advances in Technology

Adv. Technol. **2022**, *2(2), 131-138*

Full Paper

Grouping the Potential Local Feed Ingredients for Ornamental Fish Feed based on their Nutrient Composition, Cost, and Availability

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Received: 13 January 2022; Revised: 10 April 2022; Accepted:2 May 2022; Published: 25 May 2022

Abstract
In the ornamental fish industry, there is a growing concern about the higher freight cost of feed ingredients. The present study aimed to cluster the locally available ingredients based on their nutrient contents, price, and availability. A total of 15 ingredients; fish meal (FM), by-catches (BC), soybean seeds (Soy), maize (Ma), duckweed (DW), *Azolla pinnata* (Az), rice bran (RB), shrimp shells (SS), palmyrah fruit pulp (PFP), red seaweed (RS), wheat flour (WF), cassava flour (CF), cornflour (CoF), coconut copra waste (CCC), and groundnut cake (GNC) were selected as locally available ingredients. Principal Component Analysis (PCA) was performed by grouping the ingredients based on their nutrient contents, price, and availability using Minitab 16. In PCA, the two first components accounted for 57.50% of the total variance in the initial data. Protein, calcium, phosphorus, and price contribute positively with component 1, whereas carbohydrate content is related negatively to this component. Calcium and ash content correlated positively with component 2 and fat, gross energy, and availability were linked negatively to it. The PCA allowed the clustering the ingredients into four groups. Ingredients in Group 1, including Az, SS, RB, DW, and RS showed a positive correlation with component 2 and they contain more fiber although, they are not accessible throughout the year. Ingredients of Group 2, PFP, WF, CoF, and CF are excellent sources of starch but contained less protein content (0.94%, 11.00%, 0.00%, and 2.80% respectively). CCC, Ma, GNC, and SB pertain to group 3 correlating poorly with component 2. They are an excellent source of protein with the advantage that they are relatively affordable and easy to available than the other groups. Ingredients in group 4 incorporated BC and FB. Although they are high in protein, they are expensive and very poor in carbohydrates. Therefore, it is recommended that the ingredients in group 3 are the best choice for feed formulation with balanced nutrition at a low cost for fish feed preparation.

Keywords: Clustering, feed ingredients, ornamental fish, principal component analysis

Introduction

Aquaculture has experienced 7-9% growth annually for the last two decades to meet the increasing demand of fish worldwide [1]. Availability, cost, and nutrient composition of the raw ingredients for fish feed formulation are the major influencing factors in sustainable aquaculture production [2,3]. The growing concern in the aquaculture industry is the cost of fish feed formulation and nutrient contents of the diets. Fish feed cost accounts for about 40 to 50 % of the total expenditure of the total fish production [4]. The increasing cost of fish feed is due to the high cost of ingredients such as fish meal which is the main ingredient used in the formulation of commercial feeds [5] because of its high protein content and good

https://doi.org/10.31357/ait.v2i2.5445

minerals. Moreover, fish feed formulation is an applied technology on the knowledge of the nature and qualities of various feedstuff as well as nutrient requirements of fish. Several plant and animal derivatives such as fish wastes and bycatches, *Azolla* sp., duckweed, soybean seeds, coconut copra cake, and groundnut cake contain an appreciable quantity of protein with a good amino acid profile that can be utilized during feed formulation [6,7,8].

Since reducing the dependency of aquaculture on high-cost ingredients is key for sustainable development of the industry, many studies have been carried out to find out the alternative low-cost fish feed ingredient with adequate nutrition such as fish wastes [9,10], soybean seeds [11], groundnut cake [12], duckweed [13], and *Azolla sp*. [14]. To expand the ornamental fish industry, there is a need to formulate nutritious and economical diets that do not rely on a particular protein ingredient source. This would alleviate the dependence on animal and plant waste products, utilize renewable ingredients, and thus help to decrease the production costs.

However, there is limited information on the grouping of low-cost fish feed ingredients locally available in Sri Lanka. Therefore, the present study aimed to formulate a healthy diet clustering the low-cost fish feed ingredients collected from local regions.

Experimental Section/Materials and Methods

Raw feed ingredients:

A total of 15 feed ingredients such as fish meal, by-catches, shrimp shells, soybean seeds, maize, coconut copra waste, groundnut cake, red seaweed, palmyrah fruit pulp, duckweed, *Azolla pinnata*, cassava flour, rice bran, wheat flour, and corn flour were used for the present study. Information on prices, procurement areas, and availability were recorded for each raw ingredient.

