022). These environmental aspects of macroalgae are outstanding when considering the potential of algae pomace that contribute to the development of eco-friendly aquafeeds.

Approximately 37.8 million tons of algae were produced in 2022, with 99% of the biomass originating from macroalgae (FAO, 2024a). Approximately 30 to 38% of the total volume is used for direct human consumption, while 11% is used for other purposes such as agrichemicals and cosmetics (Chopin & Tacon, 2021; FAO, 2024a). In Europe, the share of macroalgae biomass used for cosmetics and wellness products is slightly greater with 17% (Araújo et al., 2021). In theory, this harvest yields a significant quantity of algae pomace (from multiple seaweed species) that could be available for aquaculture purposes. Alternative uses of algae pomace, such as fertilizer or energy production, do not create as much value as in animal feed (Figure 3.1). Economically, algae pomace could therefore represent an affordable feed additive for the aquaculture industry.

3.5.2 Weaknesses of Algae Pomace

Supplementing diets with algae pomace negatively affected growth of *L. vannamei* in clear water RAS and biofloc systems. As shown in Chapter 2.5, the reductions in growth were already evident at minimal inclusion levels (1%). These observations demonstrate that algae pomace is not a suitable feed ingredient for *L. vannamei*, at least not in the form used in the current study. Although algae pomace contains bioavailable protein and energy, the high amount of indigestible fibers presumably resulted in overall deficient nutrient uptake, elevated feed conversion ratios, and ultimately in reduced growth.

A further weakness of algae pomace is the natural variability in nutrient composition that poses challenges for standardized commercial utilizations (Buschmann et al., 2017; Diehl et al., 2023b; Wells et al., 2017). The chemical composition of *S. latissima* varies significantly depending on abiotic factors (temperature, salinity, nutrients, irradiance, currents, depth), season, and geographical location (Sæther et al., 2024). Presumably, the harvest time and location of *S. latissima* used for cosmetic purposes has been chosen to yield adequate concentrations of the compounds of interest (e.g. hydrocoloids). How this in turn affects the variability and contents of other chemical compounds (proteins, lipids, bioactive compounds) in the extraction residue remains unknown. This uncertainty presents a risk when considering algae pomace as feed ingredient or feed additive, which requires further investigation.

A safety concern in the use of seaweed is the high concentration of heavy metals, such as cadmium and arsenic, that it accumulates (FAO, 2022; Shaughnessy et al., 2023). Due to the biochemical properties of the polysaccharides present in the cell walls of the algae, heavy metals can be chelated and sequestrated from the environment (Davis et al., 2003).

The low technological development of macroalgae production is a central constraint of this sector (Araújo et al., 2021). Novel technologies with a high degree of automation throughout the nursery-, deployment-, grow-out-, harvesting-, and processing phase are needed to enable large-scale and cost-effective cultivation (Sæther et al., 2024). Furthermore, effective measures against epibionts, grazers, and algal diseases are lacking to maintain high productivity and quality of seaweeds (Stévant et al., 2017).

The harvest season of *S. latissima* is typically spring and early summer. Later in the year, biofouling of the macroalgae increases and compromises its quality (Sæther et al., 2024). The availability of fresh and plenty seaweed biomass is therefore seasonal. This poses challenges for aquafeed manufacturers as they require a steady and year-round supply of ingredients. Long term storage of stabilized material is an option. However, this could alter its quality and is an additional cost.

Algae pomace contains approximately 93% water and is an easily perishable raw material that requires quick stabilization after its production. Freezing and drying are the most common methods to prevent rapid deterioration of seaweeds (Thomas et al., 2021). Drying should be ideally done close to the location of production. Otherwise, transportation costs and associated emissions would increase markedly with the transport of (algae) water. Within the first hours of drying, the surface of the algae pomace formed a dry and rigid layer that hindered the complete drying of the material. The material had to be mixed regularly to allow thorough desiccation. The entire drying process took up to 48 h in this study (Chapter 2.5). Seaweeds contain hygroscopic polysaccharides that prevent desiccation if exposed to air at e.g. low-tide, which probably caused the observed effect during the drying process (Percival, 1979). These practical aspects of the drying of algae pomace could cause challenges in an industrial upscaling process. Drying is one of the most energy intensive processes and can significantly add to production costs and emissions during seaweed processing (Thomas et al., 2021; Wan et al., 2019).

