

## Article

# Partial Replacement of Fishmeal with Seafood Discards for Juvenile *Penaeus japonicus*: Effects on Growth, Flesh Quality, Chemical and Fatty Acid Composition

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**Abstract:** The present study was designed to assess the influence of fishmeal replacement with increasing percentages of fresh seafood discards (0, 25, 50 and 75%) in diets for *Penaeus japonicus* on growth performance, physical characteristics and the chemical and fatty acid composition of shrimp flesh. Each diet was administered for 108 days, and the trial was assayed in triplicate. The final body weight and the specific growth rate were significantly higher ( $p < 0.05$ ) in shrimps fed with 75% fishmeal replacement as compared to 25%, while the abdomen weight and the total length were the highest ( $p < 0.01$ ). Moreover, 75% replacement showed a significantly ( $p < 0.05$ ) lower value of hardness and a greater crude protein and lipid content as compared to the 0% replacement. The shrimps fed with high levels of seafood discards (50 and 75%) showed a lower saturated fatty acid concentration and, in turn, a greater amount of polyunsaturated fatty acids in shrimp meat significantly ( $p < 0.05$ ) affected the lower atherogenic and thrombogenic indices. In conclusion, replacing 75% of fishmeal with seafood discards provided satisfactory results. An economic analysis based on ESG indicators and PESTLE methodology is provided in order to show the socio-economic and governance impacts affecting the replacement of shrimps' diet with fish discard.

**Keywords:** *Penaeus japonicus*; kuruma shrimp; flesh quality; fatty acid profile; seafood discards; fishmeal replacement; ESG impacts; PESTLE; AKIS

## Key Contribution:

- Fishmeal and fish oil are considered the most important ingredients in shrimp feed, but a substitution with fresh seafood discards may have a high potential in shrimp feeding.
- Fishmeal replacement with different levels (0, 25, 50, 75%) of fresh seafood discards was investigated in kuruma shrimp farming. Shrimp growth and flesh quality were improved following 75% fishmeal replacement.

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## 1. Introduction

The shrimp aquaculture industry has grown rapidly in recent decades and represents the most important aquaculture sector in Europe [1,2]. Consumer shrimp demand has increased in recent decades, leading fish nutrition experts to study the inclusion of vegetable by-products in aquatic animal feeding [3–5]. *Penaeus japonicus*, kuruma shrimp,

is one of the major shrimp species farmed in the seas [6] that is used in the human diet since, like other shrimp species, it is highly appreciated from a gastronomic point of view as it is a good source of macronutrients (proteins and, particularly, essential amino acids) and vitamins and minerals (such as vitamin B12, iron, calcium and zinc) [7]. Additionally, shrimps contain high levels of polyunsaturated fatty acids (PUFA), e.g., C20:5 n-3 (eicosapentaenoic, EPA) and C22:6 n-3 (docosahexaenoic, DHA) acids [8], considered to be essential for human health [9]. Japan was the first country to develop breeding system technology and it has since been transferred to China, Southeast Asia, India and Latin America. Over the last decade, various strategies have been employed to expand shrimp diets and address the global expansion of shrimp farming [10].

In shrimp feed, the most nutritionally balanced and digestible ingredients are fishmeal and fish oil [11]. However, their limited source and increasing prices encouraged fish nutritionists to test several alternatives in order to reduce fishmeal use [5,12,13]. In the shrimp diet, depending on the species, from 25 to 50% fishmeal has been used; over time, both animal [13,14] and plant-based ingredients [3,15] have been investigated as alternative for fishmeal. Nevertheless, poor digestibility, an unbalanced nutritional profile, antinutritional factors, palatability and attractiveness remain challenging issues. During the juvenile stages, dietary protein content is the primary factor affecting shrimp growth [16,17]. Growth of aquatic animals occurs when, in the tissue, the quantity of protein synthesis exceeds the level of proteolysis [18,19]; therefore, the identification of sustainable alternatives having nutritional properties similar to fishmeal is ongoing. With regards to sustainability, international guidelines have been established by the European Union and the Food and Agriculture Organization of the United Nations (FAO) to manage by-catch and reduce discards from low-value marine fishes. The minimization of discards and their valorization in fish feeding as high-quality fishmeal or as a source of valuable protein, peptones, enzymatic mixtures, fish oil and bio-compounds are very interesting due to their environmental impact and to the economic feasibility of fish feeding [20].

Seafood is defined as shellfish and fish found in estuarine, marine, freshwater and brackish ecosystems; the discards, including head, shell, guts, bones, skin, fins and other items, is the result of processing for the market [21]. Furthermore, seafood discards are rich in long-chain PUFAs, essential amino acids, pigments, peptides, vitamins (B3, A, B12, B6, D) and minerals, along with a variety of nutraceuticals, including lipids, and polysaccharide-based compounds [21]. Therefore, seafood discards may be considered as nutrients rich in bioactive compounds for shrimps. The addition of a low-cost fresh food, such as pilchard, anchovy, and mussel, to a dry feed may enhance either the growth or quality traits of post larvae, reducing feeding costs. According to the New Blue Economy Strategic Guidelines issued by the European Commission [22], aquaculture has to produce food and feed with a lower climate and environmental impact than other types of farming.

