Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(2): 1249 – 263 (2025)
www.ejabf.journals.ekb.eg

Enhancing Artificial Feed Quality for the White Snapper (*Lates calcarifer*) through Mixed Microorganisms as Pre-Digestion Agents: Effects on Hydrolysis Degree, Soluble Protein, and Glucose Content

Nur Insana Salam[1,2], Siti Aslamyah[1]*, Haryati[1], and Dody Darmawan Trijuno[1]

[1]Department of Marine Science, Faculty of Marine Sciences and Fisheries, Universitas Hasanuddin. Tamalanrea Km.10, Makassar, South Sulawesi 90245, Indonesia
[2]Department of Aquaculture, Faculty of Agriculture, Universitas Muhammadiyah Makassar. Jl. Sultan Alauddin 259, South Sulawesi malanrea Km.10, Makassar, South Sulawesi 90122, Indonesia
*Corresponding Author: sitiaslamyah1@gmail.com

ARTICLE INFO
Article History:
Received: Jan. 18, 2025
Accepted: March 24, 2025
Online: March 27, 2025

Keywords:
Microbial,
Pre-digestion,
Improve,
White snapper,
Feed

ABSTRACT

The reliance on natural feeds such as rotifers and Artemia in the larval rearing of the white snapper (*Lates calcarifer*) often leads to nutritional deficiencies and elevated operational costs. This study aimed to investigate the impact of mixed microorganisms specifically *Bacillus* sp, *Aspergillus* sp., *Rhizopus* sp., *Saccharomyces* sp., and *Lactobacillus* sp., as pre-digestion agents to improve the nutritional profile of artificial feed. A factorial experiment was conducted with varying microbial doses (5, 10, and 15mL/ kg) and incubation durations (6, 12, 18, and 24 hours). The results revealed that the optimal treatment of 10mL/ kg, with an incubation period of 18 hours, significantly enhanced protein content, reduced crude fiber levels, and increased glucose concentrations, thereby improving nutrient digestibility. The microbial hydrolysis process not only elevated protein solubility but also facilitated lipid metabolism while decreasing anti-nutritional factors. These findings suggest that microbial pre-digestion is a promising strategy to enhance the efficiency and sustainability of artificial feed in aquaculture.

INTRODUCTION

Aquaculture success heavily relies on the availability of high-quality feed that meets the nutritional requirements of cultured species. For the white snapper (*Lates calcarifer*), larval rearing primarily depends on natural live feeds like rotifers (*Brachionus plicatilis*) and *Artemia nauplii* **(El-Dahhar *et al.*, 2024)**. While these live feeds provide essential nutrients; however, they frequently lack a comprehensive nutritional profile, resulting in deficiencies in essential macro- and micronutrients vital for optimal larval growth and survival **(Moroni *et al.*, 2024)**. Producing live feed on a large scale requires significant land, labor, and infrastructure, increasing operational costs in hatchery management **(Alfonso *et al.*, 2023)**. Artificial feed has emerged as an alternative to offer a nutritionally balanced and cost-effective solution for larval rearing **(Abdulla *et al.*, 2024)**. However, the effectiveness of artificial feed is limited by the

underdeveloped digestive systems found in early-stage larvae, restricting their ability to hydrolyze complex macronutrients **(Rasdi *et al.*, 2021)**.

A highly promising strategy for enhancing the digestibility of artificial feed is pre-digestion, which involves breaking down complex feed components into simpler, more bioavailable forms before consumption **(Surnawati *et al.*, 2020)**. Pre-digestion can be achieved enzymatically or microbially; each method facilitates the breakdown of proteins, lipids, and carbohydrates into smaller peptides, fatty acids, and monosaccharides. Studies have demonstrated that enzymatic hydrolysis using proteases such as papain at a concentration of 4.5% significantly increases protein solubility and enzymatic activity in feed **(Rasdi *et al.*, 2021)**. Furthermore, microbial fermentation has been widely recognized for its role in improving organic matter digestibility and nutrient bioavailability in various aquaculture feeds **(Aslamyah *et al.*, 2022)**.

Microorganisms mix including *Bacillus* sp., *Aspergillus* sp., *Rhizopus* sp., *Saccharomyces* sp., and *Lactobacillus* sp. are recognized as effective agents for feed fermentation. These microorganisms produce various hydrolytic enzymes such as proteases, amylases, cellulases, and lipases that degrade complex feed components **(Chi *et al.*, 2024)**. For example, *Bacillus* species are known to enhance protein digestibility through protease production, while *Lactobacillus* has been reported to increase lipid metabolism via lipase activity **(Niu *et al.*, 2022)**. Previous research has shown that fermentation using *Bacillus* and *Lactobacillus* can enhance the protein content of feed by promoting microbial biomass synthesis and enzymatic hydrolysis **(Rudnyckyj *et al.*, 2023)**. However, while the use of single-strain probiotics has been extensively studied, the synergistic effects of mixed microbial fermentation in aquaculture feeds remain underexplored.

Although promising results have emerged from other aquaculture species, the application of microbial pre-digestion for the white snapper larvae remains insufficiently explored. Previous studies demonstrate that microbial fermentation improves the digestibility of plant-based feed ingredients, such as soybean meal and sago pulp, by reducing anti-nutritional factors while increasing protein bioavailability **(Sumiana *et al.*, 2020)**. Moreover, mixed microbial fermentation has been reported to increase glucose content in feed, thereby enhancing its energy availability for fish larvae **(Ismail *et al.*, 2023)**. However, limited information exists regarding the optimal microbial dosage and incubation time needed to maximize pre-digestion efficiency in the white snapper larval artificial feed

Given this gap in knowledge, the present study aimed to evaluate the effect of mixed microorganism pre-digestion on artificial feed digestibility for the white snapper larvae. Specifically this study seeked to determine the optimal dosage and incubation period of microbial fermentation to enhance protein solubility, glucose availability,and

overall feed quality. By identifying the most effective pre-digestion parameters, this research could contribute to developing nutritionally superior artificial feeds that promote sustainable aquaculture practices while reducing dependency on live feeds. By implementing these corrections across all paragraphs systematically improves clarity while enhancing grammatical accuracy throughout your introduction.

