Sains Malaysiana 54(1)(2025): 9-20
http://doi.org/10.17576/jsm-2025-5401-02

Effect of Combined Prebiotics from Different Sources and Microorganism Mix on Growth Performance of Milkfish, *Chanos chanos* Forsskal, 1775

(Kesan Gabungan Prebiotik daripada Sumber Berbeza dan Campuran Mikroorganisma terhadap Prestasi Pertumbuhan Ikan Bandeng, *Chanos chanos* Forsskal, 1775)

SITI ASLAMYAH[1,*], ZAINUDDIN[1] & AYMAN ALAMEEN MOHAMMED[2]

[1]Fisheries Department, Faculty of Marine Science and Fisheries, Hasanuddin University, Jl. Perintis Kemerdekaan No 10, Tamalanrea Indah, 90245, Makassar, Indonesia
[2]Department of Fisheries and Wildlife Science, College of Animal Production Science and Technology, Sudan University of Science and Technology, Wehda street, 12217, Khartoum, Sudan

Received: 1 October 2023/Accepted: 5 November 2024

ABSTRACT

This study was conducted to determine the effect of using synbiotics in artificial feed on growth, digestive enzyme activities, glycogen levels, hepatosomatic index, body chemical composition, nutrient retention, gastric emptying rate, and blood glucose levels of milkfish. Milkfish (4.78 ± 0.16 g) were stocked at density of 20 fish per aquarium (50 × 40 × 35 cm^3) containing 48 L of water with a salinity of 20 ppt and equipped with a recirculation system. Fish were fed three times a day (7:00 AM, 12:00, and 17:00 PM) at a feeding rate of 5% for 50 days. This study used a completely randomized design (CRD) with five treatments and three replicates. Synbiotics consisting of 10 mL/kg microorganism mix and 2% different sources of prebiotics (sweet potatoes, *Kappaphycus alvarezii*, green beans, and red onion) were added to the artificial feed. The results indicated that milkfish-fed sweet potatoes and green beans experienced significant growth improvement and enhanced feed efficiency. These prebiotic sources also positively impacted digestive enzyme activities, potentially improving nutrient utilization. Additionally, green beans exhibited the most pronounced effects on liver and muscle glycogen levels, hepatosomatic index, and energy content. Body composition data showed alterations in protein, fat, ash, crude fiber, and nitrogen-free extract percentages due to prebiotic sources. Green beans demonstrated the highest nutrient retention rates. Moreover, the rate of gastric emptying and blood glucose that was observed reached its peak, and the fastest peak decreased in the prebiotic treatment, which was sourced from green beans and sweet potatoes.

Keywords: Artificial feed; growth performance; milkfish; synbiotics

ABSTRAK

Penyelidikan ini dijalankan untuk menentukan kesan penggunaan sinbiotik dalam makanan buatan terhadap pertumbuhan, aktiviti enzim pencernaan, paras glikogen, indeks hepatosomatik, komposisi kimia badan, pengekalan nutrien, kadar pengosongan gastrik dan paras glukosa darah ikan bandeng. Ikan bandeng (4.78 ± 0.16 g) distok pada ketumpatan 20 ekor setiap akuarium (50 × 40 × 35 cm^3) yang mengandungi 48 L air dengan kemasinan 20 ppt dan dilengkapi dengan sistem peredaran semula. Ikan diberi makan tiga kali sehari (7:00 AM, 12:00 dan 17:00 PM) pada kadar pemakanan 5% selama 50 hari. Kajian ini menggunakan reka bentuk rawak sepenuhnya (CRD) dengan lima rawatan dan tiga ulangan. Sinbiotik yang terdiri daripada 10 mL/kg campuran mikroorganisma dan 2% sumber prebiotik yang berbeza (ubi keledek, *Kappaphycus alvarezii,* kacang hijau dan bawang merah) telah ditambah ke dalam makanan buatan. Keputusan menunjukkan bahawa ikan bandeng yang diberi makan ubi keledek dan kacang hijau mengalami peningkatan pertumbuhan yang ketara dan kecekapan makanan yang dipertingkatkan. Sumber prebiotik ini juga memberi kesan positif kepada aktiviti enzim pencernaan yang berpotensi meningkatkan penggunaan nutrien. Selain itu, kacang hijau menunjukkan kesan yang paling ketara pada tahap glikogen hati dan otot, indeks hepatosomatik dan kandungan tenaga. Data komposisi tubuh menunjukkan perubahan dalam protein, lemak, abu, serat kasar dan peratusan ekstrak bebas nitrogen disebabkan oleh sumber prebiotik. Kacang hijau menunjukkan kadar pengekalan nutrien yang paling tinggi. Selain itu, kadar pengosongan gastrik dan glukosa darah yang diperhatikan mencapai kemuncaknya dan kemuncak terpantas menurun dalam rawatan prebiotik yang diperoleh daripada kacang hijau dan ubi keledek.

Kata kunci: Ikan bandeng; makanan buatan; prestasi pertumbuhan; sinbiotik

INTRODUCTION

The milkfish (*Chanos chanos*) holds significant culinary value within the Indo-Pacific region (Lee et al. 2022). Being a tropical marine species, it assumes a pivotal position in the field of aquaculture and is widely cultivated in diverse Asian nations such as the Philippines, Indonesia, and Taiwan. Furthermore, milkfish aquaculture is also conducted in specific places of the Americas and Africa. This fish is widely recognized for its subtle flavor and sensitive texture, making it a highly sought-after culinary choice in various countries (Marte 2010). The milkfish species is highly valued in the field of aquaculture due to its multitude of advantages when compared to other fish species. It is worth mentioning that milkfish demonstrates a remarkable rate of growth, achieving a size suitable for commercial use within a relatively short period of 6 to 8 months. In addition, the notable capacity of this organism to thrive in a variety of environmental conditions allows for its culture in a variety of setups, such as ponds, cages, and pens (Chen & Durand 2016). Intensified aquaculture has emerged as a feasible solution to suit the demands of restaurants and export markets due to the rising demand for specific milkfish sizes. Aquaculture producers encounter a notable apprehension pertaining to the expenditure associated with feed, since it comprises a considerable proportion of their operational costs (Dossou et al. 2018). The primary factor influencing the cost is the dependence of the feed on protein, predominantly obtained from fishmeal or soybeans. These protein sources are frequently imported and susceptible to market swings, resulting in escalated expenses (El Basuini et al. 2017). Despite extensive endeavors to identify alternative protein sources, none have demonstrated comparable efficacy in stimulating growth and enhancing the overall well-being of aquatic organisms. Aquafeed is crucially formulated with carbohydrates. However, the inclusion of vegetable-based carbohydrates that are rich in fiber can pose difficulties in terms of digestion, leading to reduced feed utilization and overall productivity. The issue of the exorbitant expenses associated with feed in the aquaculture sector is a prominent obstacle that requires continuous investigation and innovation to discover viable and economical substitutes for conventional feed components.