This study was designed to assess the effect of the replacement of fishmeal with different levels (0, 25, 50 and 75%) of seafood discards in diets for *Penaeus japonicus* on growth performance, physical characteristics and the chemical and fatty acid composition of shrimp meat. The effects are estimated by cross-cutting PESTLE methodology with ESG indicators in order to provide the cost benefits and impacts.

## 2. Materials and Methods

### 2.1. Animal Ethics

European animal welfare legislation does not regulate crustaceans [23]. However, the animals were kept and slaughtered under production conditions; after being immersed in ice, they were subjected to analysis. They were not subjected to any procedures during the experimental period, and all analyses were conducted post mortem.

## 2.2. Experimental Diets

In this trial, juvenile shrimps with an initial weight of 0.5 g underwent a period of acclimatization (4 weeks) to the pond environment; afterwards, a total of 720 shrimps weighing  $2.50 \pm 0.50$  g were assigned to the four isonitrogenous diets. The control diet contained 100% fishmeal, while the remaining three diets contained increasing levels of fishmeal replacement (25, 50 and 75%) by fresh seafood discards (SFs) based on a mixture of anchovy (*Engraulis encrasicolus*), pilchard (*Sardina pilchardus*) and mussel (*Mytilus galloprovincialis*) in the ratio 1:1:2 (Table 1). Discards were obtained by catching low-value fish and individuals of valuable commercial species below the minimum marketing size. The seafood discards were dehydrated at a low temperature ( $20 \pm 5$  °C) in a cool dryer to retain the chemical characteristics of the product, which were kept unaltered by removing water (Scubla Professional, Udine, Italy). In brief, in a vertical mixer, the feed ingredients, with additional vitamins and minerals, were thinly ground and mixed (M-750, Fanda, Zhengzhou, China); afterwards, fish oil was added and mixed. The diet clumped and formed a homogenous dough after slow addition of water. Then, the dough was extruded by a mill machine (SZLH-768 Shrimp Feed Pellet Making Machine, Richi machinery, Kaifeng, China) to obtain pellets with a diameter of 4 mm. For 24 h, the pellets were placed at room temperature to dry; then, they were broken and stored at  $-20$  °C. Each diet was assayed in triplicate.

**Table 1.** Ingredients, chemical and fatty acid composition in relation to different replacement levels of fishmeal with seafood discards.

Constituents (% as Fed Basis)	Replacement Levels of Fishmeal (%)				Fishmeal (FM)	Seafood Discards (SF)
	0	25	50	75		
Fishmeal	20.5	15.4	10.3	5.1	--	--
Fresh seafood	0	5.1	10.3	15.4	--	--
Corn gluten	10.3	10.3	10.3	10.3	--	--
Wheat meal	24.0	24.0	24.0	24.0	--	--
Soy protein concentrate	3.2	3.2	3.2	3.2	--	--
Wheat flour	35.1	35.1	35.1	35.1	--	--
Soybean oil	2.1	2.1	2.1	2.1	--	--
Fish oil	2.3	2.3	2.3	2.3	--	--
Premix <sup>1</sup>	2.5	2.5	2.5	2.5	--	--
Proximate composition (% on DM)						
Dry matter	94.20	94.35	94.10	94.07	91.50	93.10
Crude protein	43.30	43.32	44.38	44.73	74.35	73.81
Total lipids	11.34	11.29	11.58	11.71	10.49	12.78
Ash	11.40	10.82	10.37	11.40	16.47	8.46
Crude fibre	2.64	2.66	2.70	2.34	--	--
N-free extract	31.32	31.91	30.97	29.82	0.69	4.94
Essential Amino Acid (% on DM basis)						
Isoleucine	1.58	1.62	1.65	1.74	2.69	4.21
Leucine	2.84	2.93	3.11	3.29	5.18	6.94
Threonine	1.49	1.58	1.66	1.72	2.93	4.27
Phenylalanine	1.91	2.03	2.09	2.16	3.43	4.28
Histidine	0.88	0.79	0.77	0.74	2.12	1.97
Lysine	2.43	2.54	2.66	2.71	5.20	6.78
Arginine	3.10	3.19	3.23	3.34	4.07	5.36
Valine	1.79	1.82	1.89	1.94	3.50	4.78
Methionine	0.80	0.93	1.07	1.16	2.01	3.71
Fatty Acid profile (% total FAME)						

C14:0 (myristic)	4.49	2.92	2.87	2.84	10.30	8.41
C16:0 (palmitic)	21.61	21.32	20.97	19.99	25.61	24.03
C18:0 (stearic)	6.08	4.87	4.69	4.58	8.47	4.11
C20:0 (arachidic)	1.54	1.49	1.52	1.53	12.34	11.28
C16:1n-7 (palmitoleic)	4.15	4.30	4.54	4.69	4.82	5.94
C18:1n-9 (oleic)	22.11	22.74	22.88	23.02	16.82	19.48
C20:1n-9 (eicosanoic)	3.30	2.78	2.44	2.13	1.98	1.74
C18:2n-6 (linoleic)	24.87	24.65	24.80	24.33	9.36	8.22
C20:4n-6 (arachidonic)	0.80	0.78	0.79	0.77	0.82	0.87
C20:5n-3 (eicosapentaenoic, EPA)	7.90	8.47	9.15	9.42	11.07	13.47
C22:6n-3 (docosahexaenoic, DHA)	6.03	6.96	7.84	7.89	8.34	8.54