Samples of fish meal (FM) and by-catches (BC) were collected at the Kurunagar fish landing site and Kurunagar fish Processing factory in Jaffna, Sri Lanka. They were kept in an ice box and brought to the laboratory. Samples were allowed to thaw, gutted, cleaned and dried in an oven at 100°C to a constant weight. The dried FM and BC were ground using a laboratory-scale grinder into a fine powder.

Shrimp shells (SW) were collected from household wastes, brought to the laboratory and cleaned with tap water, followed by distilled water. Red seaweed (*Gracilaria edulis*) (RS) was collected from the Point Pedro coastal waters of the Jaffna peninsula. Duckweed (DW), *Azolla pinnata* (Az) were collected from an agricultural farm, Thirunelveli, Jaffna, Sri Lanka. SW, RSW, DW, and Az were dried in an oven at 100°C separately. Thereafter, SW, RSW, DW, Az, Soybean seeds (SB), maize (Ma), coconut copra waste (CCC), and groundnut cake (GNC) were ground into fine powder separately and stored in labelled sterilized containers and refrigerated until further use.

Fresh cassava tubers (CF) from the market, Thirunelveli, Jaffna, Sri Lanka, Rice bran (RB) from the rice mill and wheat flour (prima) (WF) and corn flour (CoF) from the grocery stores were purchased. Fresh CF was cleaned, boiled with steam to evaporate cyanide and dried in an oven at 55°C until obtained a constant weight and then ground to fine powder.

Ripe palmyrah fruits (PFP) collected in the Northern region of Sri Lanka were cleaned and removed the husks. The fruits were rubbed on a sieve to extract pulp without adding water and the pulp (PFP) was stored in a freezer at -20°C.

Proximate composition of raw feed ingredients

Proximate composition (Crude protein, fat content, ash content, gross energy content, crude fibre content, and calcium) of each raw ingredient was determined according to standard analytical procedures [15].

Crude protein

The crude protein was determined by Kjeldahl method. The dried sample approximate weight was weighed in a digestion flask and digested by heating at 420°C in the presence of sulfuric acid, hydrogen peroxide and a catalyst containing copper, selenium, and titanium compounds. The liberated ammonia was collected in boric acid solution and total nitrogen was determined titrimetrically. The percentage of protein in the sample was calculated.

Percentage of crude protein content (in dry weight) = % of Nitrogen x 6.25

Crude fat

The dried sample (5g) was placed in the thimble and it was kept in the soxhlet apparatus. Petroleum ether (90 mL) was filled in the round bottom flask and extracted for about 6 hours. The ether extract was collected in the rotary bottle (W1). The solvent was evaporated on a rotary evaporator. The rotary bottle was dried and placed in a desiccator for 2 hours and then weighed with the lipid (W2)

The lipid content (in dry weight) is given by the equation = (W2-W1)/sample weight X 100.

Ash content

The sample (1 g ± 0.5) was kept in a muffle furnace at about 550-600 ˚C for 6 hours. The ash content will be calculated by the Equation 1.

$$\text{Percentage of ash content} = \frac{\text{Weight of ash}}{\text{Weight of initial sample}} \text{ x } 100\ \% \qquad \text{Equation 1}$$

Gross energy content

Gross energy content was calculated using the bomb calorimetric method in Equation 2. The amount of heat was measured in calories that is released when the feed ingredient is completely oxidized in a bomb calorimeter (Calorie measuring unit-J, 1013-J) containing 25 to 30 atmospheres of O_2.

$$\text{Gross energy (calorie/g)} = \frac{(\text{FT}-\text{IT})(\text{W})-(\text{CV}_\text{T}+\text{CVw})-(\text{ml of Na}_2\text{CO}_3\text{ consumed})}{\text{Weight of sample (1g)}} \qquad \text{Equation 2}$$

FT- Final Temperature

IT – Initial Temperature

CV_T – Caloric value of thread

CV_W- Caloric value of ignition wire

W – Water equivalent

Crude fibre content

It was determined as that fraction remaining after digestion with standard solutions (1.25% v/v) of sulphuric acid and sodium hydroxide under carefully controlled conditions (pH neutralization).

