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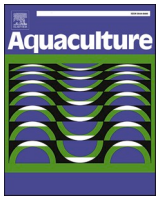

Brown shrimp (*Crangon crangon*) processing remains enhance growth of Pacific Whiteleg shrimp (*Litopenaeus vannamei*)

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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:

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

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

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):

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

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

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

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

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

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:

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

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

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

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):

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

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 (dE_{366}·min^{-1}·$mg^{-1}_{protein}$).

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 $\lambda_{ex} = 355$ nm and $\lambda_{em} = 460$ 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 (U·$mg^{-1}_{protein}$).

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 $g \cdot kg^{-1}$ "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 ($MJ \cdot kg^{-1}$)	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 ($g \cdot kg^{-1}$ 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).
[11,12] 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 $L \cdot h^{-1}$ 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; $mS \cdot cm^{-1}$: 33.1 ± 1.1; NH_4^+: 0.11 ± 0.08 $mg \cdot L^{-1}$; NO_2^-: 0.23 ± 0.17 $mg \cdot L^{-1}$; NO_3^-: 85.13 ± 35.14 $mg \cdot L^{-1}$).

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, Männedorg, 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 U·mg$^{-1}_{protein}$ to 0.06 ± 0.01 U·mg$^{-1}_{protein}$ and 0.04 ± 0.01 U·mg$^{-1}_{protein}$ to 0.07 ± 0.03 U·mg$^{-1}_{protein}$. Chitinase activities were more variable with average values ranging from 29.1 ± 13.0 U·mg$^{-1}_{protein}$ to 57.5 ± 13.5 U·mg$^{-1}_{protein}$ 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 U·mg$^{-1}_{protein}$ to 110 ± 43 U·mg$^{-1}_{protein}$. 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

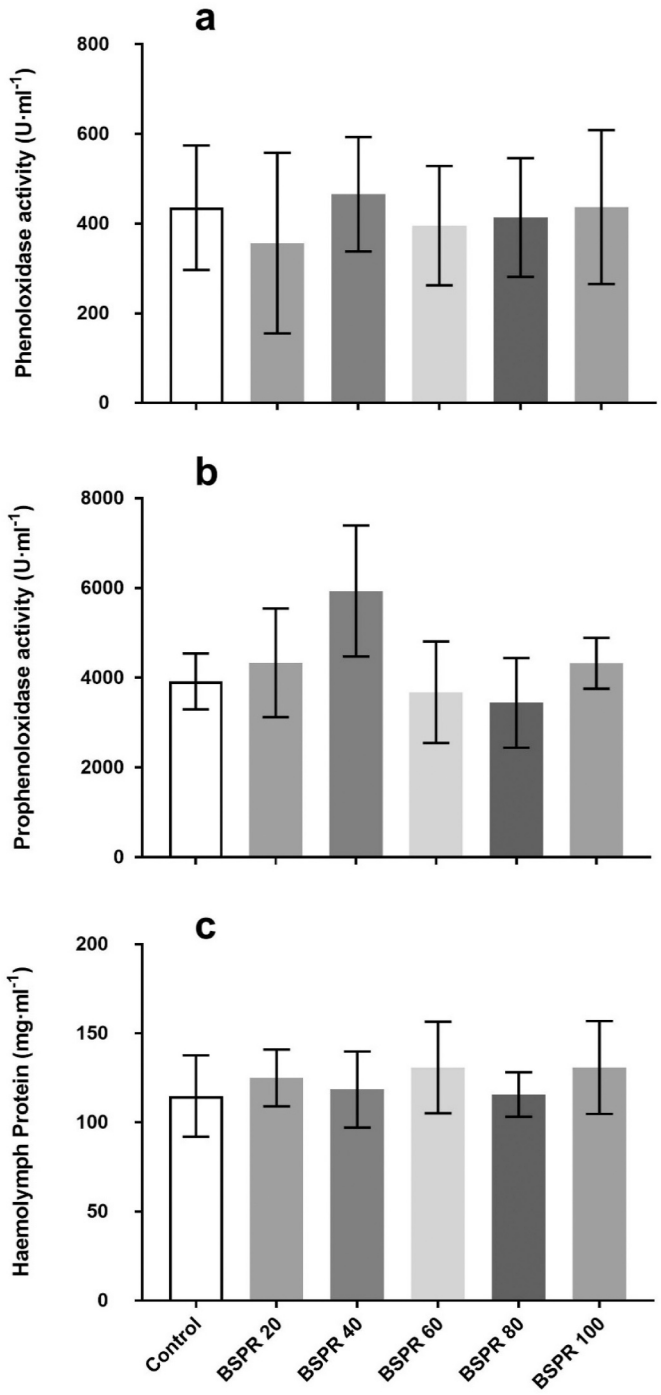

Fig. 1. Phenoloxidase, prophenoloxidase and haemolymph protein levels measured in *L. vannamei* feeding on the respective diets. Phenoloxidase and prophenoloxidase values are expressed as the mean ± sem and haemolymph protein as mean ± sd ($n = 6$). BSPR = Brown shrimp processing remains. (For interpretation 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

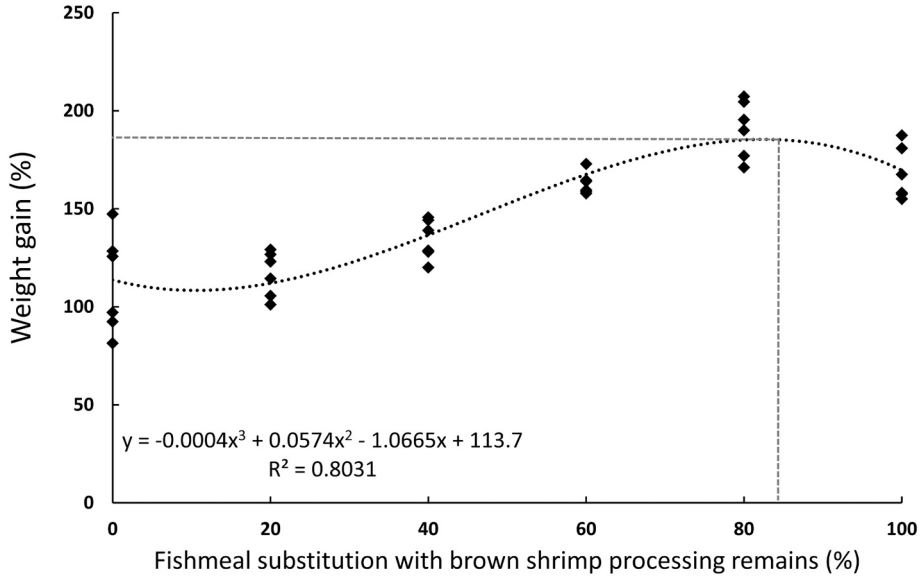

Fig. 2. Polynomial regression relating the fishmeal substitution with brown shrimp processing remains (%) and weight gain. All six data points per treatment were used for model fitting. Dashed lines indicate the local maximum (x = 85.2; y = 192.1). (For interpretation of the references to colour in this 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·kg^{-1}, unless otherwise indicated.

Essential amino acids [g·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$^{-1}_{protein}$]			
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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