<sup>1</sup> Premix provides per kg: vitamin A (8500 IU), Vitamin D3 (3000 IU), Vitamin E (30,250 mg), Vitamin K3 (9000 mg), Vitamin B1 (25 mg), Vitamin B2 (12 mg), Vitamin B3 (38.65 mg), Calcium pantothenate (25 mg), Nicotinic acid (20,000 mg), Vitamin B6 (24.5 mg), Vitamin B7 (55 mg), Vitamin B8 (350 mg), Vitamin B9 (4250 mg), Vitamin B12 (16.5 mg), Cupric sulphate (10,000 mg), Iron sulphate (12,000 mg), Potassium iodide (50 mg), Manganese oxide (85,000 mg), Sodium selenite (400 mg), Zinc sulphate (45,000 mg), Calcium carbonate (186,000 mg), Potassium chloride (24,100 mg), Sodium chloride (40,000 mg), Antioxidants (9500 mg).

The trial lasted 108 days, and the shrimps were stocked in twelve cages (60 shrimps per cage, 3 m<sup>3</sup> for each cage) divided into the testing treatments and 3 repetitions. All the shrimp experimental groups were fed four times daily (at 6:00, 11:00, 17:00 and 22:00 h) from 10% (first week) to 5% (last week) of shrimp body weight [24]. Weekly, the shrimps' total weight for each cage was recorded in order to adapt the daily amount of feed administration.

The pelleted feeds and the dehydrated seafood discards were analysed by the AOAC method [25] to evaluate the chemical analysis and fatty acid profile.

### 2.3. Growth Trial

The trial was performed in the commercial farm "Ittica Sardegna", located in Santa Caterina, near San Giovanni Sergiu (CA) (Sardinia, Italy; 39.089479 N, 8.483647 E), using 3 m<sup>3</sup> (1.5 × 2 × 1 m) cages placed on the bottom of a 1-hectare pond with a water depth of 1.0 ± 0.50 m. The cages were made of nylon with a 3–5 mm mesh diameter.

The water quality parameters were periodically measured, and the values recorded were optimal for the shrimps' growth and survival (Table 2). The temperature, salinity, pH and O<sub>2</sub> were monitored daily at 6:00 a.m. and 6:00 p.m., while total ammonia, nitrite, nitrate, and phosphate were controlled every two weeks (sensors of Softmakers S.R.L., Cittadella, PD, Italy). During the trial, a natural light–dark cycle was followed.

**Table 2.** Mean quality parameters of pond water analysed during the experimental period.

Particulars	Mean	Range
Temperature (°C)	26.46	22–30
Salinity	35.50	35–36.6
pH	7.61	7.34–7.79
O <sub>2</sub> (mg/L)	8.30	7.86–8.58
Total ammonia (mg/L)	0.33	0.50–0.21
Nitrite-N (mg/L)	<0.10	<0.01–0.16
Nitrate-N (mg/L)	0.05	<0.05–0.15
Phosphate (mg/L)	<0.03	<0.03

Pursuant to the laws in force [26], after twelve weeks of the growth trial, the shrimps were caught by net and slaughtered by immersion in ice-cold water (hypothermia). Placed on ice, the shrimps were immediately transported to the laboratory.

Shrimps were individually weighed, after the standard length was measured and the growth parameters were subsequently calculated; the cephalothorax was separated from the abdomen manually, the exoskeleton was removed to obtain the abdomen weight without the exoskeleton, while the cephalothorax and the exoskeleton were weighed together using a precision balance ( $\pm 0.01$  g). The total exoskeleton length and abdomen width were measured with a digital calliper (0.1 cm precision scale).

The following indices were calculated [27]:

- survival (%) = [final shrimp number/initial shrimp number]  $\times$  100;
- average weight gain (AWG, g/d) = (final body weight – initial body weight)/days of trial;
- weight gain (WG, %) = [(final body weight – initial body weight)/initial body weight]  $\times$  100;
- specific growth rate (SGR, g/%) = [(ln final weight – ln initial weight)/days of trial]  $\times$  100;
- meat yield (MY, %) = (weight of muscle/final weight)  $\times$  100;
- condition factor (K, g/cm<sup>3</sup>) = [final body weight/(body length)<sup>3</sup>].

#### 2.4. pH, Colour and Warner–Bratzler Shear Parameters in Shrimp Flesh

The pH values were measured on the entire shrimp abdomen without exoskeleton using a portable instrument (Model HI 9025; Hanna Instruments, Woonsocket, RI, USA) with an electrode (FC 230C; Hanna Instruments) and performing a two-point calibration (pH 7.01 and 4.01).

Instrumental colour analysis was performed by HunterLab equipment (ColorFlex, Illuminant D65) in order to measure lightness ( $L^*$ ), redness ( $a^*$ ) and yellowness ( $b^*$ ) values. To make representative measurements, colour was assessed on three different parts (proximal, central and distal) of the abdomen of each shrimp. The whiteness index ( $w^*$ ) was calculated according to Chen et al. [28]:

$$\text{whiteness} = \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}}.$$

An INSTRON 5544 texture analyser (Instron US, Norwood, MA, USA) was used to determine flesh shear force (Warner–Bratzler Shear, WBS). The shrimp's second abdominal segment was sheared in a perpendicular direction and the record of the maximum shear force was taken during cutting. All the analyses were performed in triplicate.

