Valorization of weed *Portulaca oleracea* L. as an alternative to fish feed ingredient

R Lia Rahadian Amalia[1], *Lusi H* Suryaningrum[1,*], *Sumitro* Sumitro[2], *Budiyanti* Budiyanti[2], *Sulasy* Rohmy[3], *Bastiar* Nur[3] and *Mulyasari* Mulyasari[4]

[1]Research Center for Applied Zoology, Research Organization for Life Sciences and Environment, National Research and Innovation Agency (BRIN), Cibinong, West Java 16911, Indonesia
[2]Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Universitas Dayanu Ikhsanuddin, Baubau, Sulawesi Tenggara 93724, Indonesia
[3]Research Center for Fishery, Research Organization for Earth Sciences and Maritime, National Research and Innovation Agency (BRIN), Cibinong, West Java 16911, Indonesia
[4]Research Center for Marine and Land Bioindustry, Research Organization for Earth Sciences and Maritime, National Research and Innovation Agency (BRIN), North Lombok, West Nusa Tenggara 83352, Indonesia

Abstract. Feed ingredients such as fishmeal and soybean meal are common used in the aquaculture industry. However, they have drawbacks both environmentally and economically and should switch over to more sustainable materials. Using a weed in fish feed opens up potential and environmentally conscious possibilities. The objective of this study is to explore the potential valorization of a common weed plant called *Portulaca oleracea* L., using enzymatic hydrolysis for incorporation into fish feeds. This common weed can thrive by itself without human intervention. The nutrient content of *P. oleracea* L. was 16.33% protein, 0.88% lipid, 10.19 % ash, 26.20% crude fiber, and 46.40% carbohydrates (by differences) (% dry weight). However, the high crude fiber restricts its utilization in feed formulation. Hydrolysis has been employed to reduce crude fiber and enhance its quality. The treatments in doses are as follows: 10%, 20%, 30%. and 40% (v/w). Each treatment was conducted in three triplicates for 60 hours, 55^{o}C, and pH 5.0. The result revealed that the nutrient quality of *P. oleracea* L. was improved. In conclusion, the valorized *P. oleracea* L. is feasible to be an ingredient in fish feed.

1 Introduction

The aquaculture industry faces challenges with conventional feed ingredients such as fish meal and soybean meal, which have ecological and economical drawbacks [1]. One promising and eco-friendly approach is using weed, *Portulaca oleracea* L., as an ingredient

* Corresponding author: lusi006@brin.go.id

in fish feed [2]. This innovation aligns with the circular economy principles, transforming unwanted materials into valuable feed resources.

P. oleracea L., known as common purslane, demonstrates high production efficiency with prolific growth worldwide in tropical and subtropical regions [3] [4] [5]. Its wide global distribution and ability to withstand abiotic stress [6]. Purslane is a versatile plant that can adapt and grow in various arid and moist habitats [7]. Purslane can be found in orchards, gardens, crop fields, roadsides, and even saline-alkali soils [8]. The rapid proliferation and formation of dense coverings by purslane can push aside indigenous plant species, and reducing biodiversity [7]. In agriculture, purslane will compete with cultivated crops to grow, reducing crop yield [9].

Numerous research studies have examined the nutritional profile of purslane and highlighted its potential benefits. [10] and [11] state that purslane contains various phytochemicals and bioactive substances like phenols, antioxidants, ω-3 fatty acids, minerals, flavonoids, alkaloids, organic acids, and polysaccharides. The nutrient composition of purslane can be outlined as follows 10.9% moisture, 2.45% ash, 21.63% crude fiber, 18.2% protein, 5.2% lipid, and 44.3% carbohydrates [8]. Purslane has a relatively high crude fiber content [12] [13]. This is limited its utilization in feed formulation. However, enzymatic hydrolysis could reduce the crude fiber and improve the quality of unconventional fish feed ingredients. Enzymatic hydrolysis breaks down complex fiber structures into simpler components, reducing crude fiber and improving digestibility [14]. [15] demonstrated that enzymatic hydrolysis could reduce the crude fiber content in coconut cake by 67.8%. [16] discovered that hydrolyzed corncob meal had reduced crude fiber by 57.53%, NDF fiber fraction by 38.15%, ADF fiber fraction by 6.43%, and hemicellulose by 61.96%. [17] reported that enzymatic hydrolysis reduces the fiber content of sweet sorghum bagasse. [18] stated that enzymatic hydrolysis can significantly enhance soybean hulls' nutritional value. [19] demonstrated that the bacterial enzyme extract enhances the nutrient value of sugarcane bagasse through a hydrolytic reaction.

