Hydrolysis Enzyme of Alternative Ingredients For Fish Feed: A Review

ABSTRACT

Alternative raw materials have high potential as a source of feed protein that can support growth. Alternative raw materials have advantages such as low cost, abundant availability but the use of alternative raw materials in feed formulations is often limited by the presence of anti-nutritional substances and a relatively high crude fiber content of 11-28%, so further processing is needed to improve the quality of feed ingredients from alternative materials. The purpose of this literature review study is to determine the advantages of alternative feed ingredients for fish that have been hydrolyzed by enzymes. Evaluation studies of improving the quality of feed ingredients through enzymatic hydrolysis give varying results. The best enzyme hydrolysis can reduce the crude fiber content in coconut cake by 67.8%. Enzyme hydrolysis in alternative feed ingredients can also affect growth in fish due to increased digestibility in fish. The best growth was obtained in tilapia at 4.34% by hydrolysis of the protease enzyme. Enzyme hydrolysis is proven not to have a negative impact on several types of fish so that enzyme hydrolysis can be applied to improve the quality of alternative feed ingredients in fish feed formulations. However, enzyme hydrolysis has drawbacks, namely it only works specifically and depends on temperature and pH.

Keywords : Alternative ingredients, enzymes, fish feed, growth, hydrolysis.

1. INTRODUCTION

growing activity cultivation fishery make Request feed *too* increase. However, the price of conventional feed ingredient ingredients for protein sources is still high [1] . the height price is caused because of supply limitations resources , sources of protein that are not stable [2] [3] . To resolve the Thing, you need an alternative raw ingredient that has good quality nutrition _ at an affordable price [4] . An alternative ingredient for fish feed must be worth nutrition including high protein , sufficient amino acids , taste and digestibility , as well as fiber and heavy metal must be low carb _ no dissolve , because it will affect the growth process , feed costs , feed conversion ratio , and must be in accordance with the economy production feed [5] . Fish need about 50% of the necessary protein, fat <8% for carnivorous fish, and herbivores no more than 3%, minerals such as magnesium, potassium, iodine, and iron, fiber for carnivorous fish no more than 4% while herbivorous fish 5- 10%, as well as vitamins C, A, K, B12, B1, B2, B3, B5, B6, M, H, and inositol [6] . Study about the use of alternative raw ingredients in total or some who have researched and provided influence to fish growth. Among them is use soya bean flour cake [7] [8] , leaves lamtoro [8][9] , waste vegetables [10][11] , as well as dregs know [12][13] . However, the use of local raw ingredients is still often constrained by the high rough fiber content that can interfere with fish digestion [15] , the low crude protein content, the presence of anti-nutritional substances, and the presence of a low balance in amino acids. This thing results in the need for the processing of raw ingredient feeds before they are used as ingredient feeds [16] .

Hydrolysis is the process of solving polymers into monomers for compounds that could dissolve like glucose [13] . Factors affecting the hydrolysis process are reaction time, temperature reaction, concentration acid (catalyst), starch suspension level, and mixing reactor [17] . Enzyme hydrolysis can degrade substrates as an energy source so that enzymes can hydrolyze carbohydrates, especially fiber in feed ingredients derived from high-fiber vegetable ingredients so that they can trigger rapid growth in fish [18] . The disadvantage of enzymatic hydrolysis is

that it depends on temperature and pH. Enzymes will work optimally at a certain temperature and pH. If the temperature is too high it will cause the enzyme to be denatured, while the pH which is not in accordance with the provisions will greatly affect the course of the reaction [19] . In addition, enzymatic hydrolysis takes a long time, only works specifically and cannot penetrate the lignin that binds hemicellulose and cellulose and the price of the enzyme is relatively expensive. [20] . Destination hydrolysis is to damage the structure of cellulose crystals and to increase the porosity of something ingredient [21] . This review focuses on the excellence of alternative ingredient feeds for fish that have been hydrolyzed by enzymes through search journals as reference main.