In order to mitigate the financial burden associated with feed expenses in aquaculture, the use of functional feed technology emerges as a viable approach, as proposed by Soto, Ibáñez and Godoy (2015). Functional feed technology refers to the practice of modifying feed ingredients to produce a tailored feed that addresses the precise nutritional requirements of fish, while also offering other functional advantages. This method is predicated upon a comprehension of the nutritional and physiological needs of fish, alongside the possible benefits associated with the use of specific functional components. The inclusion of probiotics, prebiotics, or their combination, known as synbiotics, in fish feed has been found to yield notable enhancements in fish growth performance, feed efficiency, and disease resistance. Moreover, these functional components contribute to the mitigation of detrimental bacteria within the gastrointestinal tract of fish, hence augmenting the efficiency of nutrient absorption. The utilization of functional feed has the potential to enhance the overall health and resilience of fish, hence fostering sustainable practices within the aquaculture industry through a decreased need on antibiotics and chemicals. Overall, the application of functional feed technology shows great promise in mitigating the challenges posed by expensive feed and contributes to the development of more environmentally friendly and efficient aquaculture practices.

Probiotics are beneficial bacteria that can have a favorable impact on the host by affecting the microbial population or interacting with the host, resulting in better feed utilization, improved disease response, and a better environment for the host (Hai 2015). To hydrolyze seaweed, Aslamyah, Karim and Badraeni (2017) used a combination of *Bacillus* sp., *Rhizopus* sp., and *Saccharomyces* sp. They supplemented the feed with 10 mL/100 g of this microorganism mix. In addition, Aslamyah, Karim and Badraeni (2018) fermented all feed ingredients with the microbe mix and added 300 mL/kg of *Lumbricus* sp. extract to milkfish feed in a separate study. The results showed that probiotic treatment boosted milkfish growth performance and feed efficiency (Aslamyah, Zainuddin & Badraeni 2022). According to them, the most effective probiotic combination contains *Bacillus* sp., *Lactobacillus* sp., *Rhizophus* sp., *Aspergillus* sp., *Saccharomyces* sp., and *Trichoderma* sp. These probiotics were found to considerably improve milkfish growth performance, feed efficiency, gastric emptying rate, and blood glucose levels.

Prebiotics are non-digestible food components that benefit the host by enhancing the activity and proliferation of specific bacteria in the colon, improving human health (Davani-Davari et al. 2019). These prebiotics are typically high in carbohydrate, such as sucrose, raffinose, oligosaccharides such as inulin, oligofructose, and galacto-oligosaccharides (Mohammadi et al. 2020). Vegetables, onions, *Jerusalem artichoke*, bamboo shoots, dahlia, banana roots, tubers, and nuts are all-natural sources of oligosaccharides.

Probiotics and prebiotics are combined to form synbiotics. Both work together to carry out their responsibilities as bioprocessors. Synbiotics, according to Marrifield et al. (2010), are a component that can increase growth, survival rate, immune system, feed efficiency, and the makeup of beneficial bacteria in the digestive tract of cultured commodities. Lesmanawati et al. (2013) achieved the greatest growth rate of SKT-b bacteria (1010 CFU/mL) using a 3% oligosaccharide extract from sweet potato. According to Marlida et al. (2014), the synbiotics *Vibrio alginolyticus* and *Pseudomonas flourescens* with sweet potato extract showed the best growth performance in duck grouper when compared to *Ewingella americana* and *Sphingomonas paucimobilis* with sweet potato extract. Furthermore, synbiotics can boost survival, growth, the immune system, and host circumstances (Cerezuela, Meseguer & Esteban 2011). Another study found that providing fructo-oligosaccharides (FOS) as a prebiotic mixed with *Bacillus subtillis* as a probiotic in feed can improve growth, survival, immunological response, and illness resistance in yellow croaker fish (Ai et al. 2011).

Numerous studies have extensively investigated the use of synbiotics in aquaculture commodities and their beneficial effect on fish growth and development. Nevertheless, there has been little research evaluating the effects of different prebiotic components, such as sweet potatoes, *Kappaphycus alvarezii*, green beans, and red onion, on the growth parameters of milkfish. This study was done to fill this gap by determining the effect of utilizing synbiotics in artificial feed on milkfish growth, digestive enzyme activities, glycogen levels, hepatosomatic index, body chemical composition, nutrient retention, stomach emptying rate, and blood glucose levels.

MATERIALS AND METHODS

The study was conducted at the Hatchery Technology Laboratory from May to July 2021. Additional analyses were carried out at the Nutrition and Feed Technology Laboratory and the Productivity and Water Quality Laboratory, Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar, Indonesia.

PREBIOTICS PREPARATION

Prebiotics were extracted from sweet potatoes, *Kappaphycus alvarezii*, green beans, and red onions. The extraction process follows the method proposed by Pollock and Jones (1979) with some modifications. Initially, 500 g of each raw material was combined with water in a 1:1 (w/v) ratio and steamed at 100 °C for 30 min. The steamed material was then oven-dried at 55 °C for 18 h, ground to a powder, and sieved. For further extraction, 10 g of each powdered prebiotic was stirred in 100 mL of 70% ethanol at room temperature for 15 h using a magnetic stirrer. The resulting mixture was filtered using filter paper, and the residue was washed with 70% ethanol. The filtrate was then concentrated using a vacuum evaporator at 40 °C. Subsequently, the concentrated solution was centrifuged at 5000 rpm for 10 min to separate contaminants, and the prebiotic extract was sterilized by filtration through a 0.2 µm filter paper. The total soluble solids of oligosaccharides in the final extract were measured at 5%.

PROBIOTICS PREPARATION

The probiotics used in this study were selected based on previous research by Aslamyah, Zainuddin and Badraeni (2022) and included *Bacillus* sp., *Lactobacillus* sp., *Rhizopus* sp., *Aspergillus* sp., *Saccharomyces* sp., and *Trichoderma* sp. These microorganisms were sourced from the Fisheries Biotechnology Laboratory at Hasanuddin University. Prior to use, the probiotic cultures were refreshed according to Afrizal and Purwanto (2011) with modifications. A substrate solution was prepared by mixing 2 L of old coconut water with 500 g of sugar. This mixture was inoculated with 2 mL of probiotic starter culture and incubated at room temperature for 24 h.

EXPERIMENTAL FEED

The experimental feeds were formulated to meet the nutritional requirements of milkfish (*Chanos chanos*). The proximate composition of the formulated feeds is presented in Table 1. All ingredients were obtained from local markets and combined in a mixer with approximately 10% warm water. The mixture was then processed into pellets and dried at room temperature before being stored in plastic bags. The feed was supplemented with a blend of synbiotics, consisting of 10 mL/kg of probiotic feed and 2% prebiotic. This mixture was diluted with distilled water to achieve a final volume of 50 mL/kg of feed, homogenized, and then coated with 2% egg white. The coated feed was dried again to reduce moisture content prior to feeding the fish.

EXPERIMENTAL FISH

The experimental fish, milkfish (4.78 ± 0.16 g) were obtained from a farmer at Maros Regency, South Sulawesi, Indonesia. The fish were acclimatized for one week and then divided randomly into five experimental groups with three replicates at a density of 20 fish/aquarium with the tank size of 50×40×35 cm^3. Each aquarium was filled with 45 mL water with 20 ppt salinity. Daily maintenance included tank cleaning, a 10% water exchange, and a complete water change every 10 days. Feeding frequencies were three times a day (7:00 AM, 12:00 PM, and 16:00 PM). The feeding rate was adjusted according to the size of the fish (5% according to body weight) for 50 days in the experimental period.

