Fishery Technology 59 (2022) : 39 - 48

Nutritional Evaluation of Fish feeds Formulated with Fish discards or Shellfish waste as Alternative Protein Sources

K. Kushani K. Premawansha[1], M. P. Kumudu S. K. de Silva[1]*, K. B. Suneetha Gunawickrama[1] and Bjørn-Steinar Sæther[2]

[1] *Department of Zoology, University of Ruhuna, Matara, Sri Lanka*

[2] *The Norwegian College of Fishery Science, Faculty of Biology, Fisheries & Economics, UiT The Arctic University of Norway, Tromsø, Norway*

Abstract

In the present study, nutritional profiling of the alternative main protein sources (AMPs) such as shellfish waste, fish offal and by-catch were carried out to evaluate the possible role to replace the fishmeal in the diet. Five diets were prepared with 40% (by weight) dried filtrate of autolyzed prawn and crab wastes (Diet A), dried fish offal (Diet B), dried by-catch (Diet C), a mixture of dried offal and shellfish waste (1:1 w/w, Diet D) and the reference diet (Diet E) using a commercial fish meal (CFM) as main protein sources. Other ingredients were common to all diets. Proximate composition of CFM, AMPs and five prepared diets were determined. Micronutrient contents, namely, total amino acid content, free amino acids, and fatty acid composition of the prepared diets were also analysed. The nutrient content of AMP was different only in the fish offal, of which the crude protein content (56.96±1.78) was significantly lower than that of the autolysed shellfish waste (71.75±5.38), dried by-catch (65.26±1.63) and CFM (70.06±1.257) (ANOVA, Tukey test, $p<0.05$). Crude lipid content of fish offal (22.81±0.57) was the highest while that was significantly different from each other (5.26±1.12, 13.55±1.61 and 18.46±0.735 for shellfish waste, dried bycatch, and CFM respectively (ANOVA, Tukey test, $p<0.05$). Mean crude protein of Diet B and C were significantly lower than that of other three diets. Crude fat, n-6 fatty acids and polyunsaturated fatty acids of four test diets were comparable to those of the reference diet while essential amino acids and n-3 fatty acids were significantly higher in reference diet. Presence of free amino acids in Diets A, C and D is an added quality to use the AMPs in formulating aqua feed. Economically viable feed can be prepared by using AMPs compared to the fishmeal diet and it confirms the possibility to replace fishmeal in formulated fish feed. Feeding trials will confirm the feed palatability, digestibility and growth performances of fish.

Keywords: Aqua feed, fish nutrition, fish meal alternatives, shellfish waste, fisheries waste

Introduction

Fish contribute a major protein source in human diets providing essential amino acids, unsaturated fatty acids (including omega-3 fatty acids) and micronutrients for a healthy life (Tacon & Metian, 2018; FAO, 2020). The global supply of wild fish however is limited, and will not be able to meet an increasing demand (Tacon & Metian, 2018). Global aquaculture therefore has become the fastest growing fish production industry especially in Asia (FAO, 2020). A major challenge to the rapid development of aquaculture industry is the availability of nutritionally balanced quality feeds at an affordable price. About 50-60% of the total production cost is allocated for fish feed (Gasco et al., 2018; FAO, 2020). Quality of harvest, growth, disease risk and the yield are affected by the quality of the feed provided to the fish (Hardy & Lee, 2010). Therefore, it is essential that cultured fish are provided with all the nutrients they need in the appropriate proportions, at relevant culture phase with adequate efficiency (NRC, 2011). Dietary requirements of most cultured fish species have been documented and these vary with aspects such as fish species, age, size, life cycle stage, and level of production (NRC,

Received 25 July 2021; Revised 22 November 2021; Accepted 01 December 2021

*E-mail: kumududs@zoo.ruh.ac.lk

2011; Craig et al., 2017; Prabu et al., 2017). In formulation of nutritionally balanced and cost-effective feed, chemical composition of feed ingredients, physical characteristics, market availability, seasonality, and their nutritional ratio should also be well considered (Hardy & Lee, 2010; NRC, 2011).

Feed manufacturers use different types of feed ingredients as the protein source. Fish meal is the most conventional main protein source in feed formulation due to high protein content, essential amino acids, fatty acid and minerals (Miles & Chapman, 2015; FAO, 2020). Fish meal is produced by whole fish or by-products of fish processing plants (Miles & Chapman, 2015). There is a high demand for the fish meal in the aqua feed industry and it has become a limiting factor. At the same time, fish meal is a primary source of protein in cattle and poultry diets creating a demand-driven competition for fish meal supply between aquaculture industry and livestock production (Khan et al., 2012). Fluctuations and limitations in raw material for fish meal production have intensified the problem, resulting in persistently high prices of fish meal in the global market (Gasco et al., 2018).

A main challenge in the aqua feed industry is therefore to identify economically viable and environmentally sustainable protein sources that can replace more traditional sources of fish meal (Gasco et al., 2018). These alternative protein sources should fulfill certain characteristics like nutrient quality, including high amounts of protein, amino acids, lipids and unsaturated fatty acids, minor nutrient contents as in fish meal. In addition, practical aspects such as easy availability, low price, low production cost, easy handling, transport, and storage are also important. Alternative protein source should also have, acceptability palatability and high digestibility by fish leading to acceptable growth rate and improved health (Tacon et al., 2006; Gasco et al., 2018).

Utilization of by-products discarded by land-based and aquatic animal processing industries has become a trend for aqua feed industry. Compared to the plant protein sources, these by-products may contain good quality proteins, lipids with essential fatty acid, and contribute with minerals and trace elements but lesser amounts of anti-nutritional factors (Tacon et al., 2006; Hua et al., 2019). Utilizing terrestrial animal by products as an alternative protein source may not be a viable option due to limited availability and complex processing mechanisms (Hua et al., 2019). As recently reported, capture fisheries by-products contribute to 10% of global fish meal production, while 25-35% supply are covered by aquaculture by-products (FAO, 2020).