2. POTENCY ENZYME FOR FISH FEED

Enzyme is a protein that has activity catalyst for lower energy Activation of something reaction so that conversion Becomes substrates product could progress faster [22] Lots of enzymes used which are protease, lipase, and amylase enzymes which are enzyme breaking down hydraulics compound macromolecule carbohydrates, fats, and proteins. A research that has been conducted with the title “Activity Test Enzyme Amylase, Lipase, and Protease from *Hermetia* Larva Intestine Extract *illucens* " aimed at knowing *Hermetia* larvae potential *illucens* which produces protease, amylase and lipase enzymes. The result is that Protease is able to break down protein into amino acids, lipase breaks down fat into fatty acids and glycerol , as well as amylase breaks up starch to maltose [23] .

Enzyme activity in the fish body depends on the eating habits of fish, for example, herbivorous fish. Herbivorous fish will tend to secrete enzymes that can accelerate the hydrolysis reactions of fats and carbohydrates such as lipase and maltase [24] . The intestines of herbivorous fish can also secrete proteases which function to accelerate protein hydrolysis reactions and break down peptide bonds [24] . In addition, the fish stage also affects the needs of fish enzymes. Adult fish tend to require exogenous enzymes to speed up the hydrolysis process because adult fish tend to produce endogenous enzymes compared to fish at fry size [25] . The addition of these exogenous enzymes to artificial feed can increase growth and reduce the feed conversion ratio in adult fish [26] .

Enzymes can also increase the growth of fish through enhancing the fish feed efficiency score [27] [28] . height the amount of feed efficiency proves that the quality of the feed is better because the efficiency of the feed has changed Becomes meaningful meat that costs required _ to produce meat that is more inexpensive [29] . Fish need good enzymes in the form of endogenous enzymes nor exogenous enzymes to speed up protein hydrolysis to amino acids and speed up the digestive process [30] . Power digest feed is influenced by enzymes present in the digestion channel and long time eaten food reacts with enzyme digestion [27] because good digestion power will add body weight to fish [31] . A number of studies mention that gift enzymes in tilapia feed add heavy of 1.26 g [32] , giving papain and protease enzymes increase growth heavy absolute catfish jambal of 4.87 g-6.63 g [33] , giving protease, lipase, and amylase enzymes in baung fish feed succeed increase growth heavy absolute of 11.06 g [34] . Increase growth heavy caused the existence of enzymes that speed up gut work _ protein hydrolysis in feed so that protein hydrolysis to more amino acids fast and more widely absorbed by the fish body [27] .

3. INGREDIENT QUALITY ALTERNATIVE AFTER HYDROLYSIS ENZYME

Hydrolysis enzyme could repair function and structure nutrition protein ingredients in short time and method _ this no need sterile and anaerobic conditions as fermentation bacteria [35] . Hydrolysis use certain ingredients could degrade rough fiber content on raw material feed [36] . Hydrolysis works by degrading crude fiber in feed ingredients including cellulose which is converted into sugar with the help of enzyme catalysts [37] . The hydrolysis of a material by enzymes is strongly influenced by the dose (quantity) and the incubation time of the hydrolysis process [38] . Enzymes have the ability to activate other compounds specifically and are able to

increase the speed of the reaction so that the hydrolysis process is faster than chemical hydrolysis [39] . Enzyme hydrolysis works specifically so it does not produce unwanted products and is more environmentally friendly [40] . The mechanism of action of enzymes on the hydrolysis of feed ingredients containing cellulose can use cellulase enzymes. Generally, cellulase enzymes consist of a mixture of several enzymes involved in hydrolysis of cellulose, namely endoglucanase which acts on the region of cellulose fibers which have low crystallinity in breaking down cellulose and forming free chains, exoglucanase which further degrades molecules by removing cellobiose units from the free end of thechain. sera glucosidase which hydrolyzes cellobiose to glucose [40] .