MATERIALS AND METHODS

Feed preparation and pre-digestion process

The feed used in this study was a commercial larval feed (Aquaxcel), which contained 38% protein, 4.35% fat, 11.44% ash, and 42.61% nitrogen-free extract. The feed was pre-digested using a mixed microorganism solution consisting of *Bacillus* sp., *Aspergillus* sp., *Rhizopus* sp., *Saccharomyces* sp , and *Lactobacillus* sp. The microbial culture was prepared by inoculating 2mL of microbial starter in a solution of 2L coconut water and 500g sugar, followed by incubation at room temperature for 24 hours. The prepared microbial culture was diluted and uniformly sprayed onto 1kg of feed. The treated feed was incubated at room temperature for varying durations (6, 12, 18, and 24 hours). The fermentation reaction was terminated by steaming the feed at 100°C for 2 minutes before further analysis.

Research design

The experimental design used was a Factorial Completely Randomized Design (CRD), comprising two factors: the dose of the microbial mixture and the incubation period. The first factor involved three treatments with varying doses of mixed microbes in artificial feed (5, 10, and 15mL/ kg). Each dosage treatment was repeated three times. The second factor was different incubation periods (6, 12, 18, and 24 hours).

Proximate analysis

The sample's protein, fat, ash, carbohydrate, fiber, and water content was determined using the proximate analysis method established by the Association of Official Analytical Chemists **(AOAC, 2005)**. The Kjeldahl technique was used to assess protein levels, while the Soxhlet method was used to evaluate fat levels. The moisture content and ash were assessed using the gravimetric method. In addition, carbohydrate levels are manually estimated using the estimated analysis results.

Degree of hydrolysis of feed nutrients

The degree of feed hydrolysis was calculated using a formula proposed by **Aslamyah (2006)**. The value obtained from analyzing artificial feed proximate before and after pre-digestion with mixed microorganisms according to dosage and incubation time is inserted into the formula below:

$$\text{DHP} = \frac{P^0 - P_t}{P^0} x\ 100$$

Where: DHP = degree of hydrolysis of protein
P0 = protein level at the initial time
Pt = protein content at the end

Glucose content

Glucose content was measured following **Yasutake (1977)** procedures. The measurement of feed glucose levels was carried out at the end of the observation. As much as 2g of the predigested feed was diluted with 5mL of aquadest. Subsequently, it was centrifuged at 3,000 rpm for 15 minutes. The resulting supernatant was used to measure used glucose content.

Soluble protein

The dissolved protein content from predigested feed samples after stopping their reactions was measured by weighing approximately 0.5g before adding it to three mL Tris HCl pH 6.5 solution followed by centrifugation at ten thousand rpm for 20 minutes. The resulting supernatant was utilized for soluble protein analysis following the method of **Bradford (1976)**.

Data analysis

Data were analyzed using factorial ANOVA, effects with a probability of $P< 0.05$ was considered to be significant. Moreover, the Tuckey post-hoc test was applied to compare differences between means that were then analyzed descriptively. The results were presented as a means with standard deviations (SD) (mean ±SD). Statistical analysis was performed using SPSS (Statistical Package for Social Sciences, Version 27, IBM Corporation, New York, USA).

RESULTS

Proximate composition of pre-digested feed

The average proximate analysis of artificial feed for white snapper larvae after adding the predigest microorganism mix is presented in Table (1). Proximate analysis showed significant variation in treatments' protein, fat, crude fiber, and ash levels. Ash, fat, and crude fiber levels decreased in all treatments compared to the control. The lowest ash content was in the A1B4 treatment at 8.52%, and the highest in the A1B3 treatment at 10.99%. The lowest fat content was in the A2B3 treatment at 2.83%, and the highest in the A3B1 treatment. The lowest crude fiber content was in the A1B3 treatment at 0.66%, and the highest was in the A1B1 dose at 1.11%. Protein and NFE levels increased

compared to the control, indicating improvements in nutritional value. Treatment A2B3 had the highest protein content at 42.27%. At the same time, A1B4 had the highest NFE content at 46.17%. These changes indicate an increase in feed quality in specific treatments compared to the control.

Table 1. Proximate analysis of predigested artificial feed using mixed microorganisms

Treatment	Ash	Fat	Protein	Crude Fiber	NEF
control	11.44	3.60	38.00	4.35	42.61
A1B1	10.74 ± 0.12^{b}	3.14 ± 0.00^{c}	40.65 ± 0.05^{a}	1.11 ± 0.01^{d}	44.35 ± 0.11^{a}
A1B2	10.51 ± 0.03^{b}	3.06 ± 0.01^{b}	41.26 ± 0.05^{a}	0.99 ± 0.01^{cd}	44.13 ± 0.06^{a}
A1B3	10.99 ± 0.33^{b}	2.88 ± 0.02^{a}	41.31 ± 0.09^{a}	0.66 ± 0.14^{a}	44.18 ± 0.10^{a}
A1B4	8.52 ± 0.12^{a}	2.84 ± 0.01^{a}	41.65 ± 0.02^{b}	0.88 ± 0.01^{c}	46.17 ± 0.01^{c}
A2B1	10.43 ± 0.09^{b}	3.09 ± 0.01^{c}	40.19 ± 0.05^{a}	0.96 ± 0.02^{d}	45.34 ± 0.05^{c}
A2B2	10.56 ± 0.21^{b}	3.03 ± 0.01^{b}	41.74 ± 0.32^{b}	0.83 ± 0.01^{b}	43.84 ± 0.11^{a}
A2B3	10.72 ± 0.24^{b}	2.83 ± 0.02^{a}	42.27 ± 0.45^{b}	0.73 ± 0.01^{a}	43.45 ± 0.35^{a}
A2B4	10.55 ± 0.13^{b}	2.84 ± 0.01^{a}	42.02 ± 0.13^{b}	0.75 ± 0.01^{a}	43.85 ± 0.01^{a}
A3B1	10.54 ± 0.03^{b}	3.14 ± 0.00^{c}	40.85 ± 0.02^{a}	0.97 ± 0.01^{d}	44.50 ± 0.04^{b}
A3B2	10.58 ± 0.05^{b}	3.02 ± 0.01^{b}	41.42 ± 0.07^{a}	0.83 ± 0.01^{b}	44.15 ± 0.02^{a}
A3B3	10.51 ± 0.06^{b}	2.84 ± 0.03^{a}	41.12 ± 0.03^{a}	0.73 ± 0.01^{a}	44.87 ± 0.06^{c}
A3B4	10.67 ± 0.04^{b}	2.86 ± 0.01^{a}	41.33 ± 0.00^{a}	0.74 ± 0.01^{a}	44.47 ± 0.02^{b}