This research aims to investigate the valorization of the weed purslane through enzymatic hydrolysis for its incorporation into fish feed. This research is expected to augment the purslane's nutritional content and improve its digestibility for fish. The research effort aspires to contribute to advancing eco-friendly and sustainable fish feed formulations, thus diminishing the dependence on conventional feed ingredients and fostering the growth of a more sustainable aquaculture industry.

2 Materials and methods

2.1 Preparation of unrefined enzyme extract

An aliquot of bacterial culture was introduced into a 10 mL Tryptic Soy Broth (TSB) medium. The cultures were subsequently incubated at 30C in an incubator for 24 hours. After this incubation, aseptically, a portion of the cultured solution, equivalent to 1 mL, was transferred to 9 mL of TSB medium, done threefold. These secondary cultures were incubated again for an additional 24-hour period. Then, the bacterial cultures underwent centrifugation at 10,000 rpm for 45 minutes at a temperature of 40^{o}C. The resulting supernatant was meticulously poured off and subsequently preserved in a refrigerated setting for further analysis.

2.2 Enzymatic hydrolysis on the purslane meal

The purslane meal was obtained from a commercial producer. Enzymatic hydrolysis was carried out by mixing 50 grams of purslane meal, buffer solution, and unrefined enzyme extract with a dose according to the treatment, namely 10%, 20%, 30%, and 40% (v/w). The mixture was diligently stirred to achieve homogenously, securely sealed, and subsequently set for a designated duration of 60 hours, pH 5.0, and temperature of 55ºC. The research was executed following a completely randomized design, with each treatment replicated three times. The mixture was heated using a conventional oven at a temperature of 105°C for 5 minutes to stop the reaction. Following that, it undergoes the drying process at 60°C for 24 hours, or until a consistent weight is achieved. The nutrient composition of purslane before and after hydrolysis were all measured.

2.3 The determination of nutrient composition in purslane

The determination of nutrient composition involved both pre- and post-enzymatic hydrolysis measurement using the proximate method, following the AOAC guidelines [20]. The moisture content was determined using the gravimetric analysis technique that entailed drying the sample material in a conventional oven at 105°C up to a constant weight. The quantification of protein content was carried out using the Kjeldahl procedure, which includes sample digestion, distillation, and titration, respectively. Lipid content was determined through organic solvent extraction. The determination of ash content involved subjecting the sample to a 600°C furnace for 3 hours, while the assessment of crude fiber content was conducted through a method with both strong acids and bases. The solubility of proteins underwent evaluation using the Bradford method [21], while the quantification of reducing sugars was conducted through the DNS method [22].

2.4 Data analysis

Data analysis was performed by tabular the obtained data using Microsoft Excel Software, followed by a study using the One-Way Analysis of Variance (ANOVA). In cases where differences were observed, Duncan's test was applied. Statistical data analysis was conducted utilizing Statistical Program for Social Science (SPSS) version 26 for Windows, while maintaining a significance level set at $p<0.05$ to ascertain statistical significance.

3 Result and Discussion

In this research, an examination and evaluation were carried out on the nutrient composition of the purslane plant, which included the levels of moisture, ash content, lipid, crude fiber, protein, and carbohydrates (by differences). Table 1 demonstrates that purslane exhibits high quantities of carbohydrates, crude fiber, and protein, accounting for 46.40%, 26.20%, and 16.33%, respectively. These findings align with [12] [13], who reported that purslane displayed elevated protein and crude fiber levels alongside comparatively lower lipid content.