Global shrimp production recently has reached 4.75 million tonnes, out of which approximately 40-50% are discarded as head and shell wastes (FAO, 2020). As a result, large amount of shellfish waste is generated by processing industries creating an environmental burden. Accumulated wastes create adverse effects on land and environmental pollution in water and aesthetic damage (Kandra et al., 2012). Shrimp waste is rich with protein, essential amino acids, lipids with high omega-3 essential fatty acids minerals and trace elements (de Silva & Senaarachchi, 2021; Kandra et al., 2012). Protein and carotenoids found in shrimp shells have been shown to be an excellent animal feed supplement. Few attempts have been made to utilize shrimp waste as a source of protein, pigments (e.g. astaxanthin) and flavour compounds in animal feed (Kandra et al., 2012; Pattanaik et al., 2020).

Fish bycatch is a protein-rich raw material for fish feed production. Trawl fishing responsible for 45.5% of bycatch discards annually (Roda et al., 2019). Fisheries by-catch is discarded in the sea or at landing sites, causing land side pollution as well as negative impact on fishery resources by overexploitation (Kasapoglu & Duzgunes, 2017). By-catch may contain high protein content, amino acids, fish oil with essential fatty acids and other biomolecules (Ghaly et al., 2013). Some of these by-catch is used for direct human consumption especially by the subsistence fishers, or is used to produce different types of value-added products such as dry fish, fish meal, or animal feed (FAO, 2020).

Fish offal discarded by processing plants and fish market sites include skin, head, fins, and internal organs This waste represents 60% of the processed fish (FAO, 2020), and there has neither been a marketable price nor a value for human consumption. However, fish offal is a rich source of protein, lipids and other biomolecules with high digestibility (Ghaly et al., 2013; Farahiyah et al., 2015). Having high amount of nutrients, enzymes and microbes in offal results in rapid degradation leading to environmental pollution (Samaddar & Kaviraj, 2014; Afreen & Ucak, 2021). Therefore, fish offal could be

converted to value-added products such as animal feed, fish meal or fertilizer (Esteban et al., 2007; Farahiyah et al., 2015).

As such, fisheries discard like shellfish waste, fish offal and by-catch seem to have a high potential to be used as alternative protein source replacing conventional fish meal in fish feed formulation. It may invariably provide a more sustainable solution to the problem of pollution in the environment due to accumulation at processing plants and thus represents an added value to fisheries and aquaculture discards. The present study aimed to evaluate the potential of fishery discards based alternative protein sources to replace commercial fish meal in fish diet without affecting the nutritional quality. The study assessed the nutrient content and the cost of four test diets formulated using shellfish waste (prawn and crab), fish offal and by-catch as fish meal replacement compared to a diet having commercially available fish meal as the main protein source.

Materials and Methods

Fresh shellfish discards of tiger prawn (*Penaeus monodon*) and blue swimming crab (*Portunus pelagicus*) and fish offal were collected from central fish market, Peliayagoda, (6096'86.7" N, 79088'90.1" E) Sri Lanka. By-catch fish Kelee shad (*Hilsa kelee*) were collected from Mannar fish market (8098'33.8" N,79092'49.8" E). A commercial fish meal and other feed ingredients, such as red rice bran, maize, soya bean, coconut poonac, wheat flour, vitamin and mineral mix were purchased from a suppler company of fish feed ingredient in Colombo, Sri Lanka.

Five diets differing only by the main protein source (approx. 40% by weight) were prepared as follows. Diet A was made with dried filtrate of autolyzed (55°C for 20 min) prawn and crab shell wastes mixed in 9:1 ratio (de Silva & Senaarachchi, 2021). Diet B had dried and ground fish offal, while diet C had dried and ground by-catch fish Kelee shad. Diet D contained 1:1 (w/w) combination of the dried ground offal and dried filtrate of autolyzed shellfish waste mixture. Diet E had commercial fish meal as the main protein source. Except for the 40% (by weight) of each of the above alternative main protein source, the other ingredients were common for each diet. Soy bean meal 30%, red rice bran 7%, maize 10%, coconut poonac 5%, wheat flour 4%, locally manufactured fish oil 2%, vitamin and mineral mix 2% were common ingredients. All ingredients were mechanically mixed while adding water until the appropriate texture is achieved, and then pellets were prepared by a pelleting machine and air dried to reduce the moisture.

Proximate composition of main protein ingredients used in the diet preparation were determined as crude protein, crude lipid, ash content and moisture. The same testing was carried out for the five different formulated diets (A, B, C, D and E) according to the standard procedure given by AOAC (2000). Direct methylation method was used for the fatty acids (FA) extraction and fatty acid profile was determined using gas chromatography (Agilent, USA) (Dulavik et al., 1998). Amino Acid profile (total amino acids and free amino acids) were analysed by ion exchange liquid chromatography using amino acid analyser (Biochrom, UK). Based on the costs of ingredients and cost of electricity during feed preparations, the price for 1 kg of formulated diets were calculated.

All the data were analysed by RStudio 1.2.1335. Statistical comparisons were made for the proximate values obtained for the main protein ingredients, five diets (A, B, C, D and E) and values of micronutrient analysis of five diets. Normally distributed data were analysed by one-way ANOVA test and pairwise comparisons were made by TukeyHSD. Data not normally distributed were analysed using nonparametric Kruskal-Wallis test and pairwise comparisons were done by Dunn Test. Adjusted $p<0.05$ level was used for both statistical analyses. Values are presented as the mean ± standard deviation of triplicate analyses (n = 3).