Note: Different letters in the same column indicate significantly different results ($P<0.05$). The same letters in the same column indicate results that are not significantly different ($P>0.05$).

Hydrolysis degree of nutrients

The data show the results of measuring the nutrient degree of artificial feed for white snapper larvae after predigestion using a mixture of microorganisms presented in Table (2); the hydrolysis degree of fat, fiber, protein, and NEF. The optimal hydrolysis degrees for fat (34.95%), fiber (79.86%), protein (-11.25%), and NEF (-1.97) were recorded at 10mL/ kg dosage with 18-hour incubation.

Table 2. Degree of hydrolysis of artificial feed of white snapper larvae predigested with mixed microorganisms

Treatment	Hydrolisis degree of Fat	Hydrolisis degree of Fiber	Hydrolisis degree of protein	Hydrolisis degree of NEF
A1B1	27.82 ± 0.07^{a}	69.02 ± 0.32^{a}	-6.98 ± 0.13^{b}	-4.08 ± 0.26^{b}
A1B2	29.76 ± 0.20^{a}	72.50 ± 0.18^{a}	-8.57 ± 0.13^{b}	-3.71 ± 0.15^{b}
A1B3	34.18 ± 0.32^{c}	81.67 ± 3.74^{d}	-8.71 ± 0.22^{b}	-3.68 ± 0.25^{b}
A1B4	33.92 ± 0.24^{b}	75.47 ± 0.25^{b}	-9.48 ± 0.06^{a}	-8.35 ± 0.01^{a}
A2B1	29.06 ± 0.06^{a}	73.51 ± 0.68^{a}	-5.76 ± 0.15^{b}	-6.40 ± 0.12^{a}
A2B2	30.44 ± 0.24^{b}	77.01 ± 0.32^{b}	-9.85 ± 0.85^{a}	-2.90 ± 0.36^{c}
A2B3	34.96 ± 0.40^{c}	79.86 ± 0.16^{c}	-11.25 ± 1.30^{a}	-1.97 ± 0.83^{c}
A2B4	34.82 ± 0.20^{c}	79.36 ± 0.18^{c}	-10.58 ± 0.37^{a}	-2.29 ± 0.01^{c}
A3B1	27.79 ± 0.10^{a}	73.15 ± 0.08^{a}	-7.51 ± 0.04^{b}	-4.43 ± 0.09^{b}
A3B2	30.69 ± 0.22^{b}	76.78 ± 0.22^{b}	-9.00 ± 0.20^{b}	-3.62 ± 0.03^{b}
A3B3	34.71 ± 0.71^{c}	79.57 ± 0.19^{c}	-8.12 ± 0.08^{b}	-5.32 ± 0.13^{a}
A3B4	34.28 ± 0.92^{c}	79.42 ± 0.16^{c}	8.67 ± 0.06^{b}	-4.38 ± 0.06^{b}

Note: Different letters in the same column indicate significantly different results ($P<0.05$).

The analysis of variance showed that the dose of mixed microorganisms, the incubation period, and the interaction of both significantly affected the average degree of hydrolysis of fat, fiber, protein, and NEF (P<0.05). The results of the W_Tukey test showed that the average dose of 10ml/ kg feed and the incubation period of 18 hours was the most optimal and significantly different from other treatments and substantially different from other treatments of 5 and 15ml/ kg feed with an incubation period of 6 and 12 hours. There was no significant difference in the treatment of doses of 5, 10, 15ml/ kg feed at a period of 18 and 24 hours. The interaction between the degree of hydrolysis of feed nutrients can be seen in Fig. (1).

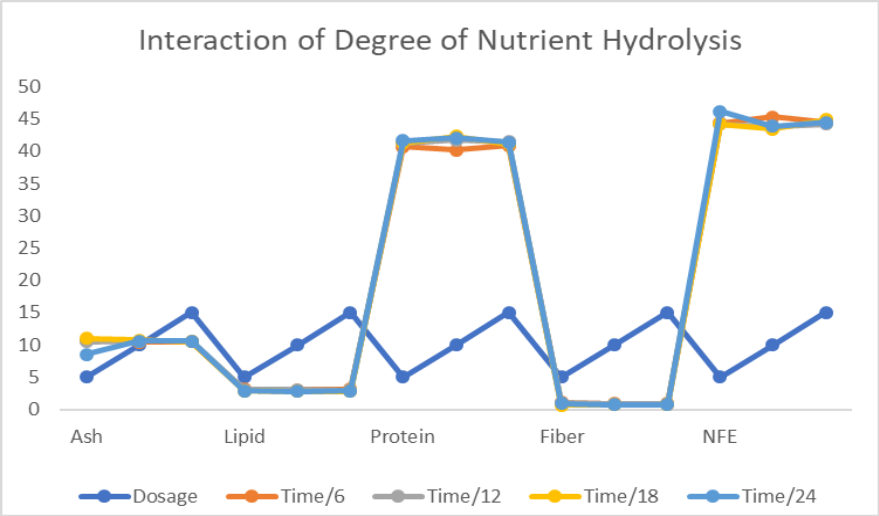

Fig. 1. Interaction of the degree of hydrolysis of predigested nutrients in artificial feed using mixed microorganisms on dose treatment and incubation period

Soluble protein and glucose content

The highest soluble protein (48mg/ 10mL) and glucose content (7.67%) were observed at 10mL/ kg dosage with 18-hour incubation.