3.5.3 Opportunities of Algae Pomace

The variable nutrient content of seaweeds poses challenges for potential applications, but also brings the opportunity to selectively harvest seaweeds with the chemical composition of interest. Under controlled culture conditions, the exposure to specific abiotic conditions could be useful to actively modulate the nutrient composition of macro algae (Wan et al., 2019). Although cosmetics is the primary purpose of the algae extraction, the selection for specific nutrient contents could also match the needs for further down-stream applications such as aquaculture feeds.

The low nutritive value of algae pomace is probably related to the high content of complex indigestible carbohydrates. Microbial fermentation or enzymatic hydrolyses could be used to break down fibers into shorter oligo-, or monosaccharides. The predominant polysaccharides in *Laminaria* spp. are alginate and laminarin (Bäumgen et al., 2021; Kadam et al., 2015; Schiener et al., 2015). These are composed of several distinct sugar monomers including glucose, galactose, mannuronic acid, and guluronic acid. The depolymerization of these structurally complex polysaccharides requires a set of specific enzymes. Several marine microorganisms and carbohydrate-active enzymes have recently been discovered that can decompose these algal carbohydrates (Bäumgen et al., 2021). The reduction of indigestible fibers could enhance the nutritional value and revoke the negative impact of algae pomace on growth of *L. vannamei*. Additionally, the mono- and oligomers resulting from the degradation of these polysaccharides could also have beneficial effects on the digestive microbiome and immune system of shrimp (Ramnani et al., 2012; Wan et al., 2019).

Spillias et al. (2023) found that approximately 650 million hectares of the global ocean could be suitable for macroalgae aquaculture, which represents 1.8% of the world’s total ocean. This area could support the production of about 6.5 billion tons of seaweed biomass, demonstrating the tremendous growth potential of this up striving industry (Spillias et al., 2023).

Along with increasing consumer awareness for food and the environmental aspects associated with its production, inclusion of sustainable seaweed ingredients such as algae pomace in aquafeeds could help to build a positive image on sustainable aquaculture production (Wan et al., 2019).

3.5.4 Threats Related to Algae Pomace

In Europe, the majority of the harvested seaweed biomass originates from wild stocks (Araújo et al., 2021). The origin of *S. latissima* used in this study is unknown, and it may have been collected from the wild. Increased collection of wild stocks raises concerns about overexploitation and possible ecological damage (Hughes et al., 2013). In the past, harvesting of wild *Gelidium* spp. for the production of microbiology-grade agar led to overexploited stocks in Morocco and Japan (Fujita et al., 2006; Givernaud et al., 2005). Despite the environmental benefits described in Chapter 3.5.1, the utilization of algae pomace originating from unsustainable and poorly managed sources could threaten coastal ecosystems, thereby diminishing the aspects of an eco-friendly feed ingredient.

The consequences of climate change on marine seaweed forests and farms are largely unclear. Rising CO_2 concentrations and acidified oceans could even result in increased macroalgae growth (Young & Gobler, 2016). However, rising sea temperatures and changes in hydrodynamic regimes are key issues for macroalgae cultivation, especially in areas where species are farmed close to the boundaries of their natural distribution (Stévant et al., 2017). In the case of *S. latissima*, marine heatwaves are presumably responsible for the reductions in abundance along the East and West coast of the North Atlantic (Filbee-Dexter et al., 2020). A northward shift of the distribution of wild *S. latissima* was already observed and this trend is expected to be more pronounced in future (Diehl et al., 2023a). Since marine seaweed farms are inevitably exposed to the environment and changing abiotic factors, climate change represents a fundamental threat to this sector.