#### 2.5. Chemical Composition and Fatty Acid Profile of Flesh Shrimp

Chemical composition of the shrimp flesh was analysed according to the AOAC procedures [25] as described in our previous work [29]. The total lipids were extracted [30] and analysed by gas chromatography (Shimadzu GC-17A) as described in Tarricone et al. [31].

#### 2.6. Economic, Social and Governance Impact

PESTLE (Political, Economic, Social, Technological, Legal and Environment) analysis is a strategic planning tool to identify the external factors affecting a particular industry or sector [32]. This analytical framework can provide valuable insights into the external factors that may impact the aquaculture sector's growth and sustainability [33,34]. In particular, conducting a PESTLE analysis of the blue economy sector can help stakeholders to identify the opportunities and challenges that arise from these external factors [33].

Environmental, social and governance (ESG) ratings provide guidance on a company or financial instrument's sustainability profile or characteristics, exposure to sustainability risks or impact on society and/or the environment. ESG investing involves analysis of the extra-financial elements of company performance. In order to make sustainability information as relevant as financial information, the practice involves identifying the sustainability issues that are financially relevant, or material, to the business model of companies [35]. These are commonly referred to in industry as key issues [36]. Materiality is based on the theory that good ESG performance brings better financial performance, but only if companies focus on the key issues that are financially material to them [37].

For this study, the indicators considered are ESG 1 energy efficiency, ESG 3 training and qualification, ESG 9 revenues from new products (shrimp with new sustainable diet), ESG 12 waste and discards, ESG 19 investment in accordance with ESG principles, ESG 20 supplies agreement and supply chain partners screened for accordance with ESG principles, ESG 21 health and safety aspects, ESG R&D expenses, ESG 28 customer retention and ESG 29 customer satisfaction. The indicators were calculated as follows using GRI 13 (Global Return investment) for fishery and aquaculture 2022, PESTLE parameters and the number of diets (4), as well as the different ratio of feed compositions (100%, 25%, 50% and 75%) with replaced fishmeal and the final eatable products.

$$ESG^x = \left( 1 + \frac{x}{1!} + \frac{x^2}{2!} + \frac{x^3}{3!} + \dots \times y \right) \times n, \quad -\infty < x < \infty$$

where  $x$  represents each ESG; 1, 2, 3, 4 represent the diets,  $y$  = PESTLE and  $n$  = number of years for scale-up.

In this study, the SGR parameters were analysed according to the three indicators by IT tools integrating artificial intelligence and managed by Aquacloud™, and the findings demonstrated the following:

- (a) the integration of feed for shrimps based on discards from both the fishery industry and aquaculture is a key driver of application in the circular economy and on sustainability;
- (b) the massive production and increasing demand for shrimps at the worldwide level can be managed by sustainable production based on a diet integrated with noble proteins from other blue resources otherwise destined for disposal and not recoverable, which are rich in functional compounds;
- (c) sustainable supply chains are able to intercept with circular economy principles, according to the General Fisheries Commission for the Mediterranean (target 2 and target 3) [38,39];
- (d) the proposed feeding system would have a positive impact in terms of ESG principles toward the SDGs (Sustainable Development Goals) because they affect cross-cutting elements between three sectors, aquaculture/fisheries, feeding and technologies, helping achieve SDGs 14, 2, 8, 10, 13, 15;
- (e) the proposed feeding system has a positive impact in terms of upskilling and reskilling because it introduces the integration of competences toward new job placements and a more sustainable workforce.

### 2.7. Statistical Analysis

Statistical analyses were performed with SAS software 9.1 2004 [40]. The Shapiro–Wilk test was conducted to verify the normal distribution of data, while the modified Levene's test was employed to assess the homogeneity of variances. In order to evaluate the effect of the diet data, a one-way analysis of variance (ANOVA) was used; Duncan's test was performed to compare differences among diets. Significance was declared at  $p < 0.05$ . Data are presented as means and standard error of means (SEM).

### 3. Results and Discussion

#### 3.1. Growth Performance

The results concerning the growth performance of *P. japonicus* fed with different replacement levels of seafood discards are presented in Table 3. The final body weight was greatest for the 75% replacement group, with significant differences ( $p < 0.05$ ) as compared to the 25% level (Table 3).

**Table 3.** Growth performance of kuruma shrimp fed diets with different replacement levels of fishmeal with fish discards.