Following the hydrolysis process, the unrefined enzyme extracts significantly improved purslane nutrient value. It recorded an improvement in protein, crude fiber, and ash content ($p<0.05$). There was a considerable rise in protein content (Figure 1). It began from 16.33% to 16.98%, 17.47%, 18.53%, and 19.28% respectively for the doses of 10%, 20%, 30%, and 40% (v/w). These results emphasize that a considerable enhancement in protein content was conducted by the hydrolyzation process. Lipid content increases from 0.88% to 2.75% (Figure 2). There was a significant reduction in the crude fiber content (Figure 3). Crude fiber decreased from its initial content of 26.20% and fell to a mere 11.43% for the 40% (v/w). A

reduction in ash content was witnessed (Figure 4). Particularly, it declined from the initial 10.19% to 8.04% (40% v/w). Reducing sugar and soluble protein also confirm the findings presented in Figure 5 and 6, respectively. There was an increase in the reducing sugar which went from 115.23 mg/L (initial) to 528.94 mg/L (40% v/w). The soluble protein of hydrolyzed purslane markedly increased from 101.28 mg/L (initial) to 427.45 mg/L (40% v/w). The doses of unrefined enzyme extract which results in an improved purslane nutrient value, follow a straight line. The results suggest that the hydrolysis process has improved the nutritional quality of purslane.

Table 1. The results of proximate analysis of purslane

Parameters	**%**
Moisture	8.27 ± 0.14
Protein	16.33 ± 0.13
Lipid	0.88 ± 0.09
Ash	10.19 ± 0.28
Crude fiber	26.20 ± 0.36
Carbohydrates (by differences)	46.40 ± 0.51

The data is presented as the mean ± standard deviation (n = 3)

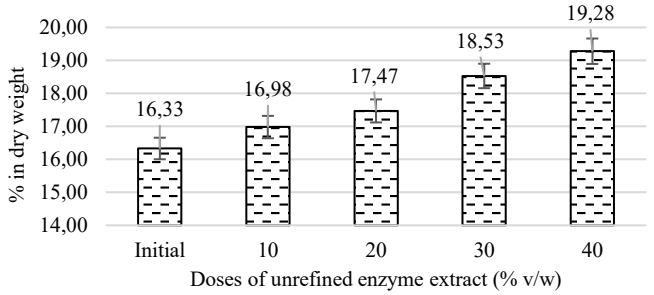

Fig. 1. Protein content of purslane (%)

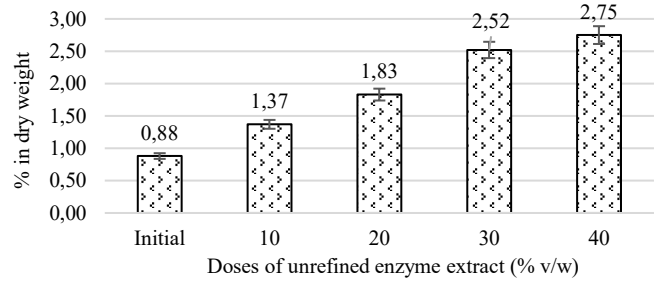

Fig. 2. Lipid content of purslane (%)

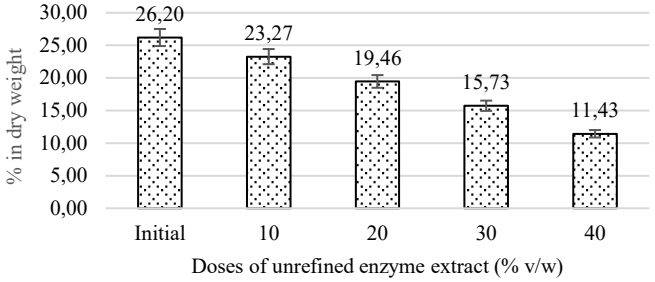

Fig. 3. Crude fiber content of purslane (%)

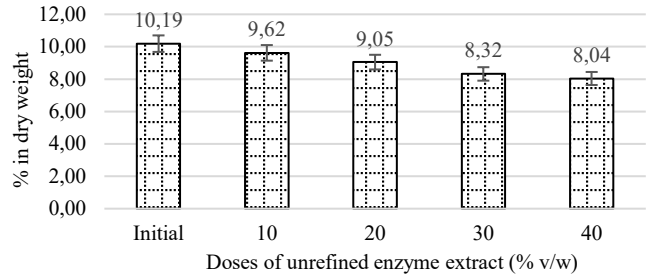

Fig. 4. Ash content of purslane (%)