Phosphorous content

It was determined by the colorimetric method of Fiske and SubbaRow [16]. Phosphorus is precipitated from the dissolved ash as ammonium phosphomolybdate. This precipitate is dissolved in base, and excess base is titrated with acid.

Calcium content

Calcium was determined by dry ashing samples of feed ingredients and dissolving the ash in dilute HCl (0.2 mol/L), precipitating the calcium as the oxalate, then dissolving the calcium oxalate and titrating with 0.1 N potassium permanganate [15].

Statistical analysis

Statistical analysis was done in Minitab 16 for descriptive statistics and averages with standard deviations. The mean values were compared using ANOVA (one way) followed by the Tukey's post-hoc test to generate homogenous subsets. Means were considered significant at $p<0.05$. Principal Component Analysis (PCA) performed on the generated data allowed grouping the ingredients on the basis of their nutritional composition such as protein, fat, carbohydrate, ash, gross energy, fibre, calcium, and phosphorous as well as their cost and availability

Results and Discussion

Raw fish feed ingredients:

Table 1 shows the locally available raw feed ingredients in the fish feed formulations according to their cost, mode of procurement, and availability. It can be seen that there is a great diversity in the local products and by-products which are able to utilize for the feed formulation. The ingredients are animal and plant derivatives which are sufficiently available in the Northern region but some are not consistently available throughout the year.

Table 1. Prices, procurement, and availability of raw fish feed ingredients

Raw feed Ingredients	**Price/1Kg (LKR)**	**Mode of procurement**	**Availability (1-Regular; 2 – Seasonal)**
FM	600	Fish landing sites and processing centers	1
BC	0	Fish landing sites and processing centers	1
Soy	280	Grocery shop	1
Ma	280	Grocery shop	1
DW	0	Agricultural farm	2
Az	0	Agricultural farm	2
RB	60	Grocery shop	1
SS	0	Household wastes	1
PFP	0	The Northern region, Sri Lanka	2
RS	0	Point Pedro coastal waters	1
WF	90	Grocery shop	1
CF	50	Market	1
CoF	100	Grocery shop	1
CCC	0	Grinding mill	1
GNC	0	Grinding mill	1

FM- Fish meal, BC – By-catches, Soy – Soybean seeds, Ma- Maize, DW – Duckweed, Az- *Azolla pinnata*, RB – Rice bran, SS – Shrimp shells, PFP – Palmyrah fruit pulp, RS - Red seaweed, WF- Wheat flour, CF- Cassava flour, CoF - Cornflour, CCC- Coconut copra waste, and GNC - Groundnut cake

The nutrient composition of the raw ingredients is presented in Table 2. The selected feedstuff contains crude protein in amounts ranging from 0.00 % for CoF to 65.24 % for FM. The higher crude protein contents of raw fish feed ingredients were 65.24 ± 6.68, 52.55 ± 5.71, 42.52 ± 3.02, 24.00 ± 0.51, 31.00 ± 7.76 and 24.00 ± 3.21 g/100g (DW) for FM, BC, SB, GNC, DW, and Az respectively. These types of feedstuff have been used as protein sources in different fish feed formulations: FWBC [9,10], SB [11], GNC [12], DW [13], and Az [14]. The dry weight of carbohydrate contents (%) of raw ingredients were determined as 66.00 ± 1.52 (RB), 56.00 ± 1.52 (Ma), 72.00 ± 3.60 (WF), and 60.00 ± 0.62 (CCC). These results have a very good proximity with the reported literature [17,18, 4]. Starchy products such as CF, WF, and CoF were used as a binder contributing to the physical quality of the extruded pellet [19,20]. The highest fat content was recorded in coconut copra cake (15.99% ± 1.65). The red seaweed recorded the highest ash content (32.56 % ± 0.39 DM) followed by Az (30.89 % ± 0.40 DM). The highest fibre content was for CCC (16 % ± 0.34 DM) while the shrimp shells had the lowest.