GROWTH PARAMETERS

Growth response parameters were assessed every 10 days throughout the experiment to evaluate the growth performance of milkfish (*Chanos chanos*). The following metrics were calculated:

a. a) Absolute growth (AG): calculated using the formula from Zonneveld, Huisman and Boon (1991):

$$\text{AG} = Wf - Wi$$

TABLE 1. Formulation and proximate composition of the experimental feeds ingredients

Ingredients	composition (%)
Fishmeal	26
Soybean meal	22
Coconut Meal Flour	16
Corn meal	18
Pollard meal	10
Fat*	4
Vitamin Mineral Mix**	4
Total	100
Proximate analysis	
Protein	26.43
Fat	8.37
Ash	24.22
Crude fiber	7.45

*) Fish oil and corn oil = 2:1
) Vitamin & mineral mix composition. Each 10 kg contains Vitamin A 12,000,000 IU; Vitamin D2,000,000 IU; Vitamin E 8,000 IU; Vitamin K 2,000 mg; Vitamin B1 2,000 mg; Vitamin B2 5,000; Vitamin B6 500 mg; Vitamin B12 12,000 µg; Ascorbic acid 25,000 mg; Calcium-D-Phantothenate 6. 000 mg; Niacin 40,000 mg; Cholin Chloride 10,000 mg; Metheonine 30,000 mg; Lysine 30,000 mg; Manganese 120,000 mg; Iron 20,000 mg; Iodine 200 mg; Zinc 100,000 mg; Cobalt 200,000 mg; Copper 4,000 mg; Santoquin (antioxidant) 10,000 mg; Zinc bacitracin 21,000 mg. *) Calculated based on energy equation (NRC 1988): 1 g carbohydrate = 2.5 kcal DE; 1 g protein = 3.5 kcal DE; 1 g fat = 8.1 kcal DE

where Wf is the Final weight of fish at the end of the experiment (g); and Wi is the Initial weight of fish at the beginning of the experiment (g).

b. Relative Growth (RG): calculated according to Takeuchi (1988):

$$RG = \frac{Wf - Wi}{Wi} \times 100$$

c. Survival rate: The survival rate was calculated as follows:

$$SR = \frac{Nt}{N0} \times 100$$

where Nt is the Final Number of fish; N0: Initial number of fish

d. Feed efficiency (FE): calculated using the following formula:

$$\frac{FE = (Wf + D) - Wi)}{Fc} \times 100$$

where D is the total weight of fish that died during the observation period (g); *Fc* is the total amount of feed consumed by the fish during the study (g).

DIGESTIVE ENZYME ACTIVITIES

At the end of the study, the activity of the digestive enzymes cellulase, α-amylase, and protease were analyzed following the protocol of Aslamyah (2006). To ensure that the enzymes remained inactive during processing, all procedures were conducted at 0 to 4 °C. Samples of the digestive system were cleaned with distilled water and dried using suction paper. Approximately 1 g of the sample was homogenized in 10 mL of cold distilled water using a mortar. The homogenate was centrifuged at 15,000 rpm for 20 min at 4 °C. The supernatant was collected for enzyme activity assays.

Cellulase activity was measured using the DNS (3,5-dinitrosalicylic acid) method. In an Eppendorf tube, 80 µL of crude enzyme extract was mixed with 720 µL of 1% carboxymethyl cellulose (CMC) solution in citrate phosphate buffer (pH 7) and incubated at 37 °C for 60 min. Following incubation, 1200 µL of DNS reagent was added, and the mixture was boiled for 15 min, then cooled in an ice bath for 20 min. A control sample was prepared by boiling 80 µL of enzyme extract for 15 min before mixing with the substrate and DNS reagent. Absorbance was measured using a UV-Vis spectrophotometer at 540 nm to quantify the reduced sugars produced, as described by Miller (1959).

Amylase activity was determined by mixing 1 mL of enzyme extract with 1 mL of 1% starch in 0.05 M citrate buffer (pH 5.7). The mixture was incubated at 37 °C for 30 min, after which the reaction was halted by adding 2 mL of DNS reagent and boiling for 5 min. The concentration of reducing sugars was measured at 540 nm using a UV-Vis spectrophotometer (Genesys 150, Thermo Scientific, China). One unit of amylase activity was defined as the amount of enzyme producing 1 µmol of glucose per minute.

Protease activity was measured based on Bergmeyer and Grassi (1983). The reaction mixture included 1 mL of 2% casein solution, 0.01 M borate buffer, 0.20 M HCl, and 0.20 mL of enzyme extract. Following 10 min of incubation at 37 °C, 2 mL of 0.1 M trichloroacetic acid (TCA) was added to stop the reaction, and the mixture was centrifuged. For quantification, 1.5 mL of filtrate was combined with 5 mL of 0.4 M disodium carbonate and 1 mL of Folin-Ciocalteu reagent, and the absorbance was measured at 578 nm after a 20-min reaction period.

GLYCOGEN LEVELS IN THE LIVER AND MUSCLE

At the end of the experiment, glycogen levels in the liver and muscle tissues of the fish were measured. Five samples were collected from each experimental unit. Whole liver samples were taken, while muscle samples were collected from the dorsal region of each fish. The tissues were dried in an oven at 50 °C for 24 h and then ground into a fine powder. Glycogen content was determined using the method described by Wedemeyer and Yasutake (1977). Approximately 100 mg of liver or muscle tissue was dissolved in 3 mL of 30% KOH, followed by the addition of 0.5 mL of saturated sodium sulfate (Na_2SO_4) and 3.5 mL of 95% ethanol. The mixture was heated to boiling to facilitate glycogen dissolution. Glycogen was then re-precipitated by adding 2 mL of distilled water and 2.5 mL of 95% ethanol. After cooling, the mixture was centrifuged to separate the precipitated glycogen from the supernatant, which was discarded. The glycogen was hydrolyzed by incubating it with 2 mL of 5 M HCl in a boiling water bath for 30 min. The neutralized hydrolysate, containing 15-150 µg glucose, was transferred into test tubes; one for the hydrolysate; one for a glucose standard (up to 5 mL containing 111 g glucose); and one distilled water as a blank control. Each tube received 10 mL of anthrone reagent, heated for 10 min in boiling water, and absorbance was measured at 635 nm using a colorimeter. Glycogen content was calculated using a standard curve derived from known glucose concentrations, with the conversion factor that 1 g of glycogen equals approximately 1.11 g of glucose in hydrolysate.