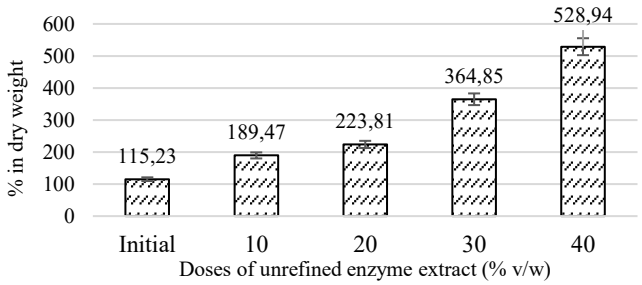

Fig. 5. Reducing sugar in purslane (mg/L)

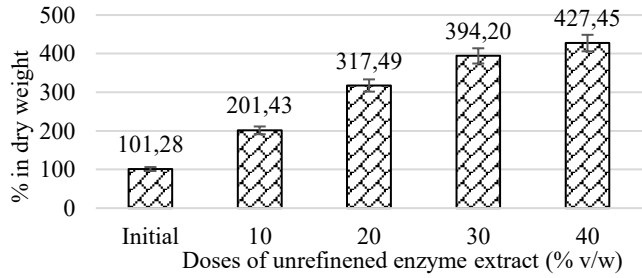

Fig. 6. Soluble protein in purslane (mg/L)

Purslane, with relatively high fiber content, necessitates preprocessing prior to its inclusion as an ingredient in fish feed. Employing enzymatic hydrolysis with an unpurified enzyme extract aims to break down the crude fiber in purslane into simpler components that are more readily digestible by fish. The enhancement in purslane quality exhibited a positive relationship with the quantity of added unpurified enzyme extract, 40% (v/w) yielding the

most favorable results. This specific outcome will be elaborated upon in the subsequent discussion.

The protein content in hydrolyzed purslane using the unrefined enzyme extract 40% (v/w) was notably elevated. The protein component in fish feed is pivotal in influencing fish growth, as it is the primary determinant. A higher protein content in the feed corresponds to an increased potential for fish growth. Protein provides amino acids that are crucial for fish growth and development. The National Research Council [23] highlights the importance of protein in fish nutrition and states that protein is the most expensive and limiting nutrient in fish feed. The protein requirements of fish depend on various factors, such as species, life stage, size, and environmental conditions. The feed formulation should consider the amino acids requirement and their bioavailability. It also must examine the balancing of both cost-effectiveness and nutrient adequacy. Using plant-based protein would increase the reliance on fish meal and fish oil, which is categorized as a non-renewable source [24]. In comparison to locally prevalent plant ingredients typically utilized in feed, such as wheat bran (11.05 %) [25], rice bran (15.23%), and fermented rice bran (17.62%) [26], sugarcane bagasse (1.49%) [27], the protein content (19.28%) in hydrolyzed purslane is relatively higher.

The hydrolyzed purslane notably increased the quantity of soluble protein, as depicted in Figure 6. Soluble proteins are either oligopeptides or amino acids that can be readily taken up by the digestive system [28]. Throughout the hydrolysis process, the interplay between the enzyme and substrate streamlined the peptide bonds, ultimately enhancing the solubility of proteins. A greater concentration of soluble proteins indicates that fish will more efficiently utilize protein for their metabolic processes and growth [29].

Purslane has a relatively significant amount of crude fiber, 26.20%. The excessive crude fiber in fish feed can pose challenges for fish as they are monogastric animals with limited ability to digest high crude fiber [30]. Fish have a relative lack of specialized microbial fermentation in their digestive tract compared to terrestrial animals. High levels of crude fiber in fish feed influence the digestion and absorption of other nutrients, leading to non-optimal nutrient utilization and fish growth [31]. Hydrolyzation process with enzyme extract from plant or microbial sources, which contains specific enzymes, such as cellulases or hemicellulases, can break down complex carbohydrates in crude fiber into more straightforward and digestible by fish [19]. In this research, the unrefined enzyme extract reduces the crude fiber of purslane from 26.20% to 11.43%. Lower than the plant-based ingredients commonly used in fish feed, such as fermented palm kernel cake (12.35%) [32] or wheat bran (13.02 %) [25].