Now, the application of hydrolysis enzymes in the ingredients feed has been done a lot. This thing is proven by a number of studies that hydrolysis by enzyme activity amylase , cellulase , protease, and lipase on coconut cake could lower Rough fiber by 67.8% [41] , in water goiter Rough fiber decreased by 19.72% [42] , on the dregs know capable of lowering fiber Rough by 22.03% [43] , on flour leaf moringa capable of lowering fiber Rough up to 4.33% [36] . Besides lowering coarse fiber, hydrolysis can also increase Crude protein content in soya bean flour cake [44] .

4. INFLUENCE HYDROLYSIS ENZYME INGREDIENT FEED ALTERNATIVE AS FISH FEED

Feed is the main factor that can influence fish growth. In addition to the nutrients contained in feed, feed consumption and feed conversion ratio also have an effect on fish growth [45] . The more high consumption of feed will increase the possibility of a lot amount consumed by fish. Ratio conversion feed show amount feed needed to produce fish weight [46] .

The nutrients needed by fish include nutrients that can produce energy in the form of proteins, fats and carbohydrates which are needed in relatively large amounts and nutrients that do not produce energy in the form of vitamins and minerals which are needed in relatively small amounts [47] . Fish feed must have good nutritional content, namely protein ranging from 20-60%, fat ranging from 4-18%, carbohydrates ranging from 20-30%, crude fiber ranging from less than 8%, and vitamins and minerals ranging from 2-5% [47] . Materials for making fish feed can be sourced from animals such as fish oil, fish bones, chicken feathers, as well as from vegetable sources such as corn, coconut soybeans, peanuts, forages, and so on.

A number of researchers have tested the influence of hydrolysis enzyme ingredient alternative for fish feed on growth without giving negative impact to a number of fish [36] , [48] , [44] , [49] , [50] However, in research using water hyacinth goiter, it does not take effect on tilapia growth. That thing is suspected of having microbes present in the material that has been hydrolyzed not yet grown optimally, so that the enzymes produced to hydrolyze the ingredients do not go well [42] .

Table 1. Influence of Hydrolysis Enzyme Ingredient Feed Alternative As Fish Feed

Fish species	EnzymeType	Alternative Ingredients	Daily Growth rate (%)	References
Clarias sp.	Proteases, lipases and amylase	Moringa Leaves	2.81	[36]
Oreochromis niloticus	protease	Feather Meal	4.31	[48]
Ictalurus punctatus	Protease, phytase	Soybean	2.73	[44]
Chanos chanos Forsskal	Protease, amylase, lipase, and cellulase	Rice bran	Increase 11.34 (g) growth	[49]
Sparus aurata	protease	Feather Meal	2.34	[50]

Osphronemus gouramy	Cellulase	Indigofera zollingeriana	3.10	[51]
Cheilinus undulatus	Papain	Trash fish	3.29	[52]

5. CONCLUSION

The high crude fiber in alternative materials, which is around 11-28%, is an obstacle in its use for fish feed, so it is necessary to increase feed ingredients, one of which is through enzyme hydrolysis. Protease, lipase, amylase, cellulase and phytase enzymes can improve the quality of alternative ingredients for fish feed. The application of enzyme hydrolysis to moringa leaf meal, feather meal, soybean meal, and refined bran has been shown to increase the growth of tilapia, catfish, milkfish, and snapper.

REFERENCES

[1] DT Afriani and U. Hasan, " Proximate Analysis of Artificial Feed With the Addition of Chicken Feather Flour Hydrolyzate as an Alternative Protein Source for Tilapia (Oreochromis sp.)," *Eksakta J. Researcher. and MIPA Learning* , vol. 5, no. 2, p. 186, 2020, doi: 10.31604/eksakta.v5i2.186-190.