Table 2. Nutrient compositions of feed ingredients

Ingredients	**Protein (%) DW**	**Fat (%) DW**	**Carbohydrate (%) DW**	**Gross Energy (Kcal/Kg)**	**Fibre (%) DW**	**Calcium (g/100g)**	**Phosphate (g/100g)**	**Ash (%)**
FM	65.24 ± 6.68^{a}	12.21 ± 0.41^{b}	0.01 ± 0.00^{i}	4520 ± 85.32^{b}	12.00 ± 0.49^{d}	2.68 ± 0.01^{a}	2.76 ± 0.06^{a}	11.23 ± 0.45^{f}
BC	52.55 ± 5.71^{b}	11.04 ± 0.68^{b}	0.03 ± 0.01^{i}	4490 ± 215.08^{b}	10.00 ± 0.32^{e}	2.65 ± 0.08^{a}	2.23 ± 0.01^{b}	13.20 ± 0.52^{e}
Soy	42.52 ± 3.02^{b}	15.99 ± 1.73^{a}	9.00 ± 1.06^{g,h}	4200 ± 55.07^{c}	9.00 ± 0.32^{f}	0.27 ± 0.01^{d}	0.51 ± 0.00^{d}	4.8 ± 0.20^{g}
Ma	9.52 ± 0.86^{e,f}	15.99 ± 0.84^{a}	56.00 ± 1.52^{d}	3900 ± 50.00^{d,e}	2.70 ± 0.05^{j}	0.02 ± 0.01^{e}	0.06 ± 0.00^{g,h}	2.76 ± 0.12^{h}
DW	31.00 ± 7.76^{c,d}	4.00 ± 0.34^{d}	21.40 ± 2.08^{f}	2006 ± 97.01^{h}	12.00 ± 0.36^{d}	0.50 ± 0.07^{b,c}	0.02 ± 0.00^{h}	5 ± 0.30^{g}
Az	24.00 ± 3.21^{c,d}	3.31 ± 1.07^{d}	10.00 ± 0.19^{g,h}	228.62 ± 8.95^{j}	14.70 ± 0.27^{c}	2.58 ± 0.03^{a}	0.26 ± 0.00^{e}	30.89 ± 0.40^{b}
RB	6.11 ± 0.66^{e,f}	0.11 ± 0.13^{c}	66.00 ± 1.52^{b}	3,494 ± 35.83^{f}	15.00 ± 0.25^{b}	0.58 ± 0.01^{b}	1.18 ± 0.00^{c}	15 ± 0.5^{d}
SS	33.12 ± 4.93^{b,c}	3.23 ± 0.21^{d}	6.00 ± 0.27^{h}	4020 ± 52.91^{c,d}	0.00^{k}	0.44 ± 0.03^{c}	0.04 ± 0.00^{h}	23.91 ± 0.31^{c}
PFP	0.94 ± 0.64^{f}	0.18 ± 0.03^{e}	21.00 ± 0.78^{f}	1028.3 ± 56.05^{i}	10.90 ± 0.18^{e}	0.09 ± 0.01^{e}	0.03 ± 0.00^{h}	0.55 ± 0.03^{j}
RS	20.84 ± 2.41^{d}	8.08 ± 0.26^{c}	12.58 ± 0.44^{g}	145 ± 5.00^{j}	8.00 ± 0.40^{g}	0.06 ± 0.00^{e}	0.12 ± 0.01^{f,g}	32.56 ± 0.39^{a}
WF	11.00 ± 1.52^{e,f}	3.50 ± 0.07^{d}	72.00 ± 3.60^{a}	339 ± 5.50^{j}	12.20 ± 0.10^{d}	0.31 ± 0.00^{d}	0.13 ± 0.00^{f}	1.8 ± 0.1^{i}
CF	2.80 ± 0.61^{e,f}	0.30 ± 0.09^{e}	78.40 ± 1.61^{a}	330 ± 5.77^{j}	3.70 ± 0.1^{i}	0.03 ± 0.00^{e}	0.55 ± 0.00^{d}	1.5 ± 0.05^{i}

CoF	0.00^{f}	0.00^{e}	30.00 ± 0.62^{e}	3810 ± 95.04^{e}	3.00 ± 0.15^{i,j}	0.00^{e}	0.00^{h}	0.00^{j}
CCC	13.00 ± 1.27^{e}	15.99 ± 1.65^{a}	60.00 ± 0.62^{c}	5872 ± 51.58^{a}	16.00 ± 0.34^{a}	0.00^{e}	0.00^{h}	5.35 ± 0.19^{g}
GNC	24.00 ± 0.51^{d}	14.84 ± 0.64^{a}	22.00 ± 1.02^{f}	2664 ± 50.64^{g}	4.80 ± 0.05^{h}	0.08 ± 0.00^{e}	0.57 ± 0.01^{d}	5.53 ± 0.06^{g}