Parameters	Replacement Levels of Fishmeal (%)				SEM <sup>1</sup>	<i>p</i> -Value
	0	25	50	75		
Initial body weight (g)	2.61	2.57	2.40	2.41	0.10	--
Final body weight (g)	20.46 <sup>ab</sup>	18.76 <sup>b</sup>	20.11 <sup>ab</sup>	23.73 <sup>a</sup>	2.85	0.045
Survival (%)	87.65	86.93	87.78	88.68	6.35	0.074
Abdomen weight (g)	9.73 <sup>B</sup>	8.04 <sup>C</sup>	9.47 <sup>B</sup>	11.23 <sup>A</sup>	1.78	0.004
Cephalothorax + exoskeleton weight (g)	10.73 <sup>B</sup>	8.72 <sup>C</sup>	10.64 <sup>B</sup>	12.50 <sup>A</sup>	2.14	0.008
Total length (mm)	150.15 <sup>B</sup>	141.14 <sup>C</sup>	149.79 <sup>B</sup>	156.74 <sup>A</sup>	8.49	0.004
Exoskeleton length (mm)	35.38 <sup>B</sup>	32.99 <sup>C</sup>	35.40 <sup>B</sup>	36.91 <sup>A</sup>	2.26	0.003
Abdomen width (mm)	14.40 <sup>B</sup>	13.44 <sup>C</sup>	14.23 <sup>B</sup>	15.09 <sup>A</sup>	1.01	0.007
AWG <sup>2</sup> (g/d)	0.21	0.17	0.21	0.25	0.03	0.062
WG <sup>3</sup> (%)	683.91	552.14	737.92	884.65	37.48	0.784
SGR <sup>4</sup> (%)	6.89 <sup>ab</sup>	6.60 <sup>b</sup>	7.01 <sup>a</sup>	7.04 <sup>a</sup>	0.41	0.047
MY <sup>5</sup> (%)	47.56 <sup>ab</sup>	47.97 <sup>a</sup>	47.09 <sup>b</sup>	47.32 <sup>b</sup>	0.38	0.017
K <sup>6</sup> (g/cm <sup>3</sup> )	0.61	0.59	0.60	0.62	0.20	0.066

<sup>1</sup> Standard error of means; <sup>2</sup> Average weight gain; <sup>3</sup> Weight gain; <sup>4</sup> Specific growth rate; <sup>5</sup> Meat yield; <sup>6</sup> Condition factor; Means having different superscripts within the row are significantly different; <sup>A, B, C</sup>;  $p < 0.01$ ; <sup>a, b</sup>;  $p < 0.05$ .

The survival rate did not differ among groups; on average, it was more than 86% following all the fishmeal replacement levels, in accordance with the findings reported by Ambasankar et al. [13], who tested the addition of Antarctic krill meal to white fishmeal for the diet of *P. vannamei*, and those reported by Wei et al. [41], who studied the influence of replacing dietary fishmeal with Antarctic krill meal in *Litopenaeus vannamei*.

The abdomen weight, cephalothorax and exoskeleton weight and total length were significantly greater ( $p < 0.01$ ) in shrimps fed with 75% seafood discard replacement, while the 25% level provided the lowest figures, with significant differences as compared to the control and 50% groups.

The SGR rate was significantly greater following the 50 and 75% replacement level diet ( $p < 0.05$ ) as compared to the 25% group. The same results are described by Renteira et al. [4], who studied the effect of replacing fishmeal with *Plukenetia volubilis* cake at different levels, and by Ambankar et al. [13].

The dietary inclusion of seafood discards at 25% positively affected the shrimps' flesh yield, which was significantly ( $p < 0.05$ ) greater in comparison with the 50 and 75% replacement levels. Also, in the study by Li et al. [27], the replacing of fishmeal with *Chlorella Sorokiniana* positively affected the meat yield of Pacific white shrimps.

The use of feed ingredients, especially of marine origin, has been reported to enhance performance in shrimps due to the higher attractability and palatability of the formulated feed [13]. Several authors stated that the ingredients used are capable of stimulating appetitive phases, which include arousal, search initiation and feed location., leading to higher ingestion and, as a consequence, to improved growth rates. Ambasankar et al. [13]

found that the inclusion of krill meal had a positive impact on shrimps' growth. Renteria et al. [3] studied the effect of fishmeal replacement with sachal inchi cake in the diet of *Litopenaeus vannamei* and reported that replacing fishmeal increased cephalothorax and abdomen weight, tending to result in an increase in final weight, as found also in the present study. Wang et al. [42] investigated the replacement of fishmeal with several types of animal products in feeding for cuneate drum (*Nibea miichthioides*) and showed that the best growth parameters were achieved when fish were fed with raw fish. In extensive ponds, shrimps may show sorting activity towards the food organisms available in the pond, while in intensive systems, the contribution of natural feed may be low or zero [43,44]. In our study, the better performance obtained following a higher ingestion of seafood discards in the diet may be mainly attributed to the nutritional benefit of the seafood mixture and to its positive influence on attractability and palatability to the shrimps. To our knowledge, other authors have investigated different seafood mixtures in other aquatic species, such as Rainbow Trout [45] and Red Drum [46,47].

### 3.2. Muscle Quality Parameters

Table 4 shows the pH, colour features and WBS of kuruma shrimp fed with different replacement levels of fishmeal with seafood discards. The four treatment groups did not differ significantly in muscle pH.

**Table 4.** pH, colour features and WBS of kuruma shrimp fed diets with different replacement levels of fishmeal with seafood discards.