HEPATOSOMATIC INDEX (HI)

Five samples were selected randomly from each experimental unit at the end of the study. The fish were first weighed and then dissected on ice. The dissection was done carefully and as quickly as possible. Then, the liver was weighed. The hepatosomatic index value was calculated based on the formula proposed by Boonanuntanasarn et al. (2018).

$$\text{Hepatosomatic index} = (100 \times \text{liver weight})/\text{body weight}.$$

BODY CHEMICAL COMPOSITION

The proximate analysis of feed and whole-body fish samples was carried out according to the standard methods by the Association of Official Analytical Chemists (AOAC 2012). The following nutrients were analyzed: moisture, crude protein (CP), crude fat (CF), ash and energy content (in Kcal/100 g). Moisture content was estimated by gravimetric analysis after oven drying at 105 °C for 12 h. Crude protein (CP) was determined by Kjeldahl method (N × 6.25) after acid hydrolysis. Crude lipid (CL) was calculated gravimetrically after extraction with petroleum ether in a soxhlet system. Total ash was determined gravimetrically by ignition at 600 °C for 6 h in a muffle furnace. Crude fiber was estimated gravimetrically after acid and alkali digestion and loss in mass by combustion at 600 °C for 3 h. Nitrogen free extract (NFE) was calculated from 1000 - (crude protein + crude lipid + crude fiber + total ash).

PROTEIN, FAT, AND ENERGY RETENTION

Protein and fat retention in the fish were assessed through proximate analysis of both the feed and fish body at the beginning and end of the experiment, following the method outlined by Takeuchi (1988). Energy retention was determined using a bomb calorimeter. The retention percentages for protein, fat, and energy were calculated by dividing the increase in protein, fat, and body energy (g) by the total amount of feed consumed (g), then multiplying the result by 100.

GASTRIC EMPTYING RATE (GER)

At the end of the study, the gastric emptying rate (GER) was determined following the methods proposed by Lee et al. (2000). Milkfish were initially fed for 24 h before being transferred to a large, aerated basin. Gastric contents were collected after feeding, with additional samples taken at one-hour intervals. Three fish were observed at each time interval for each treatment. The collection of stomach contents continued until the stomachs of the fish were empty. The percentage of feed remaining in the digestive tract was calculated by dividing the amount of feed in the stomach by the total feed consumed and multiplying by 100.

BLOOD GLUCOSE LEVELS

At the end of the study, blood glucose levels in milkfish were measured following a 24-h feeding period. The fish were then transferred to a large, aerated basin, and blood collection commenced at various time points: hour 0, and at hours 1, 2, 4, 5, 6, 8, 10, 12, 14, 16, and 20 post-prandial. For each time interval, three fish from each treatment group were sampled. Blood samples were obtained from the gills and caudal vein using a 1 mL syringe. Blood glucose levels were measured using a OneTouch Ultra Plus Flex glucometer with the strip method.

WATER QUALITY PARAMETERS

The water quality parameters such as temperature and pH were measured using a thermometer and pH meter, respectively. Dissolved oxygen and total ammonia (TAN, mg/L) were determined every week by using chemical methods according to APHA (1992). Water quality parameters were within the range of acceptable limits for milkfish, temperature (27-29 °C), pH (6.8-7.2), salinity (20-22 ppt), dissolved oxygen (5.3-6.5 ppm), and ammonia (0.001- 0.002 ppm).

STATISTICAL ANALYSIS

The growth parameters (absolute, relative, survival, and feed efficiency. average daily gain), were all subjected to one-way analysis of variance (ANOVA) to determine if significant differences occur among the dietary treatments. Tukey test was used to compare differences between means. Effects with a probability of $P < 0.05$ was considered significant. Statistical analyses were performed using SPSS (Statistical Package for Social Sciences, Version 12, IBM Corporation, New York, USA).

RESULTS AND DISCUSSION

The results of growth parameters, including absolute growth, relative growth, survival rate, feed efficiency, and digestive enzyme activity of milkfish fed functionally enriched diets with synbiotics, are presented in Table 2. The findings indicate that diets enriched with sweet potatoes and green beans significantly improved growth and digestive enzyme activity in milkfish compared to other treatments, with treatment D (green beans) yielding the highest absolute growth (25.35 ± 0.74 g) and relative growth rate (522.53 ± 23.26%), followed closely by treatment B (sweet potatoes) with 24.75 ± 1.31 g absolute growth and 510.5 ± 19.86% relative growth rate. Both treatments also showed high efficiency (72.30 ± 1.69% and 69.11 ± 2.96%, respectively). In terms of digestive enzyme activity, Treatments B and D enhanced cellulase, α-amylase, and protease activities, with α-amylase and protease levels and were significantly higher than in the control, indicating improved carbohydrate and protein digestion. Conversely, the control diet exhibited the lowest values across all parameters. The study's findings demonstrate that incorporating functional feed ingredients such as sweet potatoes and green beans into milkfish diets significantly enhances growth performance, feed efficiency, and digestive enzyme activity, exceeding the effects of other tested ingredients. Treatments with sweet potatoes and green beans achieved the highest absolute and relative growth rates, as well as notable increases in cellulase, α-amylase, and protease activities, which supports improved carbohydrate and protein digestion (Das, Mondal & Haque 2017). These enhancements align with research highlighting the benefits of prebiotic-enriched diets in aquaculture, where nutrient absorption and metabolic efficiency are substantially improved. Enhanced enzyme activities, particularly in α-amylase and protease, indicate better digestion and nutrient uptake, and are essential for growth and feed utilization (Ringø & Song 2016). Prebiotics from sweet potatoes and green beans likely facilitated microbial activity within the fish gut, providing substrates for beneficial microorganisms and promoting the production of short-chain fatty acids, amino acids, and vitamins that contribute to enhanced

TABLE 2. Absolute growth, relative growth, survival rate, feed efficiency, and digestive enzymes activity of milkfish fed functionally enriched with synbiotics

Treatments	Absolute growth (g)	Relative growth (%)	Survival rate (%)	Feed efficiency (%)	Aktivitas Enzim (IU/mL/menit)		
					Celulase	α-amylase	Protease
A (control)	11.04 ± 0.81^{a}	235.79 ± 11.54^{a}	100 ± 0	50.21 ± 2.06^{a}	0.06+0.02^{a}	7.88+0.02^{a}	0.49+0.02^{a}
B (sweet potatoes)	24.75 ± 1.31^{c}	510.5 ± 19.86^{c}	95.00 ± 8.66	69.11 ± 2.96^{b}	0.18+0.02^{c}	11.67+0.04^{c}	0.89+0.01^{c}
C (*K. Alvarezii*)	19.25 ± 0.93^{b}	406.36 ± 36.69^{b}	98.33 ±2.89	65.71 ± 3.86^{b}	0.15+0.02^{b}	10.01+0.02^{b}	0.67+0.02^{b}
D (green beans)	25.35 ± 0.74^{c}	522.53 ± 23.26^{c}	100 ± 0	72.30 ± 1.69^{b}	0.18+0.01^{c}	11.80+0.03^{c}	0.89+0.01^{c}
E (red onion)	21.27 ± 1.02^{b}	447.0 ± 28.10^{b}	98.33 ±2.89	67.26 ± 3.12^{b}	0.16+0.01^{b}	11.19+0.05^{c}	0.84+0.01^{c}

Different letters in the same column indicate significant differences between treatments at the 95% confidence level ($P<0.05$)

digestibility and nutrient utilization (Djauhari et al. 2017). The effectiveness of these synbiotics-based feed ingredients in boosting growth and feed conversion had also been demonstrated in species like tilapia and catfish, supporting their broader applicability across aquaculture. Moreover, synbiotic feeds have been shown to improve immune response and resilience against stress and disease, further underscoring their potential to enhance both growth and overall health in milkfish and other species (Puri, Sharma & Singh 2022).