Dissimilar superscripts in a column indicate significant different ($p<0.05$)

FM- Fish meal, BC – By-catches, Soy – Soybean seeds, Ma- Maize, DW – Duckweed, Az- *Azolla pinnata*, RB – Rice bran, SS – Shrimp shells, PFP – Palmyrah fruit pulp, RS - Red seaweed, WF- Wheat flour, CF- Cassava flour, CoF - Cornflour, CCC- Coconut copra waste, GNC - Groundnut cake, and DW- Dry weight

The 15 fish feed ingredients are positioned in Figure 1 as a function of the two first components, which accounted for 57.50 % of the total variance in the initial data. In interpreting the rotated component pattern, we considered a parameter as a good contributor to the variation in the dataset when its component loading was greater than 0.3 (Table 3).

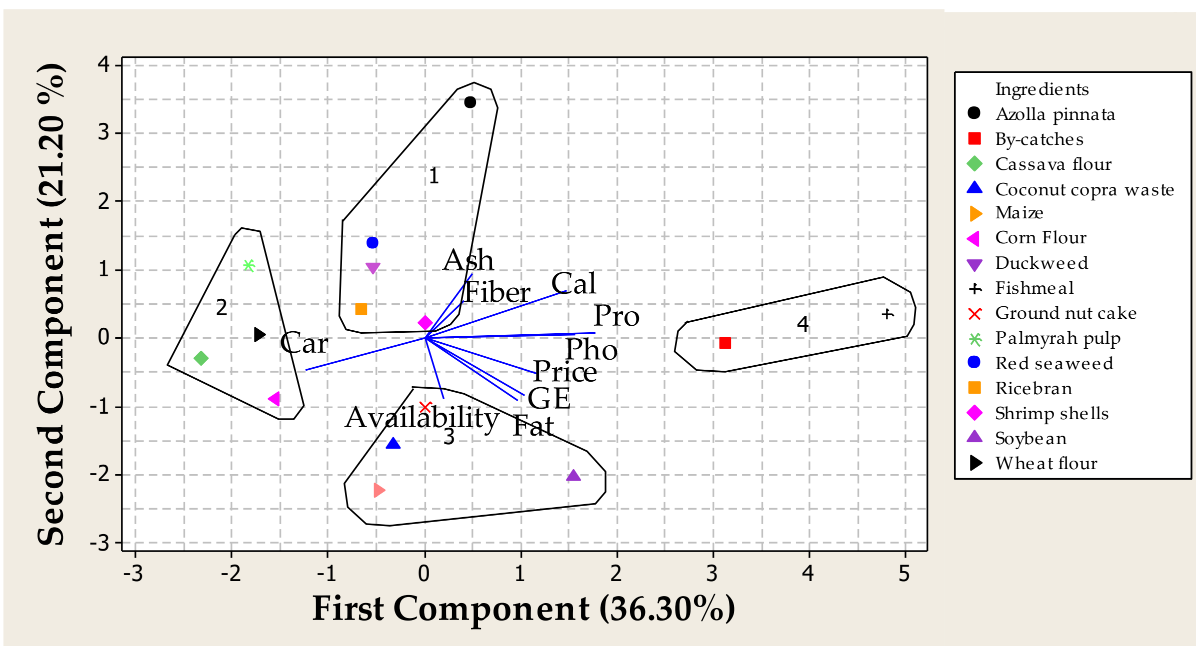

Figure 1. PCA clustering of the ingredients
Cal- Calcium, Car- Carbohydrate, GE- Gross energy, Pho- Phosphate, Pro – Protein

Table 3. Pattern matrix

	Principal components	
Variable	Component 1	Component 2
Proportion	0.363	0.212
Protein	0.490	0.042
Fat	0.286	-0.395
Carbohydrate	-0.341	-0.215
Gross energy	0.267	-0.430

Fiber	0.111	0.264
Calcium	0.409	0.330
Phosphorous	0.430	0.023
Ash	0.139	0.442
Price	0.321	-0.244
Availability	0.054	-0.421

Protein, calcium, phosphorus, and price contribute positively to component 1, whereas carbohydrate content is related negatively to this component. Calcium and ash content correlated positively with component 2 and Fat, gross energy, and availability were linked negatively to it.