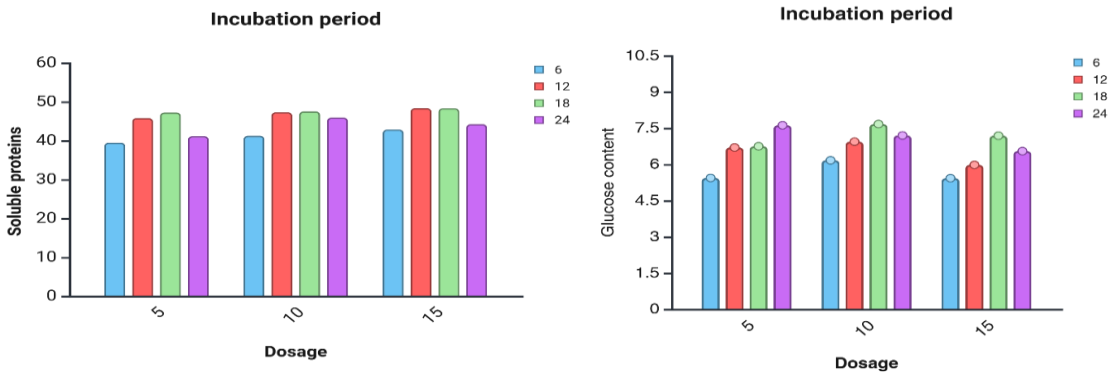

Fig. 2. Soluble proteins and glucose levels of predigest feed using microorganism mix

DISCUSSION

Proximate analysis of the pre-digested feed using mixed microorganisms revealed a significant variation in the nutritional profile of the feed treated with mixed organisms compared to the control diet. The crude protein content increased significantly in all treatments, with the highest value observed at a dose of 10mL/ kg of feed and an incubation time of 18 hours, while the feed without the addition of mixed microorganisms had the lowest protein content of 38.00%. This observed increase in protein content aligns with previous research indicating that microbial fermentation improves protein digestibility by producing exogenous enzymes, such as proteases, breaking down complex proteins into peptides and free amino acids **(Rasdi *et al*., 2021: Chi *et al*., 2024)**, which increases protein availability for fish **(Rudnyckyj *et al.*, 2023)**.

The microorganism mix contains *Bacillus* and *Lactobacillus*, known to produce protease enzymes. Previous studies have demonstrated that fermentation with *Bacillus* and *Lactobacillus* can increase feed protein content through protease enzyme synthesis and microbial biomass growth **(Li & Chen, 2024)**. Furthermore, certain microorganisms can synthesize proteins through biomass synthesis, increasing the total protein in the feed **(Anwar *et al*., 2023)**. In addition, increased protein is also associated with a reduction in crude fiber and fat, which enhances the overall nutritional composition of the feed **(Aslamyah *et al.*, 2018)**. Therefore, fermentation with the optimal dose of microorganisms can increase the protein content of feed and enhance nutrient utilization in fish **(Melianawati & Pratiwi, 2022)**.

The crude fiber content in predigested feed showed a significant reduction compared to the control (4.35%), with the lowest values observed in treatments A1B3 (0.66%) and A2B3 (0.73%). This decrease is attributed to the activity of cellulase and hemicellulase enzymes produced by the mixed microorganisms, which break down complex fibers into simpler, more digestible compounds **(Rasdi *et al*., 2021)**. Previous research has shown that fermentation with microorganisms such as *Aspergillus* and *Rhizopus* can hydrolyze crude fiber, increasing the availability of other nutrients in feed **(Fauzan *et al.*, 2022)**. Reducing crude fiber is crucial because high fiber content in fish feed can inhibit the digestion of other nutrients, especially in the larval phase with an incomplete digestive system **(Pu *et al*., 2023)**. A decrease in crude fiber enhances overall feed digestibility, enabling fish to assimilate more nutrients. Therefore, fermentation with a microorganism mix has the potential to be an effective method in optimizing the quality of fish feed through the reduction of crude fiber.

The fat content in predigested feed decreased compared to the control (3.60%), with the lowest value found in the treatment of 10mL/ kg of feed with an incubation period of 18 hours (2.83%). This decrease can be attributed to the activity of the lipase enzyme produced by the mixed microorganisms, which hydrolyzes complex fats into free fatty acids and glycerol **(Niu *et al*., 2022)**. In addition, some microorganisms, such as *Lactobacillus*, can also use lipids as an energy source, leading to reduced fat in feed **(Bendaoud *et al*., 2024)**. This reduction in fat content can mitigate the risk of fat oxidation, which can degrade feed quality and produce toxic compounds harmful to fish

(Sumiana *et al*., 2020). Therefore, fermentation with microorganisms not only enhances fat digestibility but also improves the stability of the feed.

The content of Nitrogen-Free Extract (NFE) in predigested feed increased compared to the control (42.61%). This increase reflects the effectiveness of microorganisms in hydrolyzing starch and complex carbohydrates into simple sugars, which are easier to utilize as a source of energy for fish **(Sumiana *et al.*, 2020)**. Fermentation with microorganisms such as *Saccharomyces* and *Lactobacillus* has been shown to increase NFE by converting complex carbohydrates into fermented sugars, which can increase the energy value of feed **(Ismail *et al*., 2023)**. However, treatment at a dose of 10mL/ kg of feed with an incubation period of 18 hours did not show a significant increase, likely because microorganisms, including bacteria and yeast, utilize the hydrolyzed simple sugars for their metabolic processes and growth during fermentation rather than converting them into NFE **(Chen *et al*., 2024)**. The energy obtained from hydrolyzed sugars is mainly used to synthesize microbial biomass, leading to low accumulation of NFE in fermented feeds **(Nordlund *et al*., 2024)**. The increase in microbial biomass during fermentation reduces the amount of sugar available to be converted into NFE, which affects the final yield of fermentation **(Behounek *et al*., 2024)**. Although it does not increase the NFE significantly, it improves feed quality.