Results and Discussion

Present study assessed the nutrient levels of four different test diets prepared using commonly available discards of fisheries and aquaculture and compared with a reference diet which contained conventional fish meal as the main protein source. Feed formula were calculated to contain the main protein source approximately as 40% by weight and the rest of the ingredients were common to all diets as given in the material and methods section. Hence a comparative analysis of nutrient contents of five different diets was appropriate.

Autolyzed shellfish waste filtrate and commercial fish meal had similar (70% and 71% respectively) amount of crude protein levels. Fish offal had the lowest crude protein content which was signifi-

cantly different from the other main protein sources. Autolysis process has been shown to be an efficient method to recover the proteins (Cao et al., 2009; de Silva & Senaarachchi, 2021). As reported by Gildberg et al. (2011), more than 70% of protein can be recovered in the shrimp waste as water soluble protein hydrolysate by autolysis process. Previous studies have shown that conventional commercial fish meal contain crude protein levels ranging from 60% to 70% by weight (Miles & Chapman, 2015), and the crude protein level of commercial fish meal used in the present study was within that range (70%). Generally, fish offal parts may include gills, opercula and parts of the alimentary track having low amounts of protein (Esteban et al., 2007). In the present study fish offal waste ingredient had relatively lower (56.96%) crude proteins than that of the commercial fish meal, similar to what is being reported in other studies (Esteban et al., 2007; Farahiyah et al., 2015).

The percentage of crude fat was significantly different in four main protein sources and ranged from 5.26% to 22.81%. Autolyzed shellfish waste filtrate and dried offal waste had the lowest and highest crude fat levels respectively (Table 1). Commercial fish meal had 13% higher crude fat than that of autolyzed shellfish waste filtrate. Fish lipid is mainly stored in subcutaneous tissues, belly flap, mesenteric tissues, head and liver (Khoddami et al., 2012). These parts are discarded as offal. Earlier reports show relatively lower crude fat content than that was reported for the fish offal ingredient in the present study (19.10% in Esteban et al., 2007 and 13.56% in Farahiyah et al., 2015). Crude fat content of autolyzed shellfish waste filtrate in the present study is somewhat lower than that were reported by Gildberg et al. (2011) for shrimp hydrolysate (7.9%) and da Silva et al. (2017) for freeze-dried shrimp by-product protein hydrolysate (6.25%).

Autolyzed shellfish waste filtrate obtained in the present study had significantly higher moisture level (16.90 g 100 g^{-1}) than that of the commercial fish meal ingredient (Table 1) and apparently higher than that is reported elsewhere (e.g. 4.5% by Cao et al. (2009). Autolyzed dried shellfish filtrate has a sticky jelly like texture even after drying to a constant weight. According to da Silva et al. (2017), moisture content of autolyzed shrimp waste can be reduced by freeze driying which was not used in the present study. Commercially available fish meal is dried using hot air or steamed air to reduce the moisture content sufficiently to allow it to be transported and stored for long time without depleting its quality (Miles & Chapman, 2015). Significantly higher ash content (27.50%) was found in offal waste while it was 14.8% lower in autolyzed shellfish waste filtrate. Fish offal consist of bones, hard parts like opercula with high amount of calcium carbonate and minerals resulting high amount of ash content (Afreen & Ucak, 2021). Reported value for fish offal agrees with the range of values from other studies (21.79% by Esteban et al., 2007, 27.09% by Samaddar & Kaviraj, 2014).

Proximate analysis of four test diets with alternative protein sources and the reference diet (having commercial fish meal) revealed that the crude proteins ranged from 39.74 to 46.74% (Table 2). Diets B and C had significantly lower crude protein content than the other three diets (Table 2) while the latter diets were comparable in crude protein levels. As commercial fish meal (CFM) too has derived from body parts of the fish (Khan et al., 2012; Miles & Capman, 2015), the Diet C with fish bycatch is expected to contain crude proteins comparable to CFM. Diet A containing autolyzed shellfish waste and the Diet D having the combination of shellfish waste and fish offal had crude protein content similar to that of the reference fishmeal Diet E.

Table 1. Proximate composition of four main protein ingredients used in the fish diets (g 100 g^{-1}, mean ± SD, n=3)

Feed ingredient	Nutrient Content (dry matter basis)*			
	Crude Protein	Crude Fat	Moisture	Ash
Autolyzed shellfish waste filtrate	71.75 ± 5.38^{a}	5.26 ± 1.12^{a}	16.90 ± 0.06^{a}	12.66 ± 0.37^{a}
Dried offal waste	56.96 ± 1.78^{b}	22.81 ± 0.57^{b}	13.70 ± 3.07^{ab}	27.50 ± 0.94^{b}
Dried by-catch waste	65.26 ± 1.63^{a}	13.55 ± 1.61^{c}	11.55 ± 0.11^{ab}	15.82 ± 0.07^{ab}
Commercial fish meal	70.06 ± 1.26^{a}	18.46 ± 0.74^{d}	10.01 ± 0.85^{b}	15.20 ± 1.49^{ab}

*No shared superscript letter within the same column indicates significant differences

Table 2. Proximate composition of five experimental diets and cost of feed preparation. (g per 100 g dry weight basis, mean ± SD, n=3)

Nutrient Content (Dry Matter Basis)*	Formulated Diets				
	Diet A	Diet B	Diet C	Diet D	Diet E
Crude Protein	46.38 ± 0.44^{a}	39.74 ± 0.46^{b}	42.00 ± 0.58^{b}	46.36 ± 0.09^{a}	46.74 ± 2.34^{a}
Crude Fat	7.84 ± 0.05^{a}	11.44 ± 0.06^{b}	8.70 ± 0.09^{ab}	8.61 ± 0.05^{ab}	8.16 ± 0.08^{ab}
Moisture	15.83 ± 0.14^{a}	8.63 ± 0.20^{ab}	7.88 ± 0.19^{ab}	9.70 ± 0.10^{ab}	7.40 ± 0.66^{b}
Ash	11.84 ± 3.06^{ab}	14.31 ± 0.69^{ab}	17.23 ± 0.40^{a}	13.34 ± 4.10^{ab}	10.60 ± 0.94^{b}
Cost kg^{-1} (LKR)	158.42	127.17	183.36	142.79	281.70
Cost kg^{-1} (USD) $^{\#}$	0.79	0.64	0.92	0.71	1.41