The PCA allowed the clustering of the ingredients into four groups (Figure 1). Ingredients in Group 1, including *Azolla pinnata,* shrimp shells, rice bran, duckweed, and red seaweed showed a positive correlation with component 2 and they contain more fibre than the other group ingredients however, they are not rich in fat and also not accessible throughout the year. Ingredients of Group 2, i.e. palmyrah fruit pulp, wheat flour, corn flour, and cassava flour contain a high carbohydrate content and they would be excellent sources of starch in fish feed formulation. Coconut copra waste, maize, groundnut cake, and soybean contained in group 3 correlated poorly with component 2. They are an excellent source of protein with the advantages that they are relatively affordable and available more frequently than the other groups. Ingredients in group 4 showed a positive correlation with component 1 and incorporated by-catches and fish meal. Obviously, fishmeal is the main ingredient in the formulation of commercial feeds [5]. These results seem to indicate that these raw ingredients are containing higher crude protein and poor in carbohydrate and therefore should be mixed with other nutrient components to formulate fish feed with optimum nutrient composition.

The results of the clustering analysis indicate that for local fish feed formulation, the identified raw ingredients in group 3 are possible to use as local fish feed ingredients available in the Northern region to formulate fish feeds with satisfactory nutritional composition. It is in agreement with the study of Ghosh and Mandal [12], which demonstrated the acceptable nutritional value of Groundnut oil cake as an ingredient in the diet of rohu, *Labeo rohita* (Hamilton) fingerlings and had no adverse effect on growth and feed utilization efficiency. Moreover, incorporation of GNC would be cost-effective as it is much cheaper than FM. The results obtained by Yu et al. [21] suggested that higher soybean protein concentrate in the feed mixture increases the bulk density and reduces the expansion ratio during the development of maize-based extruded products.

Conclusion

The present study characterized the local fish feed ingredients in the Northern province for their nutrient composition, price and availability. Among all ingredients, the present study identified the best cluster including the ingredients: coconut copra waste, groundnut cake, maize, and soybean for fish feed formulation in the aquaculture industry. Further investigation is needed to assess feed palatability and growth of fish for the formulated fish feed using the above cluster with a satisfactory nutritional composition.

Conflicts of Interest

The authors declare no conflict of interest

Acknowledgement
The authors wish to thank agriculture farm, Thirunelveli, Jaffna to provide *Azolla pinnata* and Duck weed.
Funding
This work was supported by authorities of University of Jaffna, Sri Lanka (Grant No: URG/2021/SEIT/30).
References
[1] J. Arnason, B.K. Larsen, B.T. Björnsson, K. Sundell, A.C. Hansen, O. Lindahl, S. Kalsdottir, R. Bjornsdottir, *Local fish feed ingredients for competitive and sustainable production of high-quality aquaculture feed, LIFF*, Nordic Innovation Publication, **2015**, 72..

[2] M.R. Hasan, in *Aquaculture in the Third Millennium,* (Eds: R.P. Subasinghe, P. Bueno, M.J. Phillips, C. Hough, S.E. McGladdery, J.R. Arthur), Technical Proceedings of the Conference on Aquaculture in the Third Millennium, Bangkok, Thailand, **2000**, 193-219.

[3] K.J. Rana, S. Siriwardena, M.R. Hasan**,** Impact of rising feed ingredient prices on aquafeeds and aquaculture production, *FAO Fisheries and Aquaculture Technical Paper*. No. 541. Rome, FAO, **2009**. 63.

[4] A. D. Adéyèmi, A. P. P. Kayodé, I. B. Chabi, Screening local feed ingredients of Benin, West Africa, for fish feed formulation. *Aquaculture Reports*, **2020**, *17*:100386.

[5] J. Ye, X. Liu, Z. Wang, K. Wang, Effect of partial fishmeal replacement by soy- bean meal on the growth performance and biochemical indices of juvenile Japanese flounder *Paralichthys olivaceus*, *Aquaculture International,* **2011**, 19 (1), 143–153.