The ash content in predigested feed decreased compared to the control (11.44%), with the lowest value observed in the treatment of 5mL/ kg of feed with an incubation period of 24 hours (8.52%). Microorganisms, such as bacteria, play a role in modifying the chemical composition of ash by solubilizing metals and other compounds, which can lead to a decrease in ash levels **(Aouad *et al*., 2006)**. Research indicates that an increased feed mixing time is related to decreased ash and crude fiber levels, suggesting that fermentation can improve feed quality **(Latief *et al*., 2023)**.

A dose of 10mL/ kg of feed, coupled with an 18-hour incubation period, yielded the highest degree of crude fat hydrolysis. This suggests that such conditions optimize microbial activity, enhancing fat digestibility. Certain microorganisms, such as *Lactobacillu*s in the mixed microbial, can augment lipid metabolism by producing short-chain fatty acids (SCFAs) during fermentation, which are essential for fat breakdown **(Niu *et al*., 2022)**. During fermentation, microbial cells multiply to produce enzymes that facilitate fat breakdown **(Sangadji, 2020)**. Optimal doses of microorganisms can enhance the enzymatic activity required for effective fat hydrolysis, thereby improving the overall nutritional quality of the feed **(Ismail *et al*., 2023)**.

An 18-hour incubation period proved optimal for achieving the highest degree of crude fat hydrolysis. Research indicates that hydrolysis processes generally last up to 18 hours, with specific enzymatic activity peaking at a given time **(Prihanto *et al*., 2021)**. This duration is also effective in hydrolyzing proteins and reducing fat content. Furthermore, an optimal incubation time can alter the composition of microbial communities, specifically increasing the abundance of fat-degrading bacteria, which is

essential for effective fat hydrolysis **(Pu *et al*., 2023)**. For example, the application of lipase enzymes has demonstrated effective fat degradation within the specified incubation period, underscoring the importance of timing for maximizing enzymatic efficiency **(Alabdalall *et al*., 2021)**.

The combination of a microbial dose of 10mL/ kg of feed and an incubation time of 18 hours resulted in optimal crude fat hydrolysis. This interaction suggests a synergistic effect, wherein the dose and incubation time together maximize enzymatic activity against fat. The optimal dose of microbial enzymes or inoculants, when combined with the appropriate incubation time, enhances hydrolysis and leads to increased fat digestibility **(Annisa *et al*., 2020)**. Conversely, insufficient doses or short incubation periods can result in inadequate hydrolysis, as demonstrated by experiments where low doses failed to achieve complete fermentation of feed components ***(*Genç *et al*., 2020)**. The extent of hydrolysis is also affected by the specific type of feed; for example, certain substrates may require longer fermentation times to achieve optimal fat breakdown **(Anwar *et al*., 2023)**. Therefore, carefully balancing dosage and incubation time is essential to maximize the efficiency of fat hydrolysis in fish feed.

Using microorganisms mixed at a dose of 10mL/ kg of feed to pre-digest the feed resulted in the highest degree of crude fiber hydrolysis, achieving a value of 79.86%. The mixed microorganism culture contains cellulase enzymes that effectively break down crude fibers into simple sugars **(Fauzan *et al*., 2022)**. Enzyme dosage is essential to maximize the hydrolysis of crude fiber in feed, improving overall feed quality **(Sumiana *et al*., 2020)**. Thus, optimizing the dosage of microorganisms is essential to improve the hydrolysis of crude fiber in feed, thereby enhancing the nutritional quality and digestibility of the feed.

An 18-hour incubation period is optimal for the hydrolysis of coarse fibers, allowing sufficient time for the enzymes to degrade the fiber components **(Guna *et al*., 2023)**. The incubation period affects the degradation of cellulose and hemicellulose, leading to a significant decrease in crude fiber content **(Anwar *et al*., 2023)**. A time period of 18 hours contributes to a reduction in fiber, which enhances the degree of hydrolysis of coarse fiber in the feed.

Enzyme dosage and incubation time play an important role in the hydrolysis of coarse fiber in feed. Increased doses of hydrolytic enzymes, such as cellulase, have been shown to significantly increase the breakdown of complex fiber structures into simpler sugars, thereby improving digestibility **(Rakhmawati *et al*., 2021)**. In addition, the length of the incubation period is equally important; the optimal incubation time allows for a wider range of enzymatic activity, which can lead to a greater reduction in crude fiber content **(Putra *et al*., 2022)**. The synergistic effect of enzyme dosage and incubation time is important to optimize feed, especially in increasing the quality of fibrous feed ingredients **(Pratiwy & Maulida, 2022)**.

The observed degree of protein hydrolysis can be attributed to the increased protein levels resulting from the enzymatic activity of microbes that break down complex protein structures into simpler, more absorbable forms. This aligns with previous research that has highlighted the role of microbial fermentation in improving protein digestibility **(Surnawati *et al*., 2020; Rasdi *et al*., 2021)**. Dosage influences the degree of protein hydrolysis, while lower doses can hinder microbial degradation, thus limiting the hydrolysis process **(Rudnyckyj *et al*., 2023)**. Conversely, higher doses can result in substrate inhibition, leading to protein damage **(Rasdi *et al.*, 2021; Li & Chen, 2024)**.

An 18-hour incubation period is optimal for protein hydrolysis, allowing the proteolytic enzymes to function optimally **(Bendaoud *et al*., 2024)**. Other studies have shown that varying fermentation durations can cause significant differences in protein levels, with specific periods correlating with higher levels of hydrolysis **(Melianawati & Pratiwi, 2022)**. The interaction between enzyme dose and incubation time affects the efficiency of protein hydrolysis in feed, which impacts the overall nutritional value and digestibility.