*No shared superscript letter in the same row indicates significant difference.
$^{\#}$ Sri Lankan rupees (LKR) converted in to USD (25.05.2021)

Protein is the most important constituent in fish feed as it directly affects the growth performance of fish by providing energy, amino acids and functional proteins (Prabu et al., 2017). Protein requirement of cultured fish generally depends on factors like fish size, life stage, feeding type, water temperature, feeding rate, reared aquaculture system, and overall digestible energy content of diet (Craig et al., 2017; Prabu et al., 2017). Therefore, protein source and the proportion given for culture species in question should be selected carefully and the present study show that Diets A and D seems to provide balanced protein replacements to match the reference diet.

Fish offal-based diet (Diet B) had apparently higher crude fat content than other three test diets but significant difference was found only with the Diet A. Except for Diet A, all other test diets had main protein ingredient derived from whole fish or fish parts. Therefore, comparable levels of crude fat in those diets can be expected. Crude fat level in Diet A and Diet E however, were not significantly different. Dietary lipids typically consist of 7 to 15% of fish diet (Craig et al., 2017; Prabu et al., 2017). Both quantity and quality of dietary lipids are important for providing energy source and essential fatty acids, for serving to transport fat soluble vitamin (Prabu et al., 2017) and for enhancing reproductive performance of brood stock (NRC, 2011). However, excessive lipid content in diets can decrease the feed consumption by fish, which in turn will reduce intake of other nutrients and decrease the health and market quality of fish (Craig et al., 2017). In agreement with Prabu et al. (2017), all the diets in the present study fulfil the crude fat requirement of fish feed.

Moisture content is a feed quality characteristic rather than a nutrient (Prabu et al., 2017). Hardy & Barrows (2002) reported that the moisture content of feeds normally should range from 6-10% for dry-compressed diets and otherwise, it can lead to microbial growth on feed. All test diets except shellfish waste Diet A in the present study had lower than 10% moisture level indicating that those diets have safer level of moisture content. Moisture content of autolyzed shellfish waste diet (Diet A) was (15.83 g 100 g^{-1}) more than twice as high as that of the fish meal incorporated diet (Diet E) (7.40 g 100 g^{-1}). Shellfish extract contains complex of carotenoproteins having, free amino acids and small polypeptide chains (de Silva & Senaarchchi, 2021). Due to their molecular nature they tend to adsorb moisture even in dried form. Ash content in test diets were not significantly different from reference diet except Diet C.

The costs of all test diets varied only by the price of the main protein source, processing method and electricity needs for processing. Based on all the expenditure including costs of ingredients and their portion in formulation, the cheapest cost (per kg diet) was found for fish offal incorporated Diet (B) (Rs. 127.17, 0.64 USD) and the highest cost was for Diet E which is the commercial fish meal incorporated diet (Rs. 281.70, 1.41 USD). Fish offal and shellfish waste did not require expenditure as these were 100% discards. At present in Sri Lanka, fish offal and shellfish waste have low commercial demand in market as a raw material and most are discarded at the harbours or market places. Fisheries by-catch used as dry fish or pet food at subsistence

level and some low cost has to be spent to use by-catch as raw material.

Total saturated fatty acids (SFA) content of five diets ranged between 31.53% and 42.49% and no significant difference observed among diets. SFA of Diet B however was more than 7-9% higher than that of the other diets (Table 3). The predominant SFA in all diets was palmitic acid and highest was found in Diet B (26.83%). Previous studies have reported that fish offal or offal-based feeds contain high amount of saturated fatty acids (SFA) than PUFAs (Khoddami et al., 2012) and that high levels of SFA decrease the digestibility of PUFAs in diets (Kaviraj et al., 2013). Total mono-unsaturated fatty acid (MUFA) of different diets ranged from 29.55% to 31.50%. The main MUFA was identified as oleic acid and the Diet A had significantly higher oleic acid (24.10%) than the Diet B.

Essential fatty acids cannot be synthesized by fish on their own. n-3 and n-6 PUFAs are considered as essential fatty acids for fish and hence fish diets should contain those in adequate level for their growth (Kaviraj et al., 2013). Total n-6 PUFAs in test diets were not significantly different from that of the

Table 3. Relative fatty acid profile composition of five experimental diets (% of total fatty acids, mean ± SD, n=3)