The results of liver and muscle glycogen levels, hepatosomatic index, and energy content (kcal/kg) of milkfish fed functionally enriched diets with synbiotics are presented in Table 3. The data indicates that synbiotic enrichment with green beans and sweet potatoes markedly improves liver glycogen storage, hepatosomatic index, and energy content in milkfish, compared to other treatments. Fish fed with green beans led to the highest hepatosomatic index (2.77 ± 0.12). Additionally, milkfish-fed green beans and sweet potatoes exhibited the highest energy content (3,706.67 ± 56.78 kcal/kg and 3,688.55 ± 70.78 kcal/kg, respectively) although liver glycogen levels were slightly higher in fish fed with *K. alvarezii* (5.75 ± 0.13 mg/g) compared to other treatments. Conversely, the control diet resulted in the lowest hepatosomatic index (1.58 ± 0.11) and energy content (3,223.53 ± 38.37 kcal/kg), underscoring the limited efficacy of a standard diet without functional synbiotics. The study's results underscore the significant benefits of enriching milkfish diets with green beans and sweet potatoes as functional synbiotic sources, as these treatments led to marked improvements in liver glycogen storage, hepatosomatic index, and overall energy content. Milkfish fed with diets enriched with green beans achieved the highest hepatosomatic index, and both green beans and sweet potatoes provided the highest energy content, supporting superior liver function and energy reserves. These findings align with previous research showing that plant-based synbiotics, like green beans and sweet potatoes, can enhance liver health and energy storage in fish by increasing liver glycogen and the hepatosomatic index, as seen in carp and tilapia fed prebiotic-rich diets (Reverter et al. 2021). Functional feeds with plant-based synbiotics have been associated with improved glucose utilization, where surplus glucose is stored as glycogen when immediate energy demands are met (Mutmainnah 2019). The liver's glycogen storage plays a critical role in regulating blood glucose, supporting efficient glucose availability across organs, while muscle glycogen supports localized energy needs (Mustakin & Tahir 2019). The study also reflects the tendency of species with higher dietary carbohydrate intake to store glucose as glycogen rather than converting it to lipids, a response regulated by metabolic factors such as SREBP-1 and ChREBP (Craig & Moon 2013). Thus, the increased energy content and stable glycogen levels in milkfish fed with green beans and sweet potatoes indicate these diets' efficacy in fulfilling energy requirements while supporting liver function, underscoring the potential of plant-based synbiotics to sustainably enhance health and metabolism in aquaculture.

The results of the body chemical composition data (%) of milkfish fed functionally enriched diets with synbiotics are presented in Table 4. The data shows that milkfish fed with synbiotic-enriched diets with sweet potatoes and green beans had the highest crude protein (69.28 ± 0.79% and 69.74 ± 0.45%, respectively) and crude fat content (14.86 ± 0.71% and 14.82 ± 0.69%, respectively). The control group had the lowest crude protein (63.09 ± 0.83%) and a significantly higher ash content (21.58 ± 1.03%). The study highlights the pronounced benefits of synbiotic-enriched diets with sweet potatoes and green beans in enhancing body composition of milkfish, with notably higher crude protein and fat content compared to other treatments. Milkfish fed with these synbiotic sources exhibited superior protein deposition and lipid accumulation, which likely stem from the positive effects of synbiotics on nutrient absorption and microbial activity. Synbiotics, combining prebiotics and probiotics, support beneficial gut microbiota that enhances digestion and nutrient assimilation, resulting in better protein and fat retention in the fish's body composition (Abdurrahman & Yanti 2018). This aligns with broader findings that plant-based synbiotics, like those from green beans and sweet potatoes, improve nutrient absorption and facilitate effective nutrient utilization by promoting beneficial gut microbiota. The control group, which lacked synbiotics, showed lower crude protein and elevated ash content, suggesting limited nutrient utilization. Enhanced protein and fat composition, as seen with sweet potato and green bean diets, has significant implications for meat quality and growth in aquaculture systems. The findings support the view that synbiotic-enriched feeds can optimize growth performance and body composition by boosting nutrient content and retention in fish, underscoring the potential of plant-based synbiotics to improve nutritional quality of aquaculture species sustainably.

The results of protein retention, fat, and energy (%) of milkfish fed with functionally enriched diets of synbiotics are presented in Table 5. The data demonstrated that green beans and sweet potatoes as functional synbiotic enrichments yielded the highest levels of protein, fat, and energy retention in milkfish, significantly outperforming other treatments. Green beans led to the most substantial retention rates across all parameters, with 60.36 ± 0.51% for protein, 41.63 ± 2.39% for fat, and 0.40 ± 0.01% for energy. Sweet potatoes also promoted strong retention rates, particularly for protein (57.13 ± 4.32%) and fat (39.82 ± 1.26). In contrast, the control group exhibited the lowest retention values, with protein, fat, and energy retention of 37.30 ± 2.43%, 22.67 ± 0.75%, and 0.28 ± 0.01%, respectively, highlighting the limited nutrient retention and metabolic efficiency without synbiotic supplementation.

The study demonstrates that synbiotic enrichment with green beans and sweet potatoes significantly enhances protein, fat, and energy retention in milkfish, supporting better nutrient utilization and metabolic efficiency compared to other treatments. High protein retention, especially seen in the green bean diet, indicates efficient conversion of dietary protein into body tissue, which is essential for growth and reflects feed quality (Halver 1989). Enhanced fat retention suggests that these synbiotic diets meet the fish's energy needs effectively, allowing surplus fat to accumulate as energy reserves. Furthermore, greater energy retention highlights improved nutrient metabolism, with proteins being allocated for growth and metabolic functions (Haryati 2011). Previous studies align with these findings, showing that prebiotics from sources like green beans and sweet potatoes promote nutrient retention in fish by improving amino acid availability and boosting essential nutrient assimilation(Masriah 2020; Qaddoori, Najim & Al-Niaeem 2022). Research on largemouth bass similarly confirms that synbiotic diets enhance growth performance and nutrient retention, underscoring the potential of plant-based synbiotics in aquaculture to optimize both growth and body composition (Yang et al. 2020). These results reinforce the role of functional feeds

TABLE 3. Liver and muscle glycogen levels, hepatosomatic index, and energy content (kcal/kg) of milkfish fed functionally enriched with synbiotics

Prebiotics source	Gilkogen content (mg/g)		Hepatosomatic index	Energy content (kcal/kg)
	Liver	Muscle		
Initial	4.45 ± 0.15	4.17 ± 0.06	0.84 ± 0.08	3048.66 ± 36.14
A (control)	5.38 ± 0.34	5.5 ± 0.19	1.58 ± 0.11ᵃ	3.223.53 ± 38.37ᵃ
B (sweet potatoes)	5.57 ± 0.11	5.08 ± 0.16	2.61 ± 0.19ᵇᶜ	3.688.55 ± 70.78ᶜ
C (*K. Alvarezii*)	5.75 ± 0.13	5.6 ± 0.02	1.93 ± 0.09ᵃ	3.397.26 ± 57.41ᵃᵇ
D (green beans)	5.6 ± 0.29	5.41 ± 0.33	2.77 ± 0.12ᶜ	3.706.67 ± 56.78ᶜ
E (red onion)	5.55 ± 0.22	5.42 ± 0.19	2.34 ± 0.07ᵇ	3.487.15 ± 87.26ᵇ