[2] R. Jannathulla, V. Rajaram, R. Kalanjiam, K. Ambasankar, M. Muralidhar, and JS Dayal, “Fishmeal availability in the scenarios of climate change: Inevitability of fishmeal replacement in aquafeeds and approaches for the utilization of plant protein sources,” *Aquac. Res.* , vol. 50, no. 12, p. 3493–3506, 2019, doi: 10.1111/are.14324.

[3] RL Olsen and MR Hasan, “A limited supply of fishmeal: Impact on future increases in global aquaculture production,” *Trends Food Sci. Technol.* , vol. 27, no. 2, pp. 120–128, 2012, doi: 10.1016/j.tifs.2012.06.003.

[4] N. Sa'adah, R. Hastuti, and NBA Prasetya, " The Effect of Formic Acid in Chicken Feathers as an Adsorbent on Reducing Levels of Remazol Golden Yellow Yellow Rnl Textile Dyes," *J. Kim. Univ. Diponegoro* , vol. 1, no. 1, pp. 202–209, 2016, [Online].

[5] S. Nagappan *et al.* , “Potential of microalgae as a sustainable feed ingredient for aquaculture,” *J. Biotechnol.* , vol. 341, no. september, pp. 1–20, 2021, doi: 10.1016/j.jbiotec.2021.09.003.

[6] RRDS Manik and J. Arleston, *Nutrition and Fish Feed* . Bandung: Widina Bhakti Persada, 2021.

[7] IGAA Agung, IM Sukerta, DN Raka, and D. Tariningsih, " Local Balinese Soybeans, Tempe Raw Materials High in Nutrition, Antioxidants and Organoleptics as well as Medicinal Efficacy," *AGRIMETA J. Pertan. Free. Eco balance.* , pp. 87–92, 2013.

[8] Z. Zhang, L. Xu, W. Liu, Y. Yang, Z. Du, and Z. Zhou, “Effects of partially replacing dietary soybean meal or cottonseed meal with completely hydrolyzed feather meal (defatted rice bran as the carrier) on production, cytokines, adhesive gut bacteria, and disease resistance in hybrid tilapia (Oreochromis nilot,” *Fish Shellfish Immunol.* , vol. 41, no. 2, pp. 517–525, 2014, doi: 10.1016/j.fsi .2014.09.039.

[9] AN Putra, AC Pradana, D. Novriansyah, and M. Mustahal, " Effect Of Dietary Fermented Lamtoro (*Leucaena leucocephala*) Leaves Flour In Feed On Digestibility And Hematological Parameters Of Catfish (Clarias sp.)," *e-Jurnal Engineering and Technol. Budid. waters.* , vol. 8, no. 1, p. 951, 2019, doi: 10.23960/jrtbp.v8i1.p951-964.

[10] R. Restiningtyas, Subandiyono, and Pinandoyo, " Utilization of Fermented Lamtoro Leaf Flour (Laucaena Gluca) in Artificial Feed on Growth of Fish Seeds," *J. Aquac. Manag. Technol.* , vol. 2, no. 3, pp. 76–85, 2013, [Online]. Available: http://ejournal-s1.undip.ac.id/index.php/jfpik

[11] AM Santoso and A. Manan, " Alternative Feed from Vegetable Waste for Black Tilapia (Oreochromis niloticus," *J. Fisheries and Marine Sciences.* , vol. 7, no. 1, pp. 35–37, 2015 .

[12] SF Afifah, . R., and . K., "Utilization of Vegetable Waste for Tilapia Fish Feed Pellets," *Gema Lingkung. healthy.* , vol. 19, no. 2, pp. 92–96, 2021, doi: 10.36568/kesling.v19i2.1535.

[13] D. NurAnggraeni and R. Rahmiati, "Utilization of Tofu Dregs as Organic Catfish (Clariasbatrachus) Feed," *Biog. J. Ilm. Bio.* , vol. 4, no. 1, pp. 53–57, 2016, doi: 10.24252/bio.v4i1.1469.