Neither the variable dose nor the incubation time significantly affected the degree of hydrolysis of NFE ($P > 0.05$). NFE hydrolysis largely depends on the activity of amylase enzymes produced by microorganisms. The amylase enzyme activity produced by the mixed microorganisms may not have been sufficient to effectively break down the NFE **(Niu *et al.*, 2022)**. Amylase has a high substrate specificity, so its effectiveness in hydrolyzing starch can vary depending on the structure and complexity of NFE in the feed **(Pu *et al.*, 2023)**. The substrate microorganisms utilize contains more fat, protein, and fiber than NFE, so microorganisms are more likely to hydrolyze these components preferentially. Several studies suggest that the presence of proteins and fibers can influence the degradation of NFE due to enzyme competition for different substrates **(Sumiana *et al*., 2020)**.

The addition of the microorganism mix increases the soluble protein content, with an incubation period of 12–18 hours proving to be the most effective for optimizing protein hydrolysis. The highest dissolved protein content observed at a dose of 15mL/ kg of feed with a 12-hour incubation (48.38mg/ 10mL) and at 10mL/ kg of feed with an 18-hour incubation (47.54mg/ 10mL) suggests that these conditions are optimal for maximizing protein digestibility. An increase in soluble protein content up to 12–18 hours of incubation indicates optimal hydrolysis of complex proteins into peptides and free amino acids **(Bendaoud *et al*., 2024)**. The results align with previous research showing that microbial fermentation using protease-producing bacteria increases protein breakdown, thereby increasing the availability of nutrients in aquaculture feed **(Ismail *et al.*, 2023)**. A decrease in soluble proteins after 24 hours of incubation suggests that prolonged fermentation can lead to the secondary degradation of peptides into non-protein nitrogen compounds, such as ammonia and volatile nitrogen metabolites **(Pu *et al.*, 2023)**.

An increase in glucose content of up to 18–24 hours of incubation demonstrates optimal hydrolysis of complex carbohydrates into simple sugars by microbial amylase **(Bendaoud *et al*., 2024)**. These results align with previous research showing that microbial fermentation can increase glucose availability by breaking down polysaccharides into monosaccharides, thereby increasing energy utilization in fish feed **(Ismail *et al*., 2023**). However, the lower glucose content observed in the treatment with a microorganism mix of 15mL/ kg of feed suggests that excessive microbial activity may lead to glucose consumption for microbial metabolism rather than accumulation in the feed **(Pu *et al*., 2023)**.

These findings suggest that a moderate microbial dose (10mL/ kg) with an incubation time of 18 hours provides the most effective hydrolysis of carbohydrates while preventing excessive glucose depletion. The increase in glucose content in feed digested by microbes contained in the mix of microorganisms, including *Bacillus*, *Lactobacillus*, and *Aspergillus*, is due to increased glucose through fermentation **(Sidar *et al*., 2023; Zhang *et al*., 2023)**. These microorganisms produce amylase and other carbohydrates that hydrolyze complex starch molecules into more straightforward and more easily digestible sugars, thereby increasing the availability of energy in feed **(Abdulla *et al*., 2024; Chi *et al*., 2024)**. However, excessive microbial doses (5mL/ kg of feed) and prolonged fermentation of more than 24 hours can lead to glucose depletion, which suggests that monitoring the dose and incubation period is essential to prevent excessive microbial sugar consumption **(Chi *et al*., 2024)** .

CONCLUSION

This study demonstrates that microbial pre-digestion significantly enhances the digestibility and nutritional quality of artificial feed for white snapper larvae. The findings reveal that a microorganism dosage of 10mL/ kg with an 18-hour incubation period results in optimal feed composition, characterized by increased protein solubility, reduced fiber content, and higher glucose availability. These results suggest that microbial fermentation effectively pre-digests feed components, improving nutrient absorption and metabolic efficiency in fish larvae. The observed improvements in nutrient availability align with previous studies on microbial fermentation, confirming that enzymatic hydrolysis by mixed microorganisms plays a crucial role in breaking down complex macronutrients. Additionally, the reduction in lipid oxidation potential and fiber content further supports the feasibility of microbial pre-digestion as a strategy to enhance artificial feed quality. The application of this technique in aquaculture could lead to reduced dependence on live feeds, lower production costs, and increased sustainability in larval fish rearing. Future research should focus on *in vivo* feeding trials to validate these findings and optimize fermentation conditions for large-scale applications.

REFERENCES

Abdulla, S.; Surendran, A. and Thatheyus, A. J. (2024). Studies on the Influence of Artificial Fish Feed on the Growth of the Fingerlings of the Spotted Tilapia, Pelmatolapia mariae. Asian Journal of Biological Sciences, 17(1), 128–137. https://doi.org/10.3923/ajbs.2024.128.137

Alabdalall, A.; H. Al-Anazi, N.; Aldakheel, L.; Amer, F. H. I.; Aldakheel, F. A.; Ababutain, I.; M. Alghamdi, A.I.; and Al-Khaldi, E. M. (2021). Application and characterization of crude fungal lipases used to degrade fat and oil wastes. Scientific Reports, 11(1), 1–10. https://doi.org/10.1038/s41598-021-98927-4

Alfonso, S.; Mente, E.; Fiocchi, E.; Manfrin, A.; Dimitroglou, A.; Papaharisis, L.; Barkas, D.; Toomey, L.; Boscarato, M.; Losasso,; C. Peruzzo, A.; Stefani, A.; Zupa, W.; Spedicato, M. T.; Nengas, I.; Lembo, G. and Carbonara, P. (2023). Growth performance, gut microbiota composition, health and welfare of European sea bass (Dicentrarchus labrax) fed an environmentally and economically sustainable low marine protein diet in sea cages. Scientific Reports, 13(1). https://doi.org/10.1038/s41598-023-48533-3

Annisa, Rizal, Y.; Mirnawati.; Suliansyah, I. and Bakhtiar, A. (2020). Determination of the appropriate inoculum dose and incubation period of Cassava Leaf Meal and Tofu Dreg mixture fermented with Rhizopus oligosporus. Journal of World's Poultry Research, 10(1), 118–124. https://doi.org/10.36380/SCIL.2020.WVJ16

Anwar, A.; Zainuddin.; Djawad, M. I. and Aslamyah, S. (2023). Fermentation of rain tree (Samanea saman) seed meal using mixed microbes to improve its nutritional quality. Biodiversitas, 24(11), 5863–5872. https://doi.org/10.13057/biodiv/d241104

A.O.A.C (2005). Analytical Chemist Inc, U.. Official methods of analysis (18th ed.). In Association Analytical Chemist Inc.