Different letters in the same column indicate significant differences between treatments at the 95% confidence level (P<0.05)

TABLE 4. Body chemical composition data (%) of milkfish fed functionally enriched with synbiotics

Prebiotics sources	Crude Protein (% wt)	Crude Fat (% wt)	Ash (% wt)	Carbohydrate (% wt)	
				Crude fiber	NFE
Initial	61.41	10.54	25.07	1.16	1.81
A (control)	63.09 ± 0.83ᵃ	11.80 ± 0.28ᵃ	21.58 ± 1.03ᶜ	1.16 ± 1.1	2.36 ± 0.05
B (sweet potatoes)	69.28 ± 0.79ᶜ	14.86 ± 0.71ᵇ	11.93 ± 1.26ᵃ	1.53 ± 0.24	2.41 ± 0.18
C (*K. Alvarezii*)	65.3 ± 0.8ᵇ	12.99 ± 0.37ᵃᵇ	17.84 ± 1.25ᵇ	1.44 ± 0.20	2.36 ± 0.37
D (green beans)	69.74 ± 0.45ᶜ	14.82 ± 0.69ᵇ	11.2 ± 0.73ᵃ	1.54 ± 0.06	2.61 ± 0.35
E (red onion)	67.12 ± 0.57ᵇ	13.30 ± 0.97ᵃᵇ	15.72 ± 1.03ᵇ	1.42 ± 0.14	2.44 ± 0.28

Different letters in the same column indicate significant differences between treatments at the 95% confidence level (P<0.05)

TABLE 5. Protein retention, fat, and energy (%) of milkfish fed functionally enriched with synbiotics

Prebiotics source	Retention (%)		
	Protein	Fat	Energy
A (control)	37.30 ± 2.43ᵃ	22.67 ± 0.75ᵃ	0.28 ± 0.01ᵃ
B (sweet potatoes)	57.13 ± 4.32ᵇᶜ	39.82 ± 1.26ᵇᵈ	0.38 ± 0.02ᵇᶜ
C (*K. Alvarezii*)	49.65 ± 2.14ᵇ	32.08 ± 1.47ᶜ	0.34 ± 0.01ᵇ
D (green beans)	60.36 ± 0.51ᶜ	41.63 ± 2.39ᵈ	0.40 ± 0.01ᶜ
E (red onion)	52.76 ± 4.22ᵇᶜ	33.80 ± 5.02ᵇ	0.35 ± 0.03ᵇ

with prebiotics and synbiotics in advancing fish growth and overall health through improved nutrient retention and metabolic efficiency.

The results of the gastric emptying rate (%) of milkfish for each observation period fed with functional feed enriched with synbiotics are presented in Table 6. The data indicates that milkfish fed with green beans and sweet potatoes had faster digestion rates, as evidenced by lower percentages of gastric content remaining across the postprandial hours, compared to other treatments. At the 4-hour mark, green beans and red onion showed gastric emptying rates of 12.02% and 11.55%, respectively, with complete emptying by the 5-hour mark. In contrast, the control group retained significantly higher gastric content (38.82% at 4 h), with remnants persisting up to the 6-hour mark. The study demonstrates that milkfish fed synbiotic-enriched diets with green beans and sweet potatoes show significantly faster gastric emptying rates, supporting more efficient digestion and nutrient uptake compared to the control diet. These faster gastric transit times, especially with green beans, can be attributed to the presence of beneficial oligosaccharides such as mannan-oligosaccharides (MOS), fructo-oligosaccharides (FOS), and galacto-oligosaccharides (GOS), which enhance digestive enzyme activity, facilitating efficient breakdown and assimilation of nutrients (Wang et al. 2022). By accelerating gastric processing, these diets allow for more frequent feeding intervals and improved growth potential. Previous research indicated that MOS supplementation can enhance glycolytic enzyme activity and alter intestinal microbiota composition, enriching glucose metabolism pathways, increasing serum glucose levels, and boosting liver glycogen reserves (Harikrishnan et al. 2023). Additionally, diets supplemented with MOS have been shown to increase lipase and trypsin activity, improving feed conversion ratios (FCR) and protein efficiency ratios (PER) (Swain et al. 2023). The prolonged enzyme interaction resulting from faster gastric emptying supports effective nutrient breakdown, aligning with studies showing that synbiotics enhance digestive efficiency and nutrient retention in aquaculture species (Aslamyah, Zainuddin & Badraeni 2022). Consequently, incorporating green beans and sweet potatoes into milkfish diets offer a promising approach for optimizing digestive efficiency, growth performance, and metabolic health in aquaculture systems.

The observed reduction in blood glucose levels (Figure 1) in fish suggests active glucose utilization to

TABLE 6. Gastric emptying rate (%) of milkfish for each observation period fed with functional feed enriched with synbiotics

Prebiotics source	Gastric emptying rate (%)					
	Post prandial hour (hour)					
	1	2	3	4	5	6
A (control)	90.18 ± 2.16	62.38 ± 5.68	42.54 ± 8.87	38.82 ± 0.98	13.36 ± 5.57	0
B (sweet potatoes)	76.95 ± 3.15	52.14 ± 3.09	30.81 ± 0.45	9.37 ± 2.41	0	
C (*K. Alvarezii*)	80.73 ± 5.53	55.56 ± 2.43	41.98 ± 4.6	23.97 ± 1.31	7.79 ± 3.28	0
D (green beans)	72.7 ± 6.55	45.06 ± 2.27	29.44 ± 1.58	12.02 ± 1.88	0	
E (red onion)	81.93 ± 6.13	44.31 ± 1.94	35.51 ± 1.02	11.55 ± 1.97	0	