Parameters	Replacement Levels of Fishmeal (%)				SEM <sup>1</sup>	p-Value
	0	25	50	75		
pH	6.96	6.99	7.00	6.98	0.14	0.241
L* (lightness)	34.81	34.66	35.54	34.89	5.65	0.201
a* (redness)	0.33 <sup>ab</sup>	0.85 <sup>a</sup>	0.31 <sup>b</sup>	0.17 <sup>b</sup>	0.19	0.035
b* (yellowness)	3.75 <sup>a</sup>	3.52 <sup>a</sup>	2.67 <sup>b</sup>	2.47 <sup>b</sup>	0.36	0.035
W (whiteness)	65.30	65.44	64.52	65.16	0.40	0.108
WBS	1.91 <sup>a</sup>	1.79 <sup>b</sup>	1.78 <sup>b</sup>	1.74 <sup>b</sup>	0.53	0.029

<sup>1</sup> Standard error of means; Means having different superscripts within the row are significantly different; a, b:  $p < 0.05$ .

With the increasing of the substitution percentage of fishmeal with seafood discards, a\* and b\* values significantly decreased ( $p < 0.05$ ). Li et al. [27] showed that increasing the quantity of *Chlorella Sorokiniana* as a substitute for fishmeal determined a higher redness and lower yellowness of white shrimp flesh. Shrimp colour is an important factor affecting consumers' evaluation in terms of price and purchase desire, because bright and appropriate colouration is associated with the freshness and quality of the product [48]. Shrimp colouration depends on the presence of carotenoid pigments in the feed, because they are not able to synthesize them [48]. In this study, lower a\* and b\* values following high levels of fishmeal replacement may be due to a lower amount of carotenoid, but the light-coloured flesh is a characteristic of this species. Similar effects were observed in another study in which fishmeal was substituted with mealworm meal [14] or *Clostridium autoethanogenum* protein [49].

The shrimp meat in the control group showed a significantly ( $p < 0.05$ ) higher value of WBS as compared to the groups in which fishmeal was replaced. Mu et al. [50] noted in aquatic animals a negative correlation between muscle texture and fat content; in this study, softer meat was found in the fishmeal replacement groups, which contained a higher amount of fat as compared to the control group. Dunajski [51] stated that the shrimps' muscle texture depended on the connective tissue and the integrity of myofibrillar proteins, which can change when the protein concentration in feed is modified [52]. The structural factors of muscle tissue are reduced by high meat levels of



moisture and lipids; previous studies documented that fish characterized by higher flesh lipids or moisture percentage was softer in flesh texture [51]. In fact, Martínez-Córdova et al. [53] noted that feeding shrimps with a high level of insects increased the meat lipid concentration and the tenderness.

### 3.3. Muscle Chemical Composition

As shown in Table 5, the dietary treatment had a significant effect on moisture, crude protein, and lipids. The moisture content of the control group was significantly ( $p < 0.05$ ) greater as compared to the other groups containing different levels of fishmeal replacement. However, the shrimp muscle moisture values found in this study are in agreement with those reported in the literature, with mean values ranging between 70 and 79% [7,41].

**Table 5.** Chemical composition of kuruma shrimp fed with different replacement levels of fishmeal with seafood discards.

Parameters	Replacement Levels of Fishmeal (%)				SEM <sup>1</sup>	p-Value
	0	25	50	75		
Moisture	75.38 <sup>a</sup>	74.31 <sup>b</sup>	74.38 <sup>b</sup>	74.03 <sup>b</sup>	1.05	0.042
Crude protein	21.48 <sup>b</sup>	22.21 <sup>a</sup>	21.90 <sup>a</sup>	22.34 <sup>a</sup>	0.75	0.036
Lipids	0.40 <sup>b</sup>	0.44 <sup>ab</sup>	0.43 <sup>ab</sup>	0.47 <sup>a</sup>	0.045	0.043
Ash	1.67	1.64	1.69	1.67	0.143	0.061
N-free extract	1.07	1.40	1.60	1.49	0.695	0.062

<sup>1</sup> Standard error of means; Means having different superscripts within the row are significantly different; <sup>a, b</sup>:  $p < 0.05$ .

As for the protein content, shrimps receiving fishmeal replaced with seafood discards showed a markedly ( $p < 0.05$ ) higher concentration as compared to the control group, regardless of the percentage of replacement. The fat content was greatest in shrimps fed 75% fishmeal replacement in comparison with the control group ( $p < 0.05$ ). The greater protein and fat content of this group, along with the better shrimp growth rate, suggests that the better performance may be attributed to the nutritional characteristics of seafood discards, which are well-balanced in amino acid profile and omega-3 phospholipids [18]. Moreover, the seafood discards may have exerted a positive effect on the attractability and palatability of feed ingestion from the shrimps. In other studies, the effect of the replacement of fishmeal provided controversial results, depending on the type of substitution ingredient; according to Yao et al. [49], replacement with different levels of *Clostridium autoethanogenum* did not affect the proximate composition of shrimp meat, as also found by Wei et al. [41], who used Antarctic krill. On the other hand, replacing fishmeal with live insects determined a decrease in the protein content, resulting in a greater fat content in shrimp muscle [53].