[14] P. Hartami and R. Rusydi, "Effectiveness of the combination of tofu dregs and pellets for the growth of sangkuriang catfish (Clarias sp)," *Acta Aquat.* , vol. 3, no. 2, pp. 40–45, 2016.

[15] AC Hansen and GI Hemre, “Effects of replacing fish meal and oil with plant resources in on-growing diets for Atlantic cod Gadus morhua L,” *Aquac. Nutr.* , vol. 19, no. 5, pp. 641–650, 2013, doi: 10.1111/anu.12078.

[16] W. Pamungkas, "Fermentation Technology, Alternative Solutions in Efforts to Utilize Local Feed Materials," *Aquaculture Media* , vol. 6, no. 1, p. 43, 2011, doi: 10.15578/ma.6.1.2011.43-48.

[17] E. Praputri, E. Sundari, F. Firdaus, and S. Sofyan, "The use of homogeneous and heterogeneous catalysts in the hydrolysis process of cassava root starch into glucose," *J. Litbang Ind.* , vol. 8, no. 2, p. 105, 2018, doi: 10.24960/jli.v8i2.4189.105-110.

[18] A. Masriah, S. Alamsyah, and Zainnudin, " Fish Feed Hydrolysis Using Cow Rumen Liquid," *J. Octopus* , vol. 7, no. 1, pp. 704–708, 2018.

[19] P. Robinson, “Enzymes: principles and biotechnological applications,” *Essay Biochem* , vol. 56, pp. 1–41, 2015.

[20] J. . Cheng and G. . Timilsina, “Status and barriers of advanced biofuel technologies: A review,” *Renew. Energy* , vol. 36, pp. 3541–3549, 2011.

[21] Asrianti, " Optimization of Temperature and Time of Enzymatic Hydrolysis of Paper Waste Using Cellulase from Candida Utilis in Bioethanol Production," Alauddinmakassar Islamic State University, 2017.

[22] Sulasi, S. Hastuti, and Subandiyono, "The Effect of Papain Enzymes and Probiotics on Artificial Feed on Protein Utilization and Growth of Goldfish," *J. Tropic Aquaculture Science.* , vol. 2, no. 1, pp. 62–71, 2018.

[23] W. Kim *et al.* , “Biochemical characterization of digestive enzymes in the black soldier fly, Hermetia illucens (Diptera: Stratiomyidae),” *J. Asia. Pac. Entomole.* , vol. 14, no. 1, pp. 11–14, 2011, doi: 10.1016/j.aspen.2010.11.003.

[24] B. Tangjaroenkul, "Ontogenic Morphology and Enzyme Activities Of The Intestinal Tract Of The Nile Tilapia," Virginia: Polytechnic Institute and State University, 2000.

[25] Hariati, Mulyadi, and I. Putra, " Addition of Hydrolytic Enzymes in Commercial Feed on the Growth and Survival of Baung Fish (*Hemibagrus nemurus*) With a Recirculation System," 2016.

[26] I. Bakhtiar, "The Effect of Addition of Hydrolytic Enzymes and Probiotics in Feed on Growth, Feed Conversion and Survival of Tilapia (*Oreochromis Niloticus Trewavas* 1983)," Gaja Mada University, 2015.

[27] D. Faizal, R. Rostika, A. Yustiati, Y. Andriani, and I. Zidni, "The Effect of Addition of a Combination of Papain and Bromelain Crude Enzyme Extracts in Artificial Feed on the Growth of Tilapia Seeds (Oreochromis Niloticus)," *J. Tropic Aquaculture Science.* , vol.

1, no. 1, pp. 10–20, 2017.

[28] IW Putri, M. Setiawati, and D. Jusadi, "Digestive enzymes and growth performance of goldfish, Cyprinus carpio Linnaeus, 1758) fed with the addition of turmeric flour Curcuma longa Linn.," *J. Iktiologi Indonesia.* , vol. 17, no. 1, p. 11, 2017, doi: 10.32491/jii.v17i1.21.