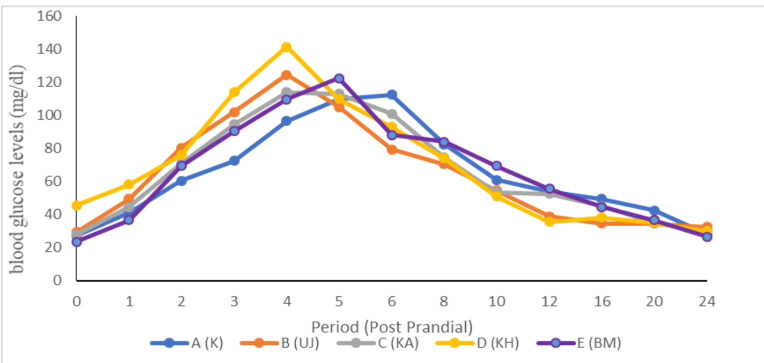

FIGURE 1. Average blood glucose levels (mg/dl) in the observation period (post prandial) of milkfish fed with functional feed enriched with synbiotics

meet energy needs and adapt to environmental changes. The liver plays a central role in maintaining blood glucose homeostasis through several metabolic pathways, including glycolysis, gluconeogenesis, glycogenesis, and glycogenolysis (Leung & Woo 2012). Additionally, insulin aids in glucose regulation by facilitating glucose uptake and stimulating protein catabolism, thereby increasing the availability of amino acids in the bloodstream (Wendelaar 1997). In this study, functional feeds enriched with synbiotics from red onion and *Kappaphycus alvarezii* demonstrated lower effectiveness compared to those with green beans and sweet potatoes across multiple parameters. This variance may be due to differences in prebiotic composition; red onion and *K. alvarezii* may contain prebiotics less conducive to fostering beneficial gut microbiota, as digestive microbial profiles can vary among fish species (Tang, Wang & Pan 2020). Further research is needed to clarify the factors influencing the efficacy of functional feeds containing synbiotics from red onion and *K. alvarezii*, as these insights could strengthen their utility in promoting animal health and growth.

CONCLUSION

This study demonstrates that synbiotic-enriched feeds with green beans and sweet potatoes significantly enhance growth and metabolic health in milkfish, showing superior results in liver glycogen storage, protein and fat retention, and gastric emptying rates compared to other treatments. Green beans in particular, led to the highest nutrient retention, efficient energy utilization, and improved metabolic adaptation, while sweet potatoes also promoted protein and fat accumulation. The fiber and bioactive compounds in these sources likely fostered beneficial gut microbiota, supporting efficient nutrient breakdown and absorption.

These results highlight green beans and sweet potatoes as highly effective, sustainable synbiotic sources for enhancing milkfish aquaculture performance. Future research should focus on optimizing synbiotic formulations using these ingredients and further investigating the mechanisms by which these plant-based synbiotics support milkfish metabolism and health. Applying these findings could provide an innovative, eco-friendly approach to improving fish growth and feed efficiency in aquaculture settings.

REFERENCES

Abdurrahman, H.Z. & Yanti, Y. 2018. Gambaran umum pengaruh probiotik dan prebiotik pada kualitas daging ayam. *TERNAK TROPIKA Journal of Tropical Animal Production* 19(2): 95-104. https://doi.org/10.21776/ub.jtapro.2018.019.02.4

Afrizal, A. & Purwanto, A. 2011. Pemanfaatan selulosa bakterial *nata de coco* sebagai adsorban logam Cu(II) dalam sistem berpelarut air. *JRSKT - Jurnal Riset Sains dan Kimia Terapan* 1(1): 27-32. https://doi.org/10.21009/jrskt.011.05

Ai, Q., Xu, H., Mai, K., Xu, W., Wang, J. & Zhang, W. 2011. Effects of dietary supplementation of *Bacillus subtilis* and fructooligosaccharide on growth performance, survival, non-specific immune response and disease resistance of juvenile large yellow croaker, *Larimichthys crocea*. *Aquaculture* 317(1-4): 155-161. https://doi.org/10.1016/j.aquaculture.2011.04.036

American Public Health Association (APHA). 1992. *Standard Methods for the Examination of Water and Waste Water*. 18th ed. Washington DC: American Public Health Association. p. 1268.

Aslamyah, S. 2006. Peningkatan peran mikroba saluran pencernaan untuk memacu pertumbuhan ikan bandeng. Dissertation. Graduate program, Institut Pertanian Bogor, Bogor.

Aslamyah, S., Zainuddin, Z. & 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

Aslamyah, S., Karim, M.Y. & Badraeni. 2018. Pengaruh dosis mikroorganisme mix. dalam memfermentasi 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., Karim, M.Y. & Badraeni. 2017. Fermentation of seaweed flour with various fermenters to improve the quality of fish feed ingredients. *Jurnal Akuakultur Indonesia* 16(1): 8-14. https://doi.org/10.19027/jai.16.1.8-14

Association of Official Analytical Chemists (AOAC). 2012. *Official Methods of Analysis of the Association of Official Analytical Chemists*. 19th ed. Arlington, VA: Association of Official Analytical Chemists.

Bergmeyer, H.U. & Grassi, M. 1983. *Methods of Enzymatic Analysis.* Volume 2. Weinheim: Verlag Chemie.

Boonanuntanasarn, S., Tiengtam, N., Pitaksong, T., Piromyou, P. & Teaumroong, N. 2018. Effects of dietary inulin and Jerusalem artichoke (*Helianthus tuberosus*) on intestinal microbiota community and morphology of Nile tilapia (*Oreochromis niloticus*) fingerlings. *Aquaculture Nutrition* 24(2): 712-722.

Cerezuela, R., Meseguer, J. & Esteban, M.A. 2011. Current knowledge in synbiotic use for fish aquaculture: A review. *Journal of Aquaculture Research and Development* S1-008. https://doi.org/10.4172/2155-9546.S1-008

Chen, H.Y. & Durand, J.D. 2016. Advances in milkfish biology and culture in Taiwan. *Reviews in Aquaculture* 8(3): 187-220.

Craig, P.M. & Moon, T.W. 2013. Methionine restriction affects the phenotypic and transcriptional response of rainbow trout (*Oncorhynchus mykiss*) to carbohydrate-enriched diets. *British Journal of Nutrition* 109(3): 402-412. https://doi.org/10.1017/S0007114512001663

Das, S., Mondal, K. & Haque, S. 2017. A review on application of probiotic, prebiotic and synbiotic for sustainable development of aquaculture. *Journal of Entomology and Zoology Studies* 5(2): 422-429.

Davani-Davari, D., Negahdaripour, M., Karimzadeh, I., Seifan, M., Mohkam, M., Masoumi, S.J., Berenjian, A. & Ghasemi, Y. 2019. Prebiotics: Definition, types, sources, mechanisms, and clinical applications. *Foods* 8(3): 92. https://doi.org/10.3390/foods8030092

Djauhari, R., Widanarni, S., Suprayudi, M.A. & Zairin, M. 2017. Growth performance and health status of common carp (*Cyprinus carpio*) supplemented with prebiotic from sweet potato (*Ipomoea batatas*) extract. *Pakistan Journal of Nutrition* 16: 155-163. doi:10.3923/pjn.2017.155.163

Dossou, S., Hossain, M.A., Haque, M.M. & Islam, M.A. 2018. Economic analysis of small-scale shrimp farming in Bangladesh: A case study in Barguna district. *Aquaculture Reports* 10: 1-7.

El Basuini, M.F., El-Haroun, E.R., Attia, E.I. & Shalaby, S.M. 2017. Evaluation of dietary lipid sources for sea bass (*Dicentrarchus labrax*) fingerlings: Growth, feed utilization, tissue composition, and histology. *Aquaculture* 473: 304-311.

Hai, N.V. 2015. The use of probiotics in aquaculture. *Journal of Applied Microbiology* 119(4): 917-935. https://doi.org/10.1111/jam.12886

Halver, J.E. 1989. *Fish Nutrition*. 2nd ed. San Diego: Academic Press.