### 3.4. Muscle Fatty Acid Composition

The fatty acid profile of meat from the shrimp abdomen is shown in Table 6. The control group and the fishmeal replacement at 25% showed a significantly ( $p < 0.05$ ) greater concentration of C18:0 and total saturated fatty acids (SFAs). Our results are similar to those found by other authors [3,53,54], who reported a greater concentration of SFA in shrimps fed with control feed or with feeds containing low levels of fishmeal replacement.

**Table 6.** Fatty acid profile of kuruma shrimp fed with different replacement levels of fishmeal with seafood discards (% Total Fatty Acid Methyl Esters).

Constituents (% as Fed Basis)	Replacement Levels of Fishmeal (%)				SEM <sup>1</sup>	<i>p</i> -Value
	0	25	50	75		
mg/g dry lipid basis	804.26	717.49	729.45	735.68	6.22	0.120
C12:0 (lauric)	0.57	0.67	0.46	0.49	0.02	0.068
C14:0 (myristic)	0.88	1.05	0.62	0.72	0.12	0.085
C16:0 (palmitic)	14.85	16.77	16.38	15.21	0.54	0.091
C17:0 (heptadecanoic)	1.46	1.49	1.01	1.06	0.03	0.131
C18:0 (stearic)	10.13 <sup>a</sup>	9.55 <sup>a</sup>	8.81 <sup>b</sup>	8.50 <sup>b</sup>	0.11	0.016
C20:0 (arachidic)	0.26	0.45	0.12	0.16	0.21	0.053
Total SFA <sup>2</sup>	28.17 <sup>a</sup>	29.98 <sup>a</sup>	27.40 <sup>b</sup>	26.14 <sup>b</sup>	0.62	0.029
C16:1n-7 (palmitoleic)	2.21	2.81	2.52	2.99	0.15	0.109
C16:1n-9 (trans <sup>9</sup> -palmitoleic acid)	0.57	0.37	0.51	0.39	0.04	0.074
C18:1n-9 (oleic)	12.56	11.40	11.52	11.89	0.35	0.087
C20:1n-9 (eicosanoic)	1.14 <sup>a</sup>	1.25 <sup>a</sup>	0.57 <sup>b</sup>	0.69 <sup>b</sup>	0.07	0.032
Total MUFA <sup>3</sup>	16.48	15.83	15.12	15.96	0.54	0.110
C18:2n-6 (linoleic)	11.75	11.64	11.65	11.72	0.19	0.087
C18:3n-3 ( $\alpha$ -linolenic)	0.95	0.90	0.98	0.86	0.03	0.066
C18:3n-6 ( $\gamma$ -linolenic)	0.25	0.24	0.13	0.27	0.03	0.079
C20:2n-6	0.74	0.82	0.78	0.61	0.06	0.112
C20:4n-6 (arachidonic)	3.44	2.11	2.22	2.18	0.12	0.085
C20:5n-3 (eicosapentaenoic, EPA)	19.05 <sup>ab</sup>	18.57 <sup>b</sup>	19.67 <sup>a</sup>	19.38 <sup>ab</sup>	0.28	0.024
C22:5n-6 (docosapentaenoic, DPA)	1.26	1.23	1.20	1.24	0.08	0.219
C22:5n-3 (docosapentaenoate)	0.88	0.93	0.93	0.91	0.07	0.087
C22:6n-3 (docosahexaenoic, DHA)	17.03 <sup>b</sup>	17.75 <sup>b</sup>	19.92 <sup>ab</sup>	20.73 <sup>a</sup>	0.72	0.035
Total PUFA <sup>4</sup>	55.35 <sup>b</sup>	54.19 <sup>b</sup>	57.48 <sup>a</sup>	57.90 <sup>a</sup>	0.95	0.023
Total n-6 <sup>5</sup>	17.44 <sup>a</sup>	16.04 <sup>ab</sup>	15.98 <sup>b</sup>	16.02 <sup>ab</sup>	0.98	0.045
Total n-3 <sup>6</sup>	37.91 <sup>b</sup>	38.15 <sup>ab</sup>	41.50 <sup>a</sup>	41.88 <sup>a</sup>	0.23	0.035
n-3/n-6	2.17	2.38	2.60	2.61	0.10	0.127
EPA/DHA	1.12	1.05	0.99	0.93	0.09	0.114
AI (Atherogenic Index)	0.22 <sup>a</sup>	0.26 <sup>a</sup>	0.19 <sup>b</sup>	0.18 <sup>b</sup>	0.02	0.047
TI (Thrombogenic Index)	0.29 <sup>a</sup>	0.28 <sup>a</sup>	0.21 <sup>b</sup>	0.22 <sup>b</sup>	0.01	0.048

<sup>1</sup> Standard error of means. <sup>2</sup> Total SFA—saturated fatty acids (C12:0 + C14:0 + C16:0 + C17:0 + C18:0 + C20:0); <sup>3</sup> Total MUFA—monounsaturated fatty acids (C16:1 n7 + C16:1 n9 + C18:1 n9 + C20:1 n9); <sup>4</sup> Total PUFA—polyunsaturated fatty acids (n-6 + n-3); <sup>5</sup> Total n-6 (C18:2 n6 + C18:3 n6 + C20:4 n6 + C22:5 n6); <sup>6</sup> Total n-3 (C18:3 n3 + C20:5 n3 + C22:5 n3 + C22:6 n3); Means having different superscripts within the row are significantly different; <sup>a, b</sup>: *p* < 0.05. Likewise, the concentration of C20:1n-9 was significantly (*p* < 0.05) higher following the control and 25% fishmeal replacement diet, while the percentage of total monounsaturated fatty acids (MUFAs) did not differ among groups.