3.6 Summarizing the Potential of the Tested By-Products

This study demonstrated that locally available by-products in northern Germany are suitable as aquafeed for *L. vannamei* (Research question 1). The inclusion of these raw materials in well-balanced aquafeeds is investigated and discussed in Chapters 2.2 -2.5 (Research question 2). To assess the potential of these by-products in view of circular bioeconomic principles in the aquatic food system, a holistic consideration of multiple influencing factors is presented in Chapters 3.1 – 3.5 (Research question 3). For each by-product, certain strengths, weaknesses, opportunities, and threats have been identified that distinctively affect their potential as aquaculture feed. The unique characteristics and different purposes as feed ingredients complicate direct comparisons. For example, adult black soldier flies and brown shrimp processing remains were investigated as dietary protein source at maximal inclusion rates of 26 to 36% of the diet. On the contrary, cocoons and algae pomace were included as functional feed additives at only 1 to 5% of the diet. Thus, both feed ingredient categories are required in different amounts and are thus subjected to distinct biomass streams, market values, and environmental impacts. This chapter aims to summarize and highlight the major factors. To provide an overview for each characteristic (strength, weakness, opportunity, threat), all arguments listed for each by-product throughout Chapters 3.2 - 3.5 were scored based on their estimated relevance and added together (Figure 3.3). A detailed description of scoring criteria is provided in the supplements (S2).

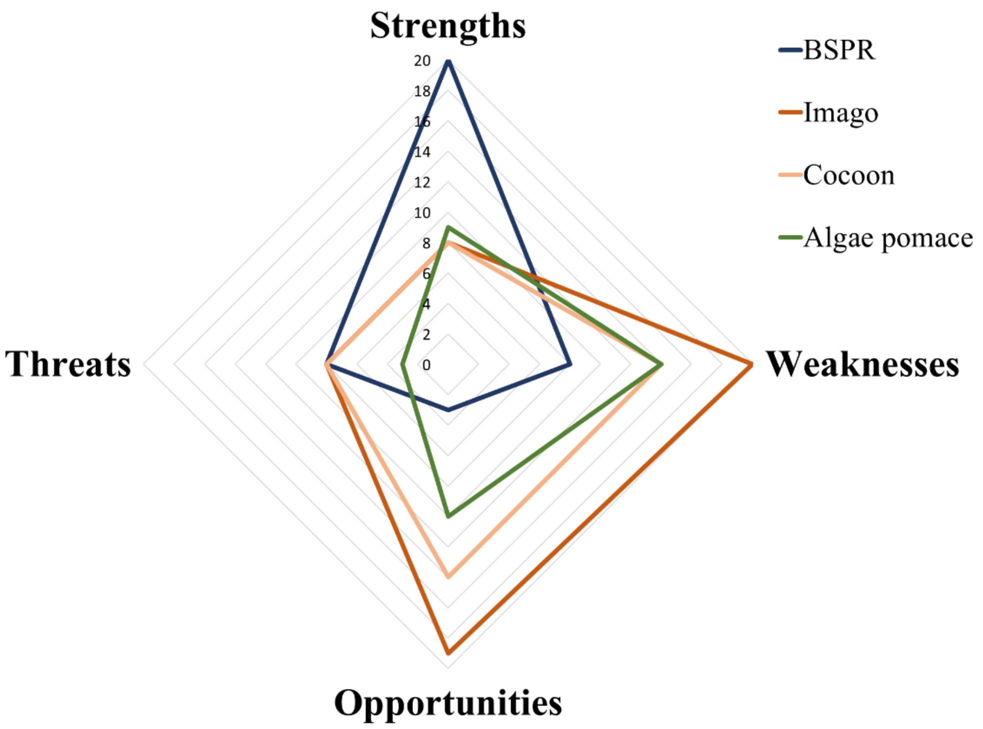

Figure 3.3. Radar chart depicting the scores regarding the strengths, weaknesses, opportuinities and threats related to each evaluated by-product. BSPR= Brown shrimp, *Crangon crangon* processing remains; Imago= Adult black soldier flies, *Hermetia illucens*; Cocoon= Cocoons of the black soldier fly, *Hermetia illucens*; Algae pomace= aqueous extraction residue of *Saccharina latissima*. Detailed information on the scores and the scoring criteria are provided in the supplements (S2).

Brown shrimp processing remains are clearly a suitable feed ingredient for *L. vannamei*. The nutritive value is optimal, the mature industry ensures stable and considerable supply and the resource is not subject to any legislative constraints. These positive aspects lead to the pronounced strengths of BSPR with a total score of 20. However, lack of scalability and uncertain future scenarios pose a limit for a widespread utilization. Landings of brown shrimp are unlikely to increase, and amounts of BSPR will not be sufficient to meet the demands of the global shrimp feed sector. Therefore, the opportunities are limited, as indicated by a score of 3. Additionally, the environmental issues of this bottom trawling fishery and the overarching threat of climate change could result in a collapse of this industry (score of 8 for Weaknesses and Threats).