Fatty Acid*	Diet A	Diet B	Diet C	Diet D	Diet E
Myristic (C14:0)	2.26±0.053^{a}	8.36±0.373^{b}	4.27±0.310^{ab}	4.05±0.212^{ab}	5.13±0.040^{ab}
Palmitic (C16: 0)	22.18±1.060^{a}	26.83±0.454^{a}	24.34±1.429^{a}	22.27±0.242^{a}	22.09±0.161^{a}
Stearic (C18:0)	6.77±0.152^{a}	7.12±0.420^{a}	7.08±0.270^{a}	6.03±0.206^{a}	4.31±0.062^{a}
Arachidic (C20:0)	0.69±0.017	0.53 $^{\#}$	ND	ND	ND
Total Saturated Fatty Acids (SFA)	31.89±1.172^{a}	42.49±0.498^{a}	35.69±1.999^{a}	32.35±0.236^{a}	31.53±0.139^{a}
Palmitoleic (C16:1 (n-7))	2.34±0.368^{a}	8.58±0.174^{b}	4.26±0.135^{ab}	3.90±0.357^{ab}	4.28±0.031^{ab}
Oleic (C18:1 (n-9))	24.10±0.266^{a}	17.05±0.218^{b}	23.18±0.236^{ab}	22.02±0.313^{ab}	21.29±0.032^{ab}
Vaccenic (C18:1 (n-7))	2.35±0.101^{a}	3.27±0.078^{a}	2.72±0.109^{a}	2.31±0.097^{a}	2.33±0.037^{a}
Gondoic (C20:1 (n-9))	0.98±0.075^{ab}	0.87±0.052^{a}	1.33±0.040^{ab}	1.32±0.074^{ab}	1.98±0.037^{b}
Total Mono Unsaturated Fatty Acids (MUFA)	29.77±0.208^{a}	29.77±0.433^{a}	31.50±0.396^{a}	29.55±0.022^{a}	29.89±0.138^{a}
Linoleic (C18:2 (n-6))	25.83±0.273^{a}	15.70±0.396^{b}	17.73±0.359^{c}	22.39±0.362^{d}	18.41±0.090^{c}
Arachidonic (C20:4 (n-6))	1.60±0.023^{ab}	1.60±0.019^{ab}	1.63±0.080^{ab}	1.79±0.119^{a}	0.97±0.005^{b}
Total Omega 6 Poly Unsaturated Fatty Acids (n-6 PUFA)	27.43±0.284^{a}	17.30±0.413^{b}	19.36±0.341^{ab}	24.18±0.480^{ab}	19.38±0.085^{ab}
alpha-Linolenic (C18:3(n-3))	2.26±0.220^{a}	2.05±0.063^{a}	1.94±0.227^{a}	3.99±0.538^{a}	3.49±0.097^{a}
Eicosatrienoic acid (C20:3 (n-3))	0.78±0.031	ND	ND	ND	ND
Eicosapentaenoic acid (C20:5 (n-3))	2.27±0.152^{a}	3.54±0.099^{b}	2.21±0.295^{a}	3.40±0.468^{b}	4.63±0.223^{c}
Docosapentaenoic acid (C22:5(n-3))	0.76±0.095^{a}	ND	0.98±0.465^{a}	ND	ND
Docosahexaenoic (C22:6 (n-3))	5.08±0.702^{a}	4.84±0.778^{a}	7.51±0.992^{b}	6.52±1.274^{a}	9.12±0.471^{b}
Total Omega 3 Poly Unsaturated Fatty Acids (n-3 PUFA)	10.64±0.631^{a}	10.43±1.195^{a}	12.63±1.881^{a}	13.91±0.267^{ab}	17.23±0.151^{b}
Total Polyunsaturated Fatty Acids (PUFA)	38.07±0.707^{a}	27.73±1.334^{b}	31.99±1.755^{ab}	38.09±0.213^{ab}	36.62±0.065^{ab}
Ratio (n3/n6)	0.40±0.026^{a}	0.61±0.041^{b}	0.65±0.103^{b}	0.58±0.023^{b}	0.89±0.012^{c}

*No shared superscript letter in the same row indicate significant differences.
#- only one measurement was available
ND–Not Detected

reference diet (Table 3). The highest total n-6 PUFA were found in Diet A differing by approximately 8% from the reference diet. Total n-6 PUFA in Diet B was the lowest and significantly different from that of the Diet A. Linoleic acid was the predominant n-6 PUFA in all diets. All experimental diets contain apparently more n-6 PUFAs than the n-3 PUFAs. Although the present study assessed the nutritional quality of diets differing only by the main protein source, all diets had more than 50% (by weight) of plant-based ingredients including soybean and coconut poonac. Vegetable oils typically contain fatty acids (FAs) with 18 carbon atoms, while most of them are also rich in unsaturated fatty acids including n-6 PUFAs. Soybean meal is also rich in n-6 fatty acid (Kaur et al., 2014). As the common ingredients did not differ in terms of the composition among experimental diets, the detected differences in fatty acids among diets can be attributed to the alternative main protein sources. n-6 PUFAs content in all test diets were comparable to that of the reference diet.

Reference diet had approximately 3-7% higher level of n-3 PUFA than four test diets and was significantly different from the test diets except Diet D. Fish meal manufacturers usually add essential fatty acids to commercial fish meal to enhance the product quality. Generally, n-3 fatty acids are mainly found in fish muscles especially in oil deposits of fish muscles (Kaur et al., 2014). Fish offal discards generally don't have large parts of skeletal muscles but contain rest of the inedible parts of fish. This can be a reason for detecting low amount of n-3 PUFA especially in Diet B. Lower n-3 PUFAs in fish offal incorporated fish diets has been reported earlier too (Kaviraj et al., 2013).

Total PUFA level varied from 27.73% (Diet B) to 38.07-38.09% (Diet A and Diet D). Diet A with autolyzed shellfish waste filtrate recorded a significantly higher PUFAs than the Diet B with high amount of n-6 PUFA. Fish absorb more favourably polyunsaturated fatty acids (PUFA) followed by monounsaturated fatty acids (MUFAs) and saturated fatty acids (SFAs) (Kaviraj et al., 2013). It is reported that dietary polyunsaturated fatty acids can be rapidly digested by fish (NRC, 2011). In general, marine fish require eicosapentaenoic acid (EPA) (20:5 n 3) and/or docosahexaenoic acid (DHA) (22:6 n 3) while freshwater fish need linoleic acid (18:2 n 6), linolenic acid (18:3 n 3) or both (Tasbozan & Gökçe, 2017). Incorporation of DHA and EPA in to feed ingredients is a convenient method to ensure adequate amounts of these essential fatty acids in fish diets but it may increase the feed costs further (Taºbozan & Gökçe, 2017). EPA level of reference diet was significantly different from that of all test diets and DHA levels also was significantly different from that of the reference diet except Diet C. Therefore, additional n-3 PUFA's may needed in those test diets to be used as a fish meal replacement according to the requirement of selected cultured fish. The n-3/n-6 ratio of experimental feeds (ranged 0.40-0.89) were not significantly different among test diets except Diet A, but a significantly higher value of n-3/n-6 was recorded in the reference diet (E) than all the test diets. Approximately 1:1 ratio of n-3/n-6 PUFAs in human diets are reported to be effective in preventing coronary heart diseases, cancer and, autoimmune diseases (Tasbozan & Gökçe, 2017).