[29] I. Effendi, "Introduction to Aquaculture," *Spreader Self Help. Jakarta* , vol. 188, 2004.

[30] W. Agustian *et al.* , “Journal of Aquaculture Management and Technology Feed Protein and Growth of Tilapia Larasati Seeds.” *J.Aquac. Manag. Technol.* , vol. 2, no. 1, pp. 1–12, 2013.

[31] I. Nurfitasari, IF Palupi, CO Sari, S. Munawaroh, N. Nafisyah, and T. Ujilestari, "Tilapia Digestive Response to Various Types of Feed," *Nectar J. Educator. Bio.* , vol. 1, no. 2, pp. 21–28, 2020.

[32] F. & RW Andini, "The Effect of Different Doses of Bromelin Enzyme on the Growth and Efficiency of Feed Utilization of Tilapia (Oreochromis niloticus)," *J. Trop Animal Science.* , vol. 9, no. 2, pp. 68–74, 2020.

[33] mustika marzah Fitriana, CN Defira, and S. Agustiana, "The Effect of a Combination of Papain Enzymes and Protease Enzymes in Commercial Feed on Feed Utilization and Seed Growth of Jambal Catfish (Pangasius SP.)," *J. Ilm. Mhs. and Perikan. Unsyiah* , vol. 3, no. 4, pp. 211–219, 2018, [Online]. Available: http://www.jim.unsyiah.ac.id/fkp/article/view/13399

[34] D. Prabarini *et al.* , "Addition of Enzyme Composition in Commercial Feed on Growth Performance and Survival of Baung Fish (Mystus Nemurus) in Tarpaulin Ponds," *J. Science Teknol. Aquaculture* , vol. 1, no. 2, pp. 120–127, 2017.

[35] J. Laporte, “Nutritional Evaluation of Animal By-Products for the Partial Replacement of Fishmeal in Diets for Gilthead Sea Bream (Sparus Aurata L.),” 2007.

[36] AN Putra, IM Maula, A. Aryati, MB Syamsunarno, and M. Mustahal, "Evaluation of Moringa Leaf Flour (Moringa oleifera) Hydrolyzed Sheep Rumen Fluid as Raw Material for Catfish (Clarias sp.) Feed," *J. Fishery. Univ. Gadjah Mada* , vol. 22, no. 2, p. 133, 2020, doi: 10.22146/jfs.57468.

[37] H. Setyoko and B. Utami, "Isolation and characterization of the cellulase enzyme in cattle rumen fluid for biomass hydrolysis," in *Proceedings of the XIII National Seminar on Biology Education. Surakarta* , 2016, pp. 863–867.

[38] D. Kamra, “Special section of microbial diversity: rumen microbial ecosystem,” *Curr. sci.* , vol. 89, no. 10, p. 124–135, 2005.

[39] L. García - Río, P. Hervés, JR Leis, JC Mejuto, and J. Pérez - Juste, “Determination of the hydrolysis rate of AOT in AOT–isooctane–water microemulsions using sodiumnitroprusside as a chemical probe,” *J. Phys. org. Chem.* , vol. 15, no. 8, pp. 576–581, 2002.

[40] A. Fuadi, K. Harismah, and A. Setiawan, " Enzymatic Hydrolysis of Used Paper With Preheating Variations," *J. Univ. Res. Colloq.* , pp. 1–8, 2015.

[41] Zuraida, D. Jusadi, and NBP Utomo, "Effectiveness of Enzyme Addition of Sheep's Rumen Fluid on Reducing Coconut Oil Crude Fiber as Raw Material for Fish Feed," *J. Indones swamp aquaculture.* , vol. 1, no. 2, pp. 117–126, 2013, [Online]. Available: https://core.ac.uk/download/pdf/267822650.pdf

[42] AN Putra, S. Ristiani, M. Musfiroh, and MB Syamsunarno, "Utilization of Water Hyacinth (Eichornia crassipes) as Tilapia Feed: Effects on Growth and Digestibility of Feed," *Leuit (Journal Local Food Secur.* , vol. 1 , no. 2, p. 77, 2020, doi: 10.37818/leuit.v1i2.10016.