The other three tested ingredients (imago, cocoon, algae pomace) originate from industries that are not fully developed. Although the technology for insect and seaweed farming are rapidly advancing, innovative solutions are still needed along the entire production chain. Furthermore, the nutritional values of imago, cocoons, and algae pomace are not adequate, and without further optimization, these ingredients cannot be recommended as feed ingredients for *L. vannamei*. As such, the weaknesses (scores of 14 - 20) overrule the strengths (score of 8 - 9) of these resources in the current situation. However, the future growth potential of these sectors, the opportunities for the optimization of the raw ingredients (extraction, enzymatic hydrolysis), and their application (modified feed formulations) could make these resources valuable candidates in the future. Especially in light of the climate crises, insect and seaweed farming, and thus their by-products, bear great potential to contribute to a resilient and sustainable food production system. The opportunities for insect and macroalgae by-products are therefore more pronounced (scores of 10-19).

In European countries including Germany, Austria, and the UK, the production of *L. vannamei* in land-based intensive aquaculture is a growing segment (Euroshrimp, 2021). The results discussed in this thesis show that industries in northern Germany generate by-products that have the potential to serve as aquafeed for *L. vannamei* raised in these systems. Prioritizing local economies for the acquisition of feed ingredients avoids long transportation routes and may help to increase the resilience of the aquatic food system. Geopolitical conflicts, the COVID-19 crisis, and recent maritime incidents in critical shipping routes have demonstrated the vulnerability of supply chains and the risks associated with globally traded commodities (Jagtap et al., 2022; Singh et al., 2021; Wu et al., 2019). The amount of BSPR that is annually generated from the North Sea regions could meet the total protein demand for the entire current land-based shrimp production in Europe (Chapter 2.1). First characterizations and attempts to include by-products of novel industries in shrimp feeds are presented, providing essential baseline information for further investigations (Chapter 2.3- 2.5). The results of this thesis thus contribute to the development of a sustainable, resilient, and circular aquaculture. Principles of the “Farm to Fork” and bioeconomy strategy of the European Union are met by reducing waste and improving the utilization efficiency of natural resources. Findings of this work directly contribute to the second (zero hunger) and 12th Sustainable Development Goal (sustainable consumption and production patterns) of the United Nations by reducing feed-food competition and addressing responsible production (UN General Assembly, 2015). Indirectly this work contributes to the protection of the climate (13th SDG), life below water (14th SDG) and life on land (15th SDG). Although the focus of this study and availability of these side streams is regional, they can serve as "light house" raw materials that demonstrate the potential of by-products that are generated worldwide.

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Supplements

Table S1. By-products generated throughout the food producing system and exemplary scientific reports on their use as aquafeed ingredients. By-products are grouped according to their origin and amounts generated per year on a global scale.

Origin	**Amount** [1]	**Group**	**Feedstuff**	**Treatment**	**Nutrient**	**Inclusion** [2]	**Species**	**References**
Crop residues	1980	Straw	No information [3]					
		Leaves	Cassava	Untreated	Protein	Medium	*Clarias gariepinus*	Falaye et al. (1999)
			Wild Tamarin	Soaked in water	Protein	Medium	*Penaeus monodon*	Vogt et al. (1986)
		Stalks	Mushroom	Hot water extract	Additive	Low	*Oreochromis niloticus*	Ahmed et al. (2017)
		Roots	No information					
		Peels, husks	Banana	Untreated	Additive	Low	*Labeo rohita*	Giri et al. (2016)
			Banana	Fermented	Protein	Medium	*Oreochromis* spp.	Intharathat et al. (2024)
			Banana	Hot water extract	Additive	Low	*Macrobrachium rosenbergii*	Rattanavichai and Cheng (2015)
			Orange	Untreated	Additive	Low	*Oreochromis niloticus*	Vicente et al. (2019)
			Lemon	Extracted oil	Additive	Low	*Oreochromis mosambicus*	Baba et al. (2016)
			Apple	Untreated	Additive	Low	*Oreochromis niloticus*	Qiang et al. (2019)
			Apple	Extract	Additive	Low	*Cyprinus carpio*	Hoseinifar et al. (2021)
			Corn silk	Untreated	Additive	Low	*Oreochromis niloticus*	Catap et al. (2015)
			Peanut	Untreated	Protein	High	*Oreochromis niloticus*	Agbo et al. (2011)
			Bambara nut	Untreated	Protein	High	*Clarias gariepinus*	Enyidi and Mgbenka (2015)
Crop processing	543	Cereal bran	Maize	Untreated	Carbohydrate	-	*Cyprinus carpio*	Fagbenro (1999)
			Sorghum	Untreated	Carbohydrate	-	*Cyprinus carpio*	Fagbenro (1999)
			Rice	Untreated	Carbohydrate	-	*Cyprinus carpio*	Fagbenro (1999)
			Wheat	Untreated	Carbohydrate	Medium	*Litopenaeus vannamei*	Vinasyiam et al. (2023)
			Wheat	Fermented	Carbohydrate	Medium	*Oreochromis niloticus*	Mohammady et al. (2023)