This approximation is supported by the formation of reducing sugars in hydrolyzed purslane, as depicted in Figure 5. The elevated levels of reducing sugars in the hydrolyzed purslane indicate the creation of simpler constituents resulting from the degradation of crude fiber by the unrefined enzyme extract [33]. Glucose, the simplest form of carbohydrate, is readily digestible by fish [34].

An excessively high ash content in fish feed can adversely affect the utilization of nutrients by fish. A high ash content can disrupt the fish's digestive system. High ash content can bind essential minerals and other nutrients in the feed, reducing their bioavailability [35]. This means that nutrients may not be efficiently absorbed by the fish, even if they are present in the feed [36].

Consequently, fish may experience nutrient deficiencies despite these nutrients in the feed. High ash content can also reduce the availability of energy in the feed [37]. If ash replaces these energy-rich components in the feed, fish may not receive sufficient energy for optimal growth and metabolism. This can lead to decreased productivity in fish farming [38]. Hydrolyzed purslane through enzymatic reaction resulted in a reduction of ash content from 10.19% to 8.04%. The ash content within the hydrolyzed purslane exhibited a notable

decrease in comparison to that of Moringa leaves meal (9.70%) and fermented Moringa leaves meal (14.05%) [39].

Utilizing hydrolyzed materials as ingredients in fish feed has garnered widespread recognition and application. [25] reported that incorporating hydrolyzed wheat bran at levels of up to 20% into Nile tilapia (*Oreochromis niloticus*) diets resulted in elevated protein content, reduced fiber content, and improved growth, feed utilization, and blood parameters as well as intestinal and liver development. [40] reported that enzyme pretreatment successfully enhances the nutritive value of high-fiber plant byproducts, leading to increased growth and feed efficiency in *Mugil cephalus* [41] presented research findings regarding the influences of a carbohydrase-rich extract obtained from hydrolyzed Brewer's spent grain (BSG). This extract was introduced into the European seabass (*Dicentrarchus labrax*) diet to improve the digestibility of dry matter, starch, cellulose, glucans, and energy. This was validated through both in vitro and in vivo assessments of digestibility.

In a separate study, [27] revealed that hydrolyzed sugarcane bagasse had a digestibility of 66.08% for dry matter, 84.35% for protein, 95.26% for lipid, and 70.49% for energy when fed to *Barbonymus schwanenfeldii*. These results suggest that hydrolyzed sugarcane bagasse is a reasonably digestible nutritional resource for *B. schwanenfeldii* and could be incorporated into its diet. Moreover, [42] reported that replacing fish meal with a hydrolyzed plant-based protein concentrate, at levels of up to 27.9% of the fish meal in juvenile olive flounder (*Paralichthys olivaceus*) diet, had no significant impact on survival rates, overall nutritional composition, hematological parameters (such as aspartate aminotransferase, alanine aminotransferase, glucose, total serum protein), and responses of non-specific immune (such as lysozyme and activity of superoxide dismutase).

4 Conclusions

Applying an unrefined enzyme extract of 40% (v/w) led to a substantial increase in protein, soluble protein, and reducing sugars while concurrently decreasing the crude fiber and ash content in purslane. The findings of this investigation indicate that the use of enzymatic hydrolysis with the unrefined enzyme extract has improved the quality of purslane to the extent that its inclusion as an ingredient in fish feed is feasible.

References

1. B. Samuel-Fitwi, S. Meyer, K. Reckmann, J. Schroeder & C. Schulz, J. Clean. Prod. **52**, 225-233 (2013). https://doi.org/10.1016/J.JCLEPRO.2013.02.031
2. Z. Sourani, S. Shirian, S. Shafiei, N. Mosayebi, A. Nematollahi, Mar. Biotechnol. **25,** 214-221 (2023). http://dx.doi.org/10.1007/s10126-022-10195-z
3. R. Srivastava, V. Srivastava, A. Singh, Environ. Manage. **72**, 309–320 (2023). https://doi.org/10.1007/s00267-021-01456-z
4. J. He, S.Y. Leng, L. Qin, Plants, **12**, 3214 (2023). http://dx.doi.org/10.3390/plants12183214
5. A. Carrascosa, J.A. Pascual, M. Ros, S.A. Petropoulos, M. del M. Alguacil, Plants, **12**, 1246 (2023). http://dx.doi.org/10.3390/plants12061246
6. K. Li, T. Xia, Y. Jiang, N. Wang, L. Lai, S. Xu, X. Yue, H. Xin, J. Ethnopharmacol. **319**, Part 2, 117211 (2024), https://doi.org/10.1016/j.jep.2023.117211
7. M. Fernandez-Poyatos, E. Llorent-Martínez, A. Ruiz-Medina, Foods, **10**, 94 (2021). https://doi.org/10.3390/foods10010094
8. S. Karakas, I. Bolat, M. Dikilitas, Hortic. **7**, 63 (2021). https://doi.org/10.3390/horticulturae7040063

