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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 $g \cdot kg^{-1}$ crude protein, 74 $g \cdot kg^{-1}$ total lipid, 15 $MJ \cdot kg^{-1}$ 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 $g \cdot kg^{-1}$). 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

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[d] Alexander Müller GbR (Herzlake, Germany)
[e] Research Diet Services (Wijk bij Duurstede, Netherlands)
[f] Merck KGaA (Darmstadt, Germany)

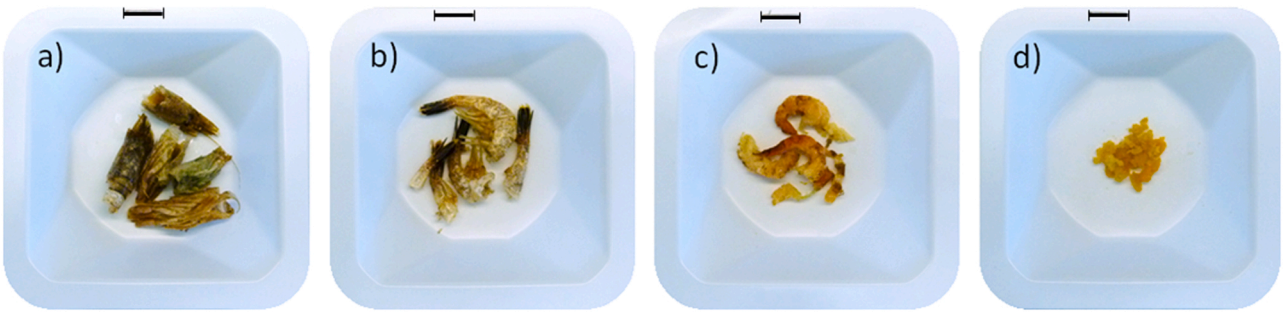

Fig. 1. Different body parts of *C. crangon* that remain after mechanical processing: a) cephalothorax, b) abdominal shell, c) abdominal muscle, and d) eggs. The scale indicates 1 cm.

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 Gefrier-trocknungsanlagen 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):

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

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):

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

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 ($g \cdot kg^{-1}$)
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 ($g \cdot kg^{-1}$)	
Calcium	90
Phosphorus	16
Potassium	7
Magnesium	3
Microminerals ($mg \cdot kg^{-1}$)	
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 ($mg \cdot kg^{-1}$)	
Arsenic	12.8
Led	< 0.2
Cadmium	0.16
Mercury	0.15
EPA-PAHs ($\mu g \cdot kg^{-1}$)	< 10
NDL-PCBs ($\mu g \cdot kg^{-1}$)	
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 ($g \cdot kg^{-1}$)
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 $MJ \cdot kg^{-1}$. The shrimp by-product meal analysed by Yang et al. (2009) showed a higher energy content of 21 $MJ \cdot kg^{-1}$, 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 $g \cdot kg^{-1}$ (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

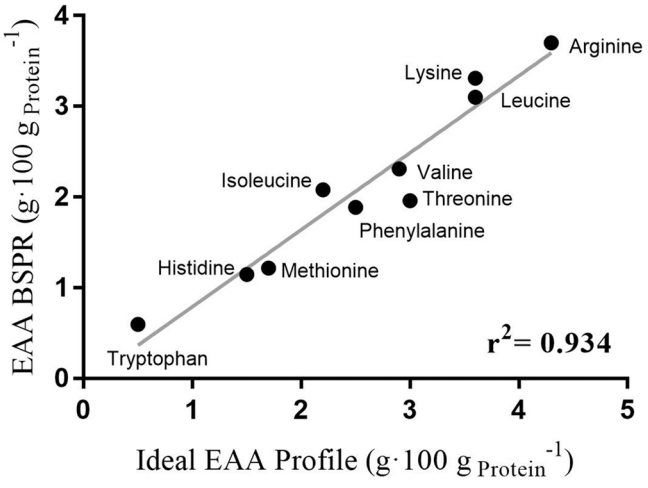

Fig. 2. Linear correlation of the ideal dietary essential amino acid profile for penaeid shrimp (NRC, 2011) and the essential amino acid content of brown shrimp processing remains (BSPR), expressed as $g \cdot 100\ g^{-1}_{Protein}$.

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 $g \cdot kg^{-1}$ on a dry matter basis. The cholesterol content of BSPR is thus in the upper range with 9 $g \cdot kg^{-1}$. 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 $mg \cdot kg^{-1}$, 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 $g \cdot kg^{-1}$ and 16 $g \cdot kg^{1}$, 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 $g \cdot kg^{-1}$ and 16.8 $g \cdot kg^{-1}$. 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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