[43] N. Nafiqoh and LH Suryaningrum, "Hydrolysis of Sugarcane Bagasse Using Cellulase

Enzymes from Bacillus subtilis Bacteria in Efforts to Use It as a Fish Feed Material," *Balai Ris. Fishery. Budid. Freshwater and Sulfur. Fishery.* , no. september, pp. 428–435, 2020, [Online]. Available: http://103.55.216.56/index.php/psb/article/view/16022

[44] X. Xuquan *et al.* , “Study of enzyme-hydrolyzed soybean replacing fish meal and/or chicken meal on the growth of channel catfish (Ictalurus punctatus),” *Aquac. Reports* , vol. 27, no. september, p. 101344, 2022, doi: 10.1016/j.aqrep.2022.101344.

[45] RO Julia, Y. Andriani, and LPS Yuliadi, “The Effect of Addition of Butterfly Pea Leaf Meal (Clitoria ternatea) in Feed on the Quality of Color of Swordtail Fish Head (Xiphophorus helleri),” vol. 26, no. July, pp. 128–137, 2019.

[46] A. Mathew Adewale, "Growth Performance and Carcass Analysis of African Catfish (Clarias Gariepinus) Juveniles Fed Varying Inclusion Levels Of Butterfly Pea (Clitoria Ternatea) Seed Meal," *J. Aquac. Livest. Prod.* , vol. 2, no. 2, pp. 1–6, 2021, doi: 10.47363/jalp/2021(2)111.

[47] V. Devani *et al.* , "Optimization of the Nutritional Content of Artificial Fish Feed Using a Multi Objective (Goal) Programming Model," *J. Science, Teknol. and Ind.* , vol. 12, no. 2, pp. 255–261, 2015, [Online]. Available: http://ejournal.uin-suska.ac.id/index.php/sitekin

[48] L. Poolsawat, H. Yang, YF Sun, XQ Li, GY Liang, and XJ Leng, “Effect of replacing fish meal with enzymatic feather meal on growth and feed utilization of tilapia (Oreochromis niloticus × O. aureus), *Anim . Feed Sci. Technol.* , vol. 274, no. December 2019, p. 114895, 2021, doi: 10.1016/j.anifeedsci.2021.114895.

[49] A. Masriah and Alpiani, "Growth and Survival of Milkfish (Chanos chanos Forsskal) Fed with Two Types of Carbohydrate Raw Material Sources of Feed Hydrolyzed Cow Rumen Liquid Waste," *Gorontalo Fish. J.* , vol. 2, no. 2, pp. 78–87, 2019.

[50] P. Psofakis, IT Karapanagiotidis, EE Malandrakis, E. Golomazou, A. Exadactylos, and E. Mente, “Effect of fishmeal replacement by hydrolyzed feather meal on growth performance, proximate composition, digestive enzyme activity, haematological parameters and growth -related gene expression of gilthead seabream (Sparus aurata),” *Aquaculture* , vol. 521, no. December 2019, p. 735006, 2020, doi: 10.1016/j.aquaculture.2020.735006.

[51] Jefry, "Utilization of Indigofera zolingeriana Leaves Hydrolyzed by Cellulase Enzymes as Feed Materials for Gouramy Seed," Bogor Agricultural University, 2021.

[52] MAS Agi, WKA Putra, and T. Yulianto, "Giving Different Doses of Papain Enzymes in Trash Feed on the Growth and Survival of Napoleon Fish (Cheilinus …) Seeds," *Intek Aquaculture* , vol. 5, pp. 82–90 , 2021, [Online]. Available: https://ojs.umrah.ac.id/index.php/intek/article/view/3089