The total polyunsaturated fatty acid (PUFA) concentration in the shrimp muscle significantly increased (*p* < 0.05) as the percentage of fishmeal replacement increased. PUFAs are essential nutrients for the growth of shrimps [55]; in a previous study carried out on *Litopenaeus vannamei*, the authors stated that this shrimp has dietary requirements for C18:2n-6 (linoleic, LOA), C18:3n-3 ( $\alpha$ -linolenic, ALA), C20:4n-6 (arachidonic, ARA), C20:5n-3 (eicosapentaenoic, EPA) and C22:6n-3 (docosahexaenoic, DHA,) [56,57]. In the present study, greater levels of inclusion of the seafood discards in

the shrimp diet led to a significant increase in the content of EPA and DHA ( $p < 0.05$ ), while the concentration of ALA and LOA remained constant. Other studies reported that shrimp are capable of prolonging and desaturating ALA to produce PUFAs like EPA and DHA [58]. Most animals cannot synthesize n-3 and n-6 PUFAs de novo but can only obtain them from food or precursors [59]; thus, diet significantly affected n-3 and n-6 PUFAs ( $p < 0.05$ ). The occurrence of cardiovascular diseases can be reduced by EPA and DHA, which are both PUFAs that have well-known beneficial effects on human health and on the regulation of lipid metabolism. Moreover, Simopoulous stated that the n-3/n-6 ratio may be more important than the content of total n-3 or n-6 PUFAs [60] and some researchers [61] proposed that an ideal value of this ratio should be about 2.5. In the present study, the n-3/n-6 ratio was lower than this value in all the experimental groups.

The shrimps fed with high levels of seafood discards (50 and 75%) showed markedly lower atherogenic and thrombogenic indices ( $p < 0.05$ ) compared with the control and 25% fishmeal replacement group. These indices are important indicators for the occurrence of coronary heart diseases [62]. Foods with low levels of myristic, palmitic and stearic acids have been proven to benefit human health. Similarly, food that has low AI and TI values can help to prevent cardiovascular diseases [63]. Our results confirm that the replacement of fishmeal with seafood discards positively affected the nutritional properties of shrimps.

### 3.5. Economic, Social and Governance Impact

The economic analyses in terms of cost/benefit regarding the replacement of the diet with fishmeal by fish discards is based on cross-cutting analysis between PESTLE methodology and ESG indicators in order to provide the measurement of impact for the replacement of diet for farm industry. The results showed the following:

- (a) A rating of 5 KPI is based on 75% replacement being optimal in terms of the balance of the impact on shrimp farming and feed produced by other blue resources in the circular economy;
- (b) Scalability and the intersection with the circular economy are key success factors in the next 5 years, according to FAO indications [33];
- (c) Replacing fishmeal and fish oil could affect the balance of n-3 and n-6 PUFAs in fish meat, certainly reducing environmental impacts but at the expense of the organoleptic and nutritional qualities of the fillet [34,64]. Indeed, in shrimp farming, this study shows a positive effect in the replacement of fishmeal in dietary patterns, as shown by the data of this study.
- (d) Recently, trash fish, fish without a commercial market, has started to be used, which is derived from the by-catch or waste of processed fish, such as heads, viscera, bones and scales [65]. The FAO predicts that this trend will continue to grow, considering that trash fish contain nutrients and PUFAs suitable for the development of farmed animals [66]. The Vietnamese production of shrimps allows this replacement but it is not yet measured with the ESG parameters [67].
- (e) Alternative methods, including PUFA supplementation through genetically engineered plant oils, such as *Camellia sativa* oil, successfully tested on farmed Sea Bass [68], do not allow the same results herewith shown for an integration with ESG principles and long-term effects.
- (f) Trash fish [69], as a key success factor in the shrimp industry, is more acceptable to consumers than the other possibilities, like the use of insect proteins as a substitute for fish oils and meal in feed [70]. Among the species allowed, the black soldier fly (*Hermetia illucens*) seems to be the most promising, even if it does not seem to excessively influence the growth and development of farmed fish [71].

## 4. Conclusions

In conclusion, feeding seafood discards provided satisfactory results with regard to performance and meat quality. Fishmeal replacement at 75% showed a higher value of

hardness, a greater crude protein and lipid content, a lower saturated fatty acid concentration and, in turn, a greater amount of polyunsaturated fatty acids in shrimp meat. In addition, the shrimps fed with high levels of seafood discards (50 and 75%) showed lower atherogenic and thrombogenic indices.

Therefore, fishmeal replacement with seafood discards may represent a promising opportunity in juvenile shrimp feeding, in addition to its great advantages in terms of sustainability and the circular economy. Further investigation is needed in order to assess the effectiveness of this feeding regimen for the achievement of prawn commercial size. The opportunity for ESG compliance is shown by integrating the effects in the long term, up to 5 years, for the implementation of the methodology and its sustainable scale-up updated with the regulatory framework.

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