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Origin	Amount [1]	Group	Feedstuff	Treatment	Nutrient	Inclusion [2]	Species	References
		Molasses	No information					
		Oilseed press cake	Soy	Untreated	Protein	Medium	*Litopenaeus vannamei*	Yao et al. (2020)
			Soy	Untreated	Protein	High	*Lateolabrax japonicus*	Zhang et al. (2018)
			Soy	Heat	Protein	Medium	*Oncorhynchus kisutch*	Arndt et al. (1999)
			Soy	Cooked	Protein	High	*Oreochromis niloticus*	Wee and Shu (1989)
			Soy	Fermented	Protein	High	*Oncorhynchus mykiss*	Barnes et al. (2014)
			Soy	Fermented	Protein	Low	*Litopenaeus vannamei*	Yao et al. (2020)
			Soy	Solvent-extracted	Protein	High	*Litopenaeus vannamei*	Sookying and Davis (2012)
			Canola	Not specified	Protein	Medium	*Oreochromis* spp.	Zhou and Yue (2010)
			Canola	Acid and heat	Protein	High	*Oreochromis niloticus*	Soltan (2005)
			Rapeseed	Untreated	Protein	Low	*Oreochromis mossambicus*	Davies et al. (1990)
			Rapeseed	Fermented	Protein	High	*Litopenaeus vannamei*	Jannathulla et al. (2019)
			Peanut	Untreated	Protein	High	*Oreochromis niloticus*	Agbo et al. (2011)
			Peanut	Fermented	Protein	High	*Labea rohita*	Ghosh and Mandal (2015)
			Cotton	Untreated	Protein	High	*Oreochromis sp.*	Mbahinzireki et al. (2001)
			Cotton	Protein concentrate	Protein	Medium	*Litopenaeus vannamei*	Wang et al. (2022)
			Cotton	Fermented	Protein	High	*Litopenaeus vannamei*	Han et al. (2022)
			Cotton	Solvent-extracted	Protein	High	*Oncorhynchus mykiss*	Luo et al. (2006)
			Olive	Untreated	Additive	Low	*Oncorhynchus mykiss*	Hoseinifar et al. (2020)
		Fruit & vegetable pomace	Apple	Fermented	Protein	High	*Oreochromis niloticus*	Vendruscolo et al. (2009)
			Apple	Extracted polyphenols	Additive	Low	*Ctenopharyngodon idella*	Yang et al. (2021)
			Grape	Untreated	Not defined	Medium	*Oncorhynchus mykiss*	Peña et al. (2020)
			Grape	Untreated	Additive	Low	*Ctenopharyngodon idella*	Baldissera et al. (2019)