[6] D.M.Gatlin, F.T. Barrows, P. Brown, K. Dabrowski, T.G. Gaylord, R.W. Hardy, E. Herman, G. Hu, A. Krogdahl, R. Nelson, K. Overturf, M. Rust, W. Sealey, D. Skonberg, E.J. Souza, D. Stone, R. Wilson, E. Wurtele, Expanding the utilization of sustainable plant products in aquafeeds: A review. *Aquaculture Research,* **2007**, 38 (6), 551–579.

[7] R.W. Hardy, Utilization of plant proteins in fish diets: Effects of global demand and supplies of fishmeal. *Aquaculture Research*, **2010**, 41 (5), 770–776.

[8] J.O. Agboola, R. Yossa, J. Verreth, J. Verreth, Assessment of existing and potential feed resources for improving aquaculture production in selected Asian and African countries Assessment of existing and potential feed resources for improving aquaculture production in selected Asian and African countri. Penang, Malaysia: *CGIAR Research Program on Fish Agri-Food Systems*. Program Report: FISH. **2019**.

[9] U.N. Wijkstrom, *The use of wild fish as aquaculture feed and its effects on income and food for the poor and the undernourished*. FAO Fisheries and Aquaculture Technical Paper, **2009**, 518, 371–407.

[10] M. Afreen, Ucak, Fish processing wastes used as feed ingredient for animal feed and aquaculture feed, *Journal of Survey in Fisheries Sciences*, **2021**, 6 (2), 55–64.

[11] S. Refstie, T. Storebakken, G. Baeverfjord, A.J. Roem, 2001. Long-term protein and lipid growth of At- lantic salmon (Salmo salar) fed diets with partial replacement of fish meal by soy protein products at medium or high lipid level, *Aquaculture*, **2001**, 193 (1–2), 91–106.

[12] K. Ghosh, S. Mandal, 2015. Nutritional evaluation of groundnut oil cake in formulated diets for rohu, Labeo rohita (Hamilton) fingerlings after solid state fermentation with a tannase producing yeast, Pichia kudriavzevii (GU939629) isolated from fish gut, *Aquaculture Reports*, **2015**, 2, 82–90

[13] C. Anthonius, A.S.K. Yong, C.F. Fui, Supplementation of duckweed diet and citric acid on growth performance, feed utilization, digestibility and phosphorus utilization of TGGG hybrid grouper (*Epinephelus fuscoguttatus x Epinephelus lanceolatus*) juvenile, *Songklanakarin Journal of Science and Technology*, **2018**, 40 (5), 1009–1016

[14] M. Das, F.I. Rahim, M.A. Hossain, Evaluation of Fresh Azolla pinnata as a Low-Cost Supplemental Feed for Thai Silver Barb Barbonymus gonionotus, *Fishes*, **2018**, 3 (1), 1–11.

[15] AOAC, Official Methods of Analysis of Official Analytical Chemists International, 16th ed. Association of Official Analytical Chemists, Arlington, VA, USA, **1995**.

[16] C.H. Fiske, Y. SubbaRow, The colometric determination of phosphorus, *Journal of Biological Chemistry*, **1925**, *66*, 375-400.

[17] A. Kouba, J. Sales, M. Sergejevová, P. Kozák, J. Masojídek, Colour intensity in angelfish (Pterophyllum scalare) as influenced by dietary microalgae addition, *Journal of Applied Ichthyology*, **2013**, *29 (1),* 193–199.

[18] S.S.K. Madage, W.U.D. Medis, Y. Sultanbawa, Fish Silage as Replacement of Fishmeal in Red Tilapia Feeds, *Journal of Applied Aquaculture*,**2015**, 27 (2).

[19] N. Kanmani, N. Romano, M. Ebrahimi, S.M. Nurul Amin, M.S. Kamarudin, A. Karami, V. Kumar, Improvement

of feed pellet characteristics by dietary pre-gelatinized starch and their subsequent effects on growth and physiology in tilapia, *Food Chemistry*, **2018**, *239*, 1037–1046.
[20] S. **S**utharshiny, D.I. Uluwaduge, K. Sivashanthini, *Improving the floatation of fish feed pellets using starch gelatinization incorporated with palmyrah fruit pulp*. *Georgia Chapter American Fisheries Society*, **2021**, 1.
[21] L. Yu, H.S. Ramaswamy, J. Boye, Protein rich extruded products prepared from soy protein isolate-corn flour blends, *Food Science and Technology*, **2013**, *50*,279–289.