Dietary amino acid compositions are significantly affected by protein content of diet, while feed with quantitatively and qualitatively high protein content has high amounts of amino acids and vice versa (Kaushik & Seiliez, 2010). Accordingly, test diets in the present study having high and comparable crude protein content to that of the reference diet also have high and comparable levels of total amino acids (TAA) contents. In four test diets, total amino acid profile was not significantly different. It varied from 26.27 mg g^{-1} to 29.27 mg g^{-1} while that of the reference diet was 31.61 mg g^{-1}. Only Diet B and Diet C showed significant difference from the reference diet (Table 4). Eighteen (18) amino acids were detected in five diets including nine essential amino acids (EAA) (tryptophan not detected) and 9 non-essential amino acids (NEAA) (glutamine not detected). Amino acids such as tryptophan and glutamine were not detected in this method of analysis, may be because these amino acids are either in undetectable levels in feed or are destroyed/denatured during preparation or extraction.

Total essential amino acid content of test diets ranged from 11.70 mg g^{-1} to 12.88 mg g^{-1} while that of the reference diet was 14.68 mg g^{-1}. Most essential amino acids were significantly higher in reference diet than some of the test diets while some EAA (histidine and arginine) had comparable levels in the four test diets and the reference diet. Leucine was the predominant amino acid among all EAA diets except arginine in Diet C (2.32 mg g^{-1}). Leucine is significantly higher in the Diet E (2.63 mg g^{-1}). It was significantly lower in Diet C (1.99 mg g^{-1}) than that

Table 4. Total Amino acid profile and free amino acid profile composition of five experimental diets. Free amino acids are given in parenthesis. (mg g^{-1}, mean ±SD, n=3)

Amino Acid*	Diet A	Diet B	Diet C	Diet D	Diet E
Essential Amino Acids					
Threonine	1.12± 0.000^{a} (1.10± 0.022^{a})	1.13± 0.036^{ab} (ND)	1.07± 0.449^{a} (ND)	1.21± 0.041^{ab} (0.87± 0.034^{b})	1.36± 0.025^{b} (ND)
Valine	1.38±0.006^{ab} (2.28± 0.107^{a})	1.18±0.029^{ab} (ND)	1.08±0.004^{a} (ND)	1.37±0.021^{ab} (1.69± 0.062^{b})	1.44± 0.052^{b} (ND)
Methionine	0.72±0.017^{ab} (1.61± 0.072^{a})	0.63±0.005^{a} (ND)	0.68±0.051^{ab} (ND)	0.61±0.167^{a} (1.40± 0.008^{b})	0.88± 0.006^{b} (ND)
Isoleucine	1.16±0.005^{a} (1.51±0.081^{a})	1.03±0.038^{b} (ND)	0.98±0.023^{b} (ND)	1.17±0.019^{a} (1.26± 0.001^{b})	1.25±0.031^{c} (ND)
Leucine	2.30±0.008^{a} (3.36±0.143^{a})	2.15±0.072^{b} (ND)	1.99±0.017^{c} (ND)	2.38±0.070^{a} (2.79± 0.055^{b})	2.63±0.064^{d} (ND)
Phenylalanine	1.53±0.015^{a} (2.07± 0.130^{a})	1.34±0.049^{b} (ND)	1.26±0.063^{b} (ND)	1.51±0.091^{a} (1.53± 0.032^{b})	1.56± 0.054^{a} (ND)
Lysine	2.22±0.007^{a} (4.58±0.140^{a})	1.88±0.056^{b} (ND)	1.68±0.045^{c} (ND)	2.13±0.044^{a} (3.09±0.096^{a})	2.43±0.050^{d} (ND)
Histidine	0.57±0.004^{a} (ND)	0.57±0.022^{a} (ND)	0.62±0.037^{a} (ND)	0.65±0.000^{a} (ND)	0.72±0.018^{a} (ND)
Arginine	1.88±0.114^{a} (2.79±0.006^{a})	1.83±0.024^{a} (ND)	2.32±0.426^{a} (ND)	1.86±0.209^{a} (1.95± 0.041^{a})	2.29±0.031^{a} (ND)
Total Essential Amino Acids	12.88±0.127^{a} (19.33±0.689^{a})	11.75±0.291^{a} (ND)	11.70±0.703^{a} (ND)	12.68±1.005^{a} (14.54±0.331^{b})	14.68±0.333^{b} (ND)
Non-Essential Amino Acids					
Aspartate	2.48±0.015^{a} (2.43±0.094^{a})	2.17±0.079^{b} (ND)	2.05±0.089^{b} (ND)	2.45±0.068^{a} (1.43±0.042^{a})	2.56±0.056^{a} (ND)
Asparagine	ND (1.07±0.066^{a})	ND (ND)	ND (ND)	ND (0.96±0.054^{a})	ND (ND)
Serine	1.15±0.015^{a} (ND)	1.31±0.035^{ab} (ND)	1.26±0.051^{ab} (ND)	1.29±0.045^{ab} (ND)	1.54±0.037^{b} (ND)
Glutamate (Glutamic Acid)	6.05±0.041^{a} (5.04±0.177^{a})	5.22±1.990^{a} (ND)	5.00±0.204^{a} (ND)	5.87±0.097^{a} (3.15±0.147^{b})	6.08±0.129^{a} (ND)
Proline	1.60±0.036^{a} (0.57±0.011^{a})	1.75±0.049^{a} (ND)	2.05±0.226^{b} (ND)	1.93±0.011^{ab} (0.75±0.039^{b})	1.80±0.012^{a} (ND)
Glycine	1.71±0.016^{a} (1.63±0.075^{a})	2.05±0.006^{ab} (ND)	2.53±2.854^{b} (ND)	2.16±0.027^{ab} (1.41±0.042^{b})	2.00±0.004^{ab} (ND)
Alanine	2.11±0.007^{a} (5.27±0.250^{a})	1.72±0.027^{b} (ND)	1.84±0.127^{ab} (0.55±0.021^{b})	2.07±0.019^{ab} (3.41±0.107^{c})	1.94±0.020^{ab} (ND)
Cysteine	0.16±0.014^{a} (ND)	0.15±0.000^{a} (ND)	0.16±0.026^{a} (ND)	0.22±0.000^{a} (ND)	0.24±0.002^{a} (ND)
Tyrosine	0.86±0.119^{a} (2.54±0.083^{a})	0.28±0.000^{b} (ND)	0.40±0.186^{ab} (ND)	1.00±0.000^{ab} (1.67± 0.040^{b})	0.87±0.174^{a} (ND)
Total Amino Acids (Total Free Amino Acids)	29.03±0.348^{ab} (34.88±1.422^{a})	26.27±0.485^{a} (ND)	27.06±1.898^{a} (0.55±0.021^{b})	29.27±1.851^{ab} (27.33±0.722^{a})	31.61±0.391^{b} (ND)