Harikrishnan, R., Devi, G., Balamurugan, P., Abdel-Warith, A.W.A., Younis, E.M., Van Doan, H. & El-Haroun, E. 2023. Immunostimulatory effect of mannan-oligosaccharides supplementation diet in milkfish (*Chanos chanos*). *Fish & Shellfish Immunology* 2023: 108568. https://doi.org/10.1016/j.fsi.2023.108568

Lee, Y.C., Lu, Y.H., Lee, J.M., Schafferer, C., Yeh, C.Y., Chu, T.W. & Huang, Y.W. 2022. A production economic analysis of different stocking density and fry size combinations of milkfish, *Chanos chanos*, farming in Taiwan. *Journal of the World Aquaculture Society* 53(2): 424-451. https://doi.org/10.1111/jwas.12842

Lesmanawati, W., Widanarni, W., Sukenda, S. & Purbiantoro, W. 2013. The potential of sweet potato oligosaccharide extract as aquaculture probiotic bacteria prebiotic. *Jurnal Sains Terapan* 3(1): 16-20. https://doi.org/10.29244/jstsv.3.1.16-20

Leung, L.Y. & Woo, N.Y.S. 2012. Influence of dietary carbohydrate level on endocrine status and hepatic carbohydrate metabolism in the marine fish *Sparus sarba*. *Fish Physiology and Biochemistry* 38(2): 543-554. https://doi.org/10.1007/s10695-011-9534-8

Marlida, R., Suprayudi, M.A., Widanarni, & Harris, E. 2014. Isolation, selection and application of probiotic bacteria for improvement the growth performance of Humback Groupers (*Cromileptes altivelis*). *International Journal of Science: Basic and Applied Research* 16(1): 367-379.

Marte, C.L. 2010. Milkfish aquaculture in the Philippines: An overview. In *Milkfish Aquaculture in Asia*, edited by Liao, I.C. & Leaño, E.M. Keelung, Taiwan: National Taiwan Ocean University, The Fisheries Society of Taiwan, Asian Fisheries Society and World Aquaculture Society. pp. 33-46.

Masriah, A. 2020. Penambahan limbah cairan rumen sapi pada berbagai level karbohidrat dalam pakan terhadap retensi nutrient dan komposisi kimia tubuh ikan bandeng (*Chanos chanos* Forsskal). *Octopus: Jurnal Ilmu Perikanan* 9(1): 33-38.

Merrifield, D.L., Dimitroglou, A., Foey, A., Davies, S.J., Baker, R.T.M., Bøgwald, J., Castex, M. & Ringø, E. 2010. The current status and future focus of probiotic and prebiotic applications for salmonids. *Aquaculture* 302(1-2): 1-18. https://doi.org/10.1016/j.aquaculture.2010.02.007

Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry* 31(3): 426-428.

Mohammadi, G., Rafiee, G., El Basuini, M.F., Abdel-Latif, H.M.R. & Dawood, M.A.O. 2020. The growth performance, antioxidant capacity, immunological responses, and the resistance against *Aeromonas hydrophila* in Nile tilapia (*Oreochromis niloticus*) fed *Pistacia vera* hulls derived polysaccharide. *Fish & Shellfish Immunology* 106: 36-43.

Mustakin, F. & Tahir, M.M. 2019. Analisis kandungan glikogen pada hati, otot, dan otak hewan. *Canrea Journal* 2(2): 75-80.

Mutmainnah, N. 2019. The effect of dissolved glucose on survival rate and performance of swimming crab larvae *Portunus pelagicus* from zoea stadia to megalopa. *International Journal of Fisheries and Aquatic Studies* 7(6): 30.

Pollock, C.J. & Jones, T. 1979. Seasonal patterns of fructan metabolism in forage grasses. *New Phytologist* 83(1): 9-15.

Puri, P., Sharma, J.G. & Singh, R. 2022. Biotherapeutic microbial supplementation for ameliorating fish health: developing trends in probiotics, prebiotics, and synbiotics use in finfish aquaculture. *Animal Health Research Reviews* 23(2): 113-135. https://doi.org/10.1017/S1466252321000165

Qaddoori, M.S., Najim, S.M. & Al-Niaeem, K.S. 2022. Effects of some probiotics and synbiotic dietary supplementation on growth performance and digestive enzymes activity of common carp, *Cyprinus Carpio*. *Journal of Pharmaceutical Negative Results* 13(7): 175-184. https://doi.org/10.47750/pnr.2022.13.S07.027

Reverter, M., Tapissier-Bontemps, N., Sarter, S., Sasal, P. & Caruso, D. 2021. Moving towards more sustainable aquaculture practices: A meta-analysis on the potential of plant-enriched diets to improve fish growth, immunity and disease resistance. *Reviews in Aquaculture* 13(1): 537-555. https://doi.org/10.1111/raq.12485

Ringø, E. & Song, S.K. 2016. Application of dietary supplements (synbiotics and probiotics in combination with plant products and β-glucans) in aquaculture. *Aquaculture Nutrition* 22(1): 4-24. https://doi.org/10.1111/anu.12349

Soto, C., Ibáñez, A.L. & Godoy, F.A. 2015. Functional feed in aquaculture: A review. *Revista MVZ Córdoba* 20(2): 4879-4890.

Takeuchi, T. 1988. Laboratory work chemical evaluation of dietary nutrients. In *Fish Nutrition and Mariculture,* edited by Watanabe, T. Department of Aquatic Bioscience, Tokyo University of Fisheries. JICA. pp. 179-226.

Tang, Y., Wang, J. & Pan, L. 2020. Effect of prebiotics and synbiotics on intestinal microbiota and metabolic biomarkers in ruminants: A systematic review and meta-analysis. *Journal of Animal Science and Biotechnology* 11(1): 1-14.

Wang, T., Wu, H.X., Li, W.J., Xu, R., Qiao, F., Du, Z.Y. & Zhang, M.L. 2022. Effects of dietary mannan oligosaccharides (MOS) supplementation on metabolism, inflammatory response and gut microbiota of juvenile Nile tilapia (*Oreochromis niloticus*) fed with high carbohydrate diet. *Fish & Shellfish Immunology* 130: 550-559.

Wedemeyer, G.A. & Yasutake, W.T. 1977. *Clinical Methods for the Assessment of the Effects of Environmental Stress on Fish Health*. Technical Paper of the US Fish and Wildlife Service. Volume 89. USA Washington DC: US Department of the Interior Fish and Wildlife Service.

Wendelaar, S.E. 1997. The stress response in fish. *Physiological Reviews* 77(3): 591-625. https://doi.org/10.1152/physrev.1997.77.3.591

Yang, P., Yang, W., He, M., Li, X. & Leng, X.J. 2020. Dietary synbiotics improved the growth, feed utilization and intestinal structure of largemouth bass (*Micropterus salmoides*) juvenile. *Aquaculture Nutrition* 26(2): 590-600. https://doi.org/10.1111/anu.13020

Zonneveld, N., Huisman, E.A. & Boon, J.H. 1991. *Principles of Fish Farming*. Jakarta: PT. Gramedia Pustaka Utama. p. 318.

*Corresponding author; email: siti.aslamyah@unhas.ac.id