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Origin	Amount [1]	Group	Feedstuff	Treatment	Nutrient	Inclusion [2]	Species	References
			Tomato	Untreated	Not defined	High	*Cyprinus carpio*	Amirkolaie et al. (2015)
			Olive	Untreated	Lipid	Low	*Sparus aurata*	Nasopoulou et al. (2011)
			Olive	Untreated	Lipid	Low	*Dicentrarchus labrax*	Nasopoulou et al. (2011)
			Olive	Untreated	Not defined	Medium	*Acipenser baerii*	Banavreh et al. (2019)
			Pineapple	Untreated	Carbohydrate	Medium	*Clarias gariepinnus*	Oduntan et al. (2022)
			Papaya	Fermented	Protein	High	*Litopenaeus vannamei*	Kang et al. (2010)
Livestock	63	Meat, bone	Mixed	Heat	Protein	Medium	*Oncorhynchus mykiss*	Bureau et al. (2000)
			Poultry	Not specified	Protein	High	*Litopenaeus vannamei*	Cruz-Suárez et al. (2007)
		Blood	Not specified	Acid	Protein	Low	*Litopenaeus vannamei*	Dominy and Ako (1988)
			Pork	Not specified	Protein	Low	*Mylopharyngodon piceus*	Twahirwa et al. (2021)
		Feathers	Mixed	Heat and pressure	Protein	Medium	*Oncorhynchus mykiss*	Bureau et al. (2000)
			Not specified	Not specified	Protein	Low	*Litopenaeus vannamei*	Cheng et al. (2002)
		Viscera (liver)	Pork	Enzyme hydrolysis	Protein	Low	*Steindachneridion melanodermatum*	Lewandowski et al. (2013)
Food & Beverage processing	>28	Food processing	Cookie	Untreated	Carbohydrate	Medium	*Litopenaeus vannamei*	Cruz-Suarez et al. (1994)
			Pasta	Untreated	Carbohydrate	Medium	*Litopenaeus vannamei*	Cruz-Suarez et al. (1994)
		Fermentation	Distillery grain	Untreated	Protein	Medium	*Litopenaeus vannamei*	Novriadi et al. (2022)
			Distillery grain	Enzyme hydrolysis	Protein	Medium	*Litopenaeus vannamei*	He et al. (2020)
			Distillery yeast	Untreated	Protein	High	*Oreochromis niloticus*	Nhi et al. (2018)
			Distillery yeast	Untreated	Protein	Medium	*Macrobrachium rosenbergii*	Nguyen et al. (2019)
			Bakery yeast	Enzyme hydrolysis	Additive	Low	*Oreochromis niloticus*	Hassaan et al. (2018)
Fisheries, Aquaculture	10	Carcass	Tuna (head)	Enzyme hydrolysis	Protein	High	*Litopenaeus vannamei*	Nguyen et al. (2012)
			Pollock	Enzyme hydrolysis	Additive	Low	*Scophthalmus maximus*	Zheng et al. (2013)
			Tilapia	Enzyme hydrolysis	Protein	Low	*Steindachneridion melanodermatum*	Lewandowski et al. (2013)

(continued on next page)

Origin	**Amount** [1]	**Group**	**Feedstuff**	**Treatment**	**Nutrient**	**Inclusion** [2]	**Species**	**References**
			Salmon	Enzyme hydrolysis	Additive	Low	*Litopenaeus vannamei*	Grey et al. (2009)
		Viscera	Squid	Untreated	Protein	Medium	*Litopenaeus vannamei*	Navarro et al. (2020)
			Scallop	Untreated	Protein	Medium	*Litopenaeus vannamei*	Navarro et al. (2020)
			Not specified	Cooked, defatted	Protein	High	*Clarias gariepinus*	Jimoh et al. (2022)
			Tuna	Enzyme hydrolysis	Additive	Low	*Lates calcarifer*	Chotikachinda et al. (2013)
		Blood	Not specified	Untreated	Protein	Low	*Litopenaeus vannamei*	Pranama et al. (2018)
		Shrimp head	Not specified	Untreated	Protein	High	*Litopenaeus schmitti*	Jaime-Ceballos et al. (2009)
			Pacific Whiteleg	Untreated	Protein	Medium	*Lutjanus guttatus*	Osuna-Salazar et al. (2023)

[1] Theoretical available amount globally in million tons, based on Sandström et al. (2022).

[2] Inclusion levels tested in aquaculture diets with low < 10%; medium = 10 - 25%; and high > 25%.

[3] No information was found regarding the use of this group of by-products as aquaculture feed ingredient.

Supplements 2 (S2)

To summarize the potential of the different by-products, each argument listed throughout Chapters 3.2 – 3.5 was scored and combined to obtain an overarching value of each material for the respective category (Strength, Weakness, Opportunity, Threat). Arguments were scored on a scale from one to three (1 = low relevance, 2 = medium relevance, 3 = high relevance). For example, the macronutrient profile of brown shrimp processing remains (BSPR) received a score of 3 due to its well-balanced protein, lipid, and amino acid contents. Adult black soldier flies received a score of 2 for their macronutrient profile because of excessive lipid amounts and an amino acid profile that did not completely match the ideal dietary EAA profile for penaeid shrimp. Other arguments, such as the relevance of environmental impacts and technological innovations, were more difficult to assess. The scores are based on personal judgment of each argument's importance, is not an absolute value, and should be considered a careful estimate. The objective was to present an overview of the potential by summarizing all the listed arguments and visually presenting them in a radar chart.