Aouad, G.; Crovisier, J.; Damidot, D.; Stille, P.; Meyer, J. and Geoffroy, V. (2006). Action bactérienne sur un mâchefer d ' incinération d ' ordure ménagère. 12–16.

Aslamyah, S. (2006). Penggunaan mikroflora saluran pencernaan sebagai probiotik untuk meningkatkan pertumbuhan dan kelangsungan hidup ikan bandeng (Chanos chanos Forsskal).

Aslamyah, S.; Karim, M. and Badraeni. (2018). Pengaruh Dosis Mikroorganisme Mix Dalam Memfermetasi Bahan Baku Pakan Yang Mengandung Sargassum sp. Terhadap Kinerja Pertumbuhan Komposisi Kimia Tubuh Dan Indeks Hepatosomatik Ikan Bandeng (Chanos chanos Forsskal). Torani Jurnal of Fisheries and Marine Science, 1(2), 59–70.

Aslamyah, S.; Zainuddin, Z. and Badraeni, B. (2022). The effect of microorganisms combination as probiotics in feed for growth performance, gastric evacuation rates, and blood glucose levels of milkfish, Chanos chanos (Forsskal, 1775). Jurnal Iktiologi Indonesia, 22(1), 77–91. https://doi.org/10.32491/jii.v22i1.583

Behounek, M.; Cochran, D.; Motta-Romero, H.A.; Yang, Q.; Ding, W.; Morton, M.; Majumder, K.; Powers, R.; and Rose, D. J. (2024). In Vitro Fermentation of Animal and Plant Protein Isolates by the Human Gut Microbiota Under High and Low Carbohydrate Conditions. Molecular Nutrition and Food Research, 68(14), 1–15. https://doi.org/10.1002/mnfr.202300555

Bendaoud, A.; Hmamou, A.; Moussaoui, F.; Eloutassi, N. and Lahkimi, A. (2024). Optimization of Lignocellulosic Enzyme production by Microbial Strains: Study of Kinetics and Environmental Influences. Research Journal of Chemistry and Environment, 28(9), 1–8. https://doi.org/10.25303/289rjce0108

Chen, Jinmeng.; Cai, Y.A.; Zhi, Wang.; Zhengzhong, Xu.; Wei, Zhuang.; Dong, Liu.; Yongkun, L.v.; Shilei, Wang.; Jingliang, Xu. and Hanjie Ying. (2024). Solid-state fermentation of corn straw using synthetic microbiome to produce fermented feed: The feed quality and conversion mechanism. Science of The Total Environment, 920. https://doi.org/https://doi.org/10.1016/j.scitotenv.2024.171034

Chi, Z.; Feng, Y.; Wei, X.; Yang, H.; Fang, X.; Cheng, B.; Li, Z.; Shi, B. and Gu Z. (2024). A co-fermentation technology based on small peptide production and protein structure modification increases the edible value of corn gluten meal. Lwt, 198(600), 116054. https://doi.org/10.1016/j.lwt.2024.116054

El-Dahhar, A. A.; Khaleefa, G. A.; El-Zaeem, S. Y.; Mourad, M. M. and Raheem, M. M. A. (2024). Impact of organic and nanoparticle-selenium fortified larval weaning diet on survival, growth, gastrointestinal, and (GPx) activity of European Sea Bass (Dicentrarchus labrax) Larvae. Aquaculture International. https://doi.org/10.1007/s10499-024-01576-3

Fauzan.; Suprayudi, M. A.; Fauzi, I. A. and Ekasari J. (2022). Utilization of hydrolyzed corncob as a carbohydrate source in diets for red Nile tilapia Oreochromis niloticus. Jurnal Akuakultur Indonesia, 21(1), 32–40. https://doi.org/10.19027/jai.21.1.32-40

Genç, B.; Salman, M.; Bölükbaş, B.; Kaya, İ. and Açici M. (2020). The effects of fumaric and malic acids on the in vitro true digestibility of some alternative feedstuffs for ruminants. Ankara Universitesi Veteriner Fakultesi Dergisi, 67(2), 185–192. https://doi.org/10.33988/auvfd.623821

Guna D. (2005). Sebagian Yang Untuk Derajat and Peternakan, S.. F a k u l t a s p e t e r n a k a n universitas mataram mataram 2023.

Ismail, R.; Trisna, A.; Tati, V. S.; and Amran, M. (2023). Evaluation Nutrient of Larvae Black Soldier Fly (Hermetia Illucens) on Fermented Palm Oil Sludge Culture Media. IOP Conference Series: Earth and Environmental Science, 1286(1). https://doi.org/10.1088/1755-1315/1286/1/012021

Latief, M. F.; Hasrin, H.; Amal, I.; Chadija, S. and Aini, F. N. (2023). Analisis Kualitas Nutrisi Konsentrat Pakan Sapi Potong Dengan Variasi Waktu Pencampuran Pakan Menggunakan Mixer Vertical. Jurnal Nutrisi Ternak Tropis, 6(2), 90–

97. https://doi.org/10.21776/ub.jnt.2023.006.02.3

Li, M. and Chen H. (2024). Enhanced short-chain fatty acid production from sludge anaerobic fermentation by combined pretreatment with sodium pyrophosphate and thermal hydrolysis. Bioresource Technology, 406, 131067. https://doi.org/10.1016/j.biortech.2024.131067

Melianawati, R. and Pratiwi, R. (2022). PENGARUH PAKAN EKSOGEN AWAL TERHADAP AKTIVITAS ENZIM PENCERNAAN DAN PERTUMBUHAN LARVA Epinephelus fuscoguttatus (Forsskal, 1775). Jurnal Ilmu Dan Teknologi Kelautan Tropis, 14(1), 131–146. https://doi.org/10.29244/jitkt.v14i1.39801

Ming, M.Q.; Yu, Q. R.; Er,-C. L.; Ying, S.; Xin.-Y. C.; Yu.-H.; Chuan,-J. Q.; Xiao,-D. W.; Jian,-G. Q. and Chen, L.-Q. (2024). Leucine improves dietary protein use efficiency by regulating protein synthesis by activating amino acid transporters and the mTORC1 pathways in Chinese mitten crab (Eriocheir sinensis). Aquaculture, 581, 740423.