*No shared superscript letter in the same row indicate significant differences. ND - not detected

of the other test diets. Fish require amino acids for protein synthesis and many other functions including cell signalling, appetite regulation, osmoregulation, growth and development, and immunity and survival. Hence formulated feed should have required amounts of EAAs (Kaushik & Seiliez, 2010). Accordingly, these alternative protein sources can replace the conventional fish meal without compromising the total EAA content. Of the NEAA, glutamic acid (glutamate) was the predominant but not significantly different among five diets.

Total free essential amino acid content was higher in Diet A than Diet D (Table 4). Only Diet A and Diet D contained more than one free amino acids, whereas, only alanine was detected as a free amino acid in Diet C (by-catch diet). Diet A and D consist with autolyzed shellfish extract fully and partially respectively. During the autolysis process proteins breakdown to peptide fragments making it easier to release (de Silva & Senaarachchi, 2021) and therefore most of the FAA were perhaps detected in those shellfish waste diets. This free amino acid profile of autolyzed shellfish waste incorporated diet (Diet A) is relatively similar to what is been reported by Gildberg et al. (2011). Due to the oven drying (60°C) of ingredients of fish offal and by-catch in feed preparation, heat may have affected the free amino acids in those two diets. Generally, aquaculture industry seeks feed that provide fast growth of fish to get more profit. When diet has free amino acids fish can easily utilize the amino acids and show fast growth which is more suitable for fry stages of fish (Craig et al., 2017; Prabu et al., 2017). The higher free amino acid content (34.88 mg g^{-1}) in autolyzed shellfish waste incorporated diet (A) therefore make it more suitable to use as the fry/juvenile fish feed. When comparing the free amino acid level with the reference diet (E) there is high potential to replace the fish meal due to the high amount of free amino acid level found in shellfish waste incorporated feeds (A and D).

Present study concludes that all four test diets having alternative main protein sources could be used as low cost alternatives for conventional fish meal incorporated diet. Having good nutrient levels, low cost, and easy availability, these alternative protein ingredients provide better candidates for high-cost conventional fish meal ingredient in fish feed. Low cost aqua feed with comparable nutrients to commercial feed is an essential need especially for small scale fish farmers and diets tested have shown to meet the requirement. Feeding trials need to be done to evaluate the test diets for their acceptability, digestibility, feed conversion ratio and growth performances for selected culture fish. Accumulation of fisheries and aquaculture waste creates a huge problem to the environment. Utilization of these discards is important for cleaner production opportunities for the fishery industry and to reduce the disposal cost. It also provides a green solution to prevent environment pollution and a value addition to the aquaculture and fisheries.

Acknowledgements

NOR-LANKA BLUE (NORPART) Partnership Project of UiT-The Arctic University of Norway is acknowledged for funding support. Guro Kristine Edvinsen, Nuwan P. P. Liyanage and W. A. R. K Senarachchi are acknowledged for their support in laboratory work and feed formulation.

References

Afreen, M. and Ucak, I. (2020) Fish processing wastes used as feed ingredient for animal feed and aquaculture feed. J. Surv. Fish. Sci. 6(2): 55-64

AOAC (2000) Official methods of analysis of association of official analytical chemist, 17th edn., Washington DC

Cao, W., Zhang, C., Hong, P., Ji, H., Hao, J. and Zhang, J. (2009) Autolysis of shrimp head by gradual temperature and nutritional quality of the resulting hydrolysate. LWT - Food Sci. Technol. 42(1): 244-249

Craig, S., Kuhn, D. and Schwarz, M. (2017) Understanding fish nutrition, feeds, and feeding. Virginia Coop. Ext. pp 1-6

da Silva, C. P., Bezerra, R. S., dos Santos, A. C. O., Messias, J. B., de Castro, C. R. O. B. and Junior, L. B. C. (2017) Biological value of shrimp protein hydrolysate by-product produced by autolysis. LWT - Food Sci. Technol. 80: 456-461

de Silva, M. P. K. S. K. and Senaarachchi, W. A. R. K. (2021) Efficiency of biotransformation of shellfish waste to carotenoprotein by autolysis and crab-shrimp endo-enzymes. J. Aquat. Food Prod. Technol. 30(5): 526-534