S2. Arguments of the four different categories (strength, weakness, opportunity, threat), the designated score of each argument (1 = low relevance, 2 = medium relevance, 3 = high relevance), and the resulting sum of the category.

By product:	**Brown shrimp, *Crangon crangon* processing remains (BSPR)**	
Category	**Argument**	**Score**
Strength	Macronutrient profile	3
	Bioavailability	3
	Presence of EPA, DHA	2
	Micronutrients (Copper, chitin, minerals)	2
	Growth promoting	3
	Preservation (Cooking)	1
	Low contamination	2
	Mature industry	3
	Moderate price	1
	Sum	***20***
Weakness	Cooking reduces nutrient content	1
	Stagnating and variable catches	2
	Negative ecological impact	3
	High emissions	2
	Sum	***8***

(*continued on next page)*

Opportunity	Growth factor	2
	Technological innovations	1
	Sum	***3***
Threat	Competition from other sectors	2
	Future of fishery uncertain	3
	Climate change	3
	Sum	***8***

By product:	**Adult black soldier fly, *Hermetia illucens* (imago)**	
Category	**Argument**	**Score**
Strength	Macronutrient profile	2
	Micronutrients	2
	Low moisture content	1
	Good environmental footprint	2
	No exploitation of wild stocks	1
	Sum	***8***
Weakness	Reduces growth	3
	Low palate	2
	Low bioavailability	2
	High and unsuitable lipid content	2
	High biomass requirement	3
	High energy demand	2
	Not developed industry	2
	High price	2
	Regulatory framework	2
	Sum	***20***
Opportunity	Pre-processing	2
	Modulate nutrient profiles through substrate	2
	Re-introduce nutrients into human food system	3
	Reduced CO_2 emissions	2
	Regulatory changes to unlock potential	2
	Positive consumer perception	2
	Controlled production, steady supply	2
	Controlled production mitigates contamination	1
	Resilient to extreme climate	2
	Use of renewable energy	1
	Sum	***19***

Threat	Competition from other sectors	1
	Competition for biomass	2
	Risk of contamination (unhygienic biomass streams)	1
	High energy prices	2
	Welfare – mass rearing of insects	1
	Invasive potential	1
	Sum	***8***

By product:	**Cocoons of the black soldier fly, *Hermetia illucens***	
Category	**Argument**	**Score**
Strength	Macronutrient profile	1
	Micronutrients	2
	Low moisture content	1
	Good environmental footprint	2
	No exploitation of wild stocks	1
	Can increase survival	1
	Sum	***8***
Weakness	Low bioavailability	2
	Can reduce growth	2
	High biomass requirement	2
	High energy demand	2
	Not developed industry	2
	High price	2
	Regulatory constraints	2
	Sum	***14***
Opportunity	Pre-processing	3
	Re-introduce nutrients into human food system	1
	Regulatory changes to unlock potential	2
	Positive consumer perception	2
	Controlled production, steady supply	2
	Controlled production mitigates contamination	1
	Resilient to extreme climate	2
	Use of renewable energy	1
	Sum	***14***
Threat	Competition from other sectors	2
	Competition for biomass	1
	Risk of contamination (unhygienic biomass streams)	1
	High energy prices	2
	Welfare – mass rearing of insects	1
	Invasive potential	1
	Sum	***8***

By product:	**Algae pomace, *Saccharina latissima***	
Category	**Argument**	**Score**
Strength	Can increase survival	1
	Bioactive compounds	1
	Primary producer, photoautotroph	2
	Ecological benefits	3
	Availability / Price	2
	Sum	***9***
Weakness	Can reduce growth	3
	Low bioavailability	2
	High natural variability	2
	Risk of contamination	1
	Low technological development	2
	Seasonal variability	2
	High water content	2
	Sum	***14***
Opportunity	Modulation of nutrient profile	2
	Pre-processing	3
	Large scalability	3
	Support positive aquaculture image	2
	Sum	***10***
Threat	Could contribute to exploitation of wild stocks	1
	Production affected by climate change	2
	Sum	***3***

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