Moroni, F.; Carvalho, M.; Di, Rosa A. R.; Torrecillas, S.; Fontanillas, R.; Haffray, P.; Allal, F.; Bajek, A.; Chiofalo, B.; Terova, G. and Montero D. (2024). Genetic selection and novel feeds containing single cell protein as a substitute for fishmeal in European sea bass: Effects on growth, fatty acid profile and E-sensing analysis of fillets. Aquaculture Reports, 35. https://doi.org/10.1016/j.aqrep.2024.102021

Niu, X.; Zhang, N;. Li, S.; Li, N.; Wang, R.; Zhang, Q.; He, J.; Sun, E.; Kang, X. and Zhan, J. (2022). Bifidobacterium animalis subsp. lactis MN-Gup protects mice against gut microbiota-related obesity and endotoxemia induced by a high fat diet. Frontiers in Nutrition, 9(November), 1–12. https://doi.org/10.3389/fnut.2022.992947

Nordlund, E.; Silventoinen-.;Veijalainen, P.; Hyytiäinen-Pabst, T.; Nyyssölä, A.; Valtonen, A.; Ritala, A.; Lienemann, M. and Rosa-Sibakov N. (2024). In vitro protein digestion and carbohydrate colon fermentation of microbial biomass samples from bacterial, filamentous fungus and yeast sources. Food Research International, 182(December 2023). https://doi.org/10.1016/j.foodres.2024.114146

Pratiwy, F. M. and Maulida Y. (2022). Hydrolysis Enzyme of Alternative Ingredients for Fish Feed: A Review. Asian Journal of Fisheries and Aquatic Research, 20(6), 95–101. https://doi.org/10.9734/ajfar/2022/v20i6521

Prihanto, A. A.; Nurdiani, R. and Sari, L.W. (2021). Production and characteristics of sailfin catfish (Pterygoplichthys pardalis) protein hydrolysate. F1000Research, 10, 1089. https://doi.org/10.12688/f1000research.73335.1

Pu, X.X.; Zhang, X.M.; Li, Q. S.; Wang, R.; Zhang, M.; Zhang, S. Z.; Lin, B.; Tan, B.; Tan, Z. L. and Wang, M. (2023). Comparison of in situ ruminal straw fiber degradation and bacterial community between buffalo and Holstein fed with high-roughage diet. Frontiers in Microbiology, 13(January), 1–8. https://doi.org/10.3389/fmicb.2022.1079056

Putra, A. N.; Indayah, U.; Nokiyah, N. and Syamsunarno, M. B. (2022). Improving quality of cassava peel meal as raw material for tilapia feed. Depik, 11(3), 319–326. https://doi.org/10.13170/depik.11.3.24620

Rakhmawati, R.; Sofiana, A.; Indariyanti, N.; and Bokau, R. J. M. (2021). Decreasing of Crude Fibre in Indigofera Leaves Flour Hydrolysed with Cellulase Enzyme as a Source of Feed Protein. IOP Conference Series: Earth and Environmental Science, 1012(1). https://doi.org/10.1088/1755-1315/1012/1/012060

Rasdi.; Latama, G. and Tandipayuk H. (2021). The Effectiveness Of Additional Papain Enzyme In Artificial Diets On Protein Hydrolysis And Protease Enzyme Activity, White Snapper Larva (Lates calcarifer, Bloch 1790). International Journal of Scientific and Research Publications (IJSRP), 11(12), 31–36. https://doi.org/10.29322/ijsrp.11.12.2021.p12007

Rudnyckyj, S.; Chaturvedi, T. and Thomsen, M.H. (2023). Low-dosage enzymatic hydrolysis of organic municipal waste for sugar and ethanol production. Biomass Conversion and Biorefinery. https://doi.org/10.1007/s13399-023-04858-7

Sangadji, I. S. (2020). Nutritional Quality And Fiber Fraction Of Complete Feed Silage Based On Sago By-Products, Imperata Cylindrica And Leucaena Leucocephala. Buletin Peternakan, 44(4), 203–208. https://doi.org/10.21059/buletinpeternak.v44i4.58960

Sidar, A.; Voshol, G. P.; Vijgenboom, E. and Punt, P. J. (2023). Novel Design of an α-Amylase with an N-Terminal CBM20 in Aspergillus niger Improves Binding and Processing of a Broad Range of Starches. Molecules, 28(13). https://doi.org/10.3390/molecules28135033

Sumiana, I. K.; Ekasari, J.; Jusadi, D. and Setiawati M. (2020). Utilization of fermented sago pulp as a source of carbohydrate in feed for Nile tilapia Oreochromis niloticus. Jurnal Akuakultur Indonesia, 19(2), 106–117. https://doi.org/10.19027/jai.19.2.106-117

Surnawati, N. and Azhar, F. (2020). PERTUMBUHAN DAN KELANGSUNGAN HIDUP BENIH IKAN KAKAP PUTIH Lates calcarifer, Bloch DENGAN PEMBERIAN DOSIS PROBIOTIK YANG BERBEDA. Jurnal Ruaya : Jurnal Penelitian Dan Kajian Ilmu Perikanan Dan Kelautan, 8(1), 38–44. https://doi.org/10.29406/jr.v8i1.1449

Yasutake W. (1977). This is a reproduction of a library book that was digitized by Google as part of an ongoing effort to preserve the information in books and make it universally accessible . Biologia Centrali-Americaa, 2, v–413.

Zhang, D.; Liu, L. and Chen B. S. (2023). Marine-Derived Fungi as a Valuable Resource for Amylases Activity Screening. Journal of Fungi, 9(7). https://doi.org/10.3390/jof9070736