9. M. Camalle, D. Standing, M. Jitan, R. Muhaisen, N. Bader, M. Bsoul, Y. Ventura, A. Soltabayeva, M. Sagi, Agron. **10**, 656 (2020). https://doi.org/10.3390/agronomy10050656
10. M. Uddin, A.S. Juraimi, M.S. Hossain, M. Nahar, A. Un, M. Ali, M. Rahman, Sci. World. J 951019 (2014). https://doi.org/10.1155/2014/951019
11. C.O. Montoya-García, R. García-Mateos, E. Becerra-Martínez, R. Toledo-Aguilar, V.H. Volke-Haller, J.J. Magdaleno-Villar, Sci. Hortic. **308**, 111584 (2023). https://doi.org/10.1016/j.scienta.2022.111584
12. D.A. Al-Quwaie, A. Allohibi, M. Aljadani, A.M. Alghamdi, A.A. Alharbi, R.S. Baty, S.H Qahl, O. Saleh, A.O. Shakak, F.S. Alqahtani, O.S.F. Khalil, M.T. El-Saadony, A.M. Saad, Molecules, **28**, 5859 2023. https://doi.org/10.3390/ molecules28155859
13. W. Obied, E. Mohamoud, O. Mohamed, Small. Rumin. Res. **48**, 31–36 (2003)
14. S. Borges, J. Odila, G. Voss, R. Martins, A. Rosa, J.A. Couto, A. Almeida, M. Pintado, Molecules, **28**, 1155 (2023). https://doi.org/10.3390/molecules28031155
15. F.M. Pratiwy, Y. Maulida, Asian J. Fish. Aquat. Res. **20**, 95-101 (2022). https://doi.org/10.9734/ajfar/2022/v20i6521
16. M.A. Fauzan, A. Suprayudi, A. Fauzil, J. Ekasari. JAI, **21**, 1 (2022), 32-40. https://doi.org/10.19027/jai.21.1.32-40
17. R. Haiwei, S. Ruifeng, W. Huiyuan, W. Li, G. Xiaopeng, L. Dong, L. Ruiyuan, L. Jinping, (Transactions of the CSAE), **39**, 224-236 (2023). https://doi.org/10.11975/j.issn.1002-6819.202211017
18. G. Lili, Z. Weiyun, H.H. Suqin, Chinese J. Anim. Nutr. **35**, 3361-3371 (2023). https://doi.org/10.12418/CJAN2023.312
19. L.H. Suryaningrum, R. Samsudin, 2021. *Improvement quality of sugar cane bagasse as fish feed ingredient.* in Proceedings of the International Conference on Biotechnology and Food Sciences, INCOBiFS, 11 September 2020, Surabaya, Indonesia (2020)
20. AOAC International. Guidelines for Standard Method Performance Requirements. AOAC Official Methods of Analysis. Appendix F, pp. 1-18 (2016). http://www.eoma.aoac.org/app_f.pdf
21. M.P. Spinola, M.M. Costa, J.A.M. Prates, Agricult. **13**, 221 (2023). https://doi.org/10.3390/agriculture13010221
22. S. Wonorahardjo, D.P. Sari, A. Salsabila, E. Estiyawati, D. Yuliani, A.R. Wijaya, S. Suharti, I.K. Kusumaningrum, C.A. Maharani, T.A. Noviyanti, Makara J. Sci. **27,** 148-159 (2023)
23. National Research Council. Nutrient requirements of fish and shrimp. Washington, DC: The National Academies Press (2011)
24. A.G. Tacon, M. Metian, Aquacult. **285**, 146-158 (2008)
25. E.Y. Mohammady, A.M. Aboseif, M.R. Soaudy, E.A. Ramadan, M.S. Hassaan, Aquacult. **575**, 739755 (2023). https://doi.org/10.1016/j.aquaculture.2023.739755
26. J.J.D. Manlapig, T. Ban-Tokuda, H. Matsui, Anim. Sci. J. **94**, e13860 (2023). https://doi.org/10.1111/asj.13860
27. L.H. Suryaningrum, R. Samsudin, Mulyasari, A. Saputra, *The digestibility of improved sugar cane bagasse on Barbonymus schwanenfeldii,* in Proceedings of the International and National Symposium on Aquatic Environment and Fisheries, ISAEF, 24 September 2020, Banda Aceh, Indonesia (2020)
28. E.T. Moran, E.T. Anim. Feed Sci. Technol. **221**, 284-303 (2016). https://doi.org/10.1016/J.ANIFEEDSCI.2016.05.015
29. Y. Wei, M. Liang, H. Xu, Aquacult. Nutr. 2019; 00: 1–11. https://doi.org/10.1111/anu.12976