Dulavik, B., Sørensen, N. K., Barstad, H., Horvli, O. and Olsen, R. L. (1998) Oxidative stability of frozen light and dark muscle of saithe (*Pollachius virens L.*). J. Food Lipids 5(3): 233-245

Esteban, M. B., García, A. J., Ramos, P. and Márquez, M. C. (2007) Evaluation of fruit–vegetable and fish wastes as alternative feedstuffs in pig diets. Waste Management. 27(2): 193-200

FAO (2020) The State of World Fisheries and Aquaculture 2020. Sustainability in action. Food and agriculture organization of the United Nations, Rome, Italy

Farahiyah, I. J., Wong, H. K., Zainal, A. A. and Ahmad, A. (2015) Fish offal meal as an alternative protein source of fish meal for Tilapia, *Oreochromis sp*. Malaysian J. Anim. Sci. 18(2): 81-86

Gasco, L., Gai, F., Maricchiolo, G., Genovese, L., Ragonese, S., Bottari, T. and Caruso, G. (2018) Fishmeal alternative protein sources for aquaculture feeds. In: Feeds for the Aquaculture Sector. Springer Briefs in Molecular Science. Springer, Cham. pp 1-28

Ghaly, A. E., Ramakrishnan, V. V., Brooks, M. S., Budge, S. M. and Dave, D. (2013) Fish processing wastes as a potential source of proteins, amino acids and oils: a critical review. J. Microb. Biochem. Technol. 5: 107-129

Gildberg, A., Arnesen, J. A., Sæther, B. S., Rauø, J. and Stenberg, E. (2011) Angiotensin I-converting enzyme inhibitory activity in a hydrolysate of proteins from Northern shrimp (*Pandalus borealis*) and identification of two novel inhibitory tri-peptides. Process Biochem. 46(11): 2205-2209

Hardy, R. W. and Barrows, F. T. (2002) Diet formulation and manufacture. In Fish nutrition (Halver, J.E. and Ronald, W.H., Eds.), pp 505-600 SanDiego, CA: Academic Press

Hardy, R. W. and Lee, C. (2010) Aquaculture Feed and Seafood Quality. Bull. Fish Res. Agen. 31: 43-50

Hua, K., Cobcroft, J.M., Cole, A., Condon, K., Jerry, D.R., Mangott, A., Praeger, C., Vucko, M. J., Zeng, C., Zenger, K. and Strugnell, J. M. (2019) The Future of Aquatic Protein: Implications for Protein Sources in Aquaculture Diets. One Earth. 1(3): 316-329

Kandra, P., Challa, M. M. and Jyothi, H. K. P. (2012) Efficient use of shrimp waste: Present and future trends. Appl. Microbiol. Biotechnol. 93(1): 17-29

Kasapoglu, N. and Duzgunes, E. (2017) The common problem in the Black Sea fisheries: By-catch and its effects on the fisheries economy. TrJFAS 17(2): 387-394

Kaur, N., Chugh, V. and Gupta, A. K. (2014) Essential fatty acids as functional components of foods- a review. J. Food Sci. Technol. 51(10): 2289-2303

Kaushik, S. J. and Seiliez, I. (2010) Protein and amino acid nutrition and metabolism in fish: Current knowledge and future needs. Aquac. Res. 41(3): 322-332

Khan, T. A., Khan, N., Ashraf, M., Qureshi, N. A., Mughal, M. S. and Abbas, G. (2012) Source, Production and Chemical Composition of Fish Meal in Pakistan. JVAS. 2(2): 65-71

Khoddami, A., Ariffin, A. A., Bakar, J. and Ghazali, H. M. (2012) Quality and fatty acid profile of the oil extracted from fish waste (head, intestine and liver) (*Euthynnus affinis*) Afr. J. Biotechnol. 11(7): 1683-1689

Kaviraj, A., Mondal, K., Mukhopadhyay, P. K. and Turchini, G. M. (2013) Impact of fermented mulberry leaf and fish offal in diet formulation of Indian major carp (*Labeo rohita*). In Proc. Zool. Soc. 66(1): 64-73

Miles, R. D. and Chapman, F. (2015) The Benefits of Fish Meal in Aquaculture Diets. University of Florida IFAS extension. pp 1-6

NRC (2011) Nutrient requirements of fish and shrimp. National Research Council. Washington, DC: The National Academies Press

Pattanaik, S. S., Sawant, P. B., Xavier, K. A. M., Dube, K., Srivastava, P. P. and Dhanabalan, V. (2020) Characterization of carotenoprotein from different shrimp shell waste for possible use as supplementary nutritive feed ingredient in animal diets. Aquaculture. 515: 734594

Prabu, E., Felix, S., Felix, N., Ahilan, B. and Ruby, P. (2017) An overview on significance of fish nutrition in aquaculture industry. Int. J. Fish Aquat. 5(6): 349-355

Roda, M. A. P. (ed.), Gilman, E., Huntington, T., Kennelly, S. J., Suuronen, P., Chaloupka, M. and Medley, P. (2019) A third assessment of global marine fisheries discards. FAO Fisheries and Aquaculture Technical Paper No. 633. Rome. 78 p

Samaddar, A. and Kaviraj, A., (2014) Processing of fish offal waste through fermentation utilizing whey as inoculum. Int. J. Recycl. Org. Waste Agricult. 3: 45

Tacon, A. G. and Metian, M. (2018) Food matters: fish, income, and food supply a comparative analysis. Rev. Fish Sci. 26(1): 15-28

Tacon, A. G. J., Hasan, M. R. and Subasinghe, R. P. (2006) Use of fishery resources as feed inputs for aquaculture development: trend and policy implications. FAO Fisheries Circular No. 1018. Rome, FAO. pp 37-53

Tasbozan, O., and Gökçe, M. A. (2017) Fatty Acids in Fish. Fatty Acids. 1: 143-159