30. Y. Sun, X. Zhao, H. Liu, Z. Yang, J. Aquac. Res. Dev. **10**, 1-7 (2019). https://doi.org/10.35248/2155-9546.19.10.577
31. F. Gai, P. Peiretti, A. Brugiapaglia, L. Gasco., J. World. Aquac. Soc. **47**, 496-507 (2016). https://doi.org/10.1111/JWAS.12294
32. M. Firdaus, M.H. Rohaya, M.S. Miskandar, A.A. Astimar,, J. Oil. Palm. Res. **34**, 92-103 (2022). https://doi.org/10.21894/jopr.2021.0022
33. Lusi H. Suryaningrum, D. Jusadi, M. Setiawati, M.T.D Sunarno, AACL Bioflux, **10**, 77-86 (2017)
34. L.H. Suryaningrum, R. Samsudin, EJFA. **32**, 7, 488–494. https://doi.org/10.9755/ejfa.2020.v32.i7.2131
35. S. Sugiura, V. Raboy, K. Young, F. Dong, R. Hardy, Aquacult, **170**, 285-296 (1999). https://doi.org/10.1016/S0044-8486(98)00414-1
36. V. Kaiza, M. Yildiz, V. Eldem, S. Golzaradabi, S. Ofori-Mensah, J. Anim. Physiol. Anim. Nutr. **107**, 1517–1529. https://doi.org/10.1111/jpn.13858
37. R. Ruiji. W. Wang, L. Liu, F. Ma, Q. Li, P. Zhao, W. Ma, J. Cen, X. Liu, Int. J. Biol. Macromol. **250**, 126155. https://doi.org/10.1016/j.ijbiomac.2023.126155
38. R. Defaix, J. Lokesh, M. Ghislain, M. Le Bechec, M. Marchand, V. Veron, A. Surget, S. Biasutti, F. Terrier, T. Pigot, S. Panserat, K. Ricaud, Aquacult, **578**, 740049, https://doi.org/10.1016/j.aquaculture.2023.740049
39. S. Helmiati, A. Rustadi, Isnansetyo, Zuprizal, AACL Bioflux **13**, 1622-1632 (2020)
40. F.P. Martínez-Antequera, I. Barranco-Ávila, J.A. Martos-Sitcha, F.J. Moyano, Fishes, **7**, 4 (2022). https://doi.org/10.3390/fishes7010004
41. H. Fernandes, F. Moyano, C. Castro, J. Salgado, F. Martínez, M. Aznar, N. Fernandes, P. Ferreira, M.M. Goncalves, I. Belo, A. Oliva-Teles, H. Peres, Sci. Rep. **11**, 22946 (2021). https://doi.org/10.1038/s41598-021-02393-x
42. M. Seong, S. Lee, Y. Song, J. Bae, K. Chang, S.C. Bai, Aquacult. **483**, 196–202 (2018). http://dx.doi.org/10.1016/j.aquaculture.2017.10.023