FISHERIES & AQUATIC LIFE (2024) 32: 34 - 43
Archives of Polish Fisheries
DOI 10.2478/aopf-2024-0004

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RESEARCH ARTICLE

Effects of dietary supplementation with mannan-rich oligosaccharides and solid-state fermented *Aspergillus niger* on the growth performance of juvenile Asian seabass, *Lates calcarifer*

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Received – 20 November 2023/Accepted – 11 March 2024. Published online: 31 March 2024;
Citation: Felix, G. P., Lyons, P., Wong, H., Lin, G., Noordin, N. M., Sung, Y. Y., Danish-Daniel, M., Wong, L. L. (2024). Effects of dietary supplementation with mannan-rich oligosaccharides and solid-state fermented *Aspergillus niger* on the growth performance of juvenile Asian seabass, *Lates calcarifer*. Fisheries & Aquatic Life 32, 34-43.

Abstract. Feed additives are gaining popularity as dietary supplements with the potential to support growth, immune competence, and the general health of aquaculture species. This study aimed to evaluate growth performance and feed utilization in juvenile Asian seabass, *Lates calcarifer*, fed experimental diets containing enzymes derived from the solid-state fermentation (SSF) of *Aspergillus niger* and mannan oligosaccharides (MOS), both individually and in combination. Fish were fed six experimental diets: control (no additives), SSF (0.025% & 0.05%), MOS (0.1% & 0.2%), and combined SSF (0.025%) + MOS (0.05%). The feeding trial was conducted for ten weeks in triplicate for each experimental diet consisting of 51 fish in 120 L tanks. Following the collection of growth performance metrics, proximate analysis of muscle and feces samples was performed, and all datasets were statistically analyzed with One-way ANOVA. The results showed that the highest specific growth rate (SGR = 1.29%) and lowest feed conversion rate (FCR = 1.0) were obtained by fish fed the diet supplemented with SSF (0.025%) + MOS (0.05%). The synergistic effects of SSF as an exogenous enzyme source and MOS in supporting gut health likely facilitated enhanced nutrient digestibility and absorption, which supported the growth of juvenile Asian seabass.

Keywords: aquafeed, dietary supplement, growth promoter, nutrient utilization

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Introduction

The importance of euryhaline Asian seabass, *Lates calcarifer* (Bloch, 1790), for both food security and

recreational purposes resulted in an increase in its production by 54% from 68.7K metric tons in 2015 to 105.8K metric tons in 2020 (FAO 2022). While the aquaculture protocol for this species is reasonably well-established, the assessment of growth performance, feed conversion ratio (FCR), and survival rates still requires improvement. The aquaculture sector of Asian seabass has also faced common global challenges, such as constraints on feed raw materials like soybean meal and fish oil, alongside a declining fish meal supply, prompting nutritionists to seek alternative plant-based components (Boonyaratpalin et al. 1998, Ghosh and Ray 2017, Azaza et al. 2023). Given that most plant-based ingredients possess antinutrient properties that are indigestible and detrimental to fish gut health, enzymes and immunomodulators have been adopted as promising strategies to enhance fish immunity (Ringø et al. 2014, Hoseinifar et al. 2020).

Many feed additives, specifically those derived from plant-based substances, are utilized. Feed additives are nutritive or non-nutritive compounds that are incorporated into the diet in small quantities, and they have a wide range of functions, such as enhancing the nutritional value of aquafeed, increasing immunomodulation and stress-alleviation, enhancing feed palatability, improving growth performance, and maintaining water quality (Gasco et al. 2018). Although the benefits of feed additives have been recorded for various aquaculture species, most assays were conducted using single feed additives. Therefore, the present study explored the potential combined effects of two feed additives, namely mannan oligosaccharide (MOS) and enzymes derived from the solid-state fermentation of *Aspergillus niger* (SSF), in the diet of juvenile Asian seabass. MOS, originating from the cell wall of *Saccharomyces cerevisiae*, is resistant to digestive enzyme degradation and can reach the distal intestine to promote the colonization of beneficial gut bacteria (Sudewi et al. 2023). On the other hand, SSF produces various hydrolytic enzymes that break down complex substrates to promote nutrient release (Jannathulla and Dayal 2022). This study aimed to determine the optimal dosage and individual/synergistic effects for both single or combined additives in relation to the growth and survival of juvenile *L. calcarifer*.

Methodology

Ethical Statement

The handling of animals complied with ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments) and was approved by the UMT Research Ethics Committee (UMT/JKEPHMK/2023/104) of the University Malaysia Terengganu.

Supplemented Diet Preparation

In this feeding trial, commercial feed that contained 49.60 ± 0.39% CP, 7.15 ± 0.02% moisture, 13.50 ± 0.11% ash, 9.27 ± 0.08% CL, 1.79 ± 0.06% fiber (B5003, Uni-President Vietnam Co., LTD) was coated with single or combined feed additives at corresponding concentration levels (SSF 0.025%, SSF 0.05%, MOS 0.1%, MOS 0.2%, and SSF 0.025%+MOS 0.05%). The MOS product Actigen® and the SSF product Allzyme® SSF were obtained from Alltech Biotechnology Malaysia Sdn Bhd. The coating process involved two steps, where the additives were first mixed with distilled water at ratios of 1:10 using a Planetary Mixer (Model Mix: B20-A) for 3 min, followed by 2 g kg^{-1} feed of diluted agar (Fluka Analytical, Germany) to prevent the homogeneously coated additives from leaching. The coated feeds were then oven-dried for 5 h at 45°C and air-dried at room temperature before they were vacuum-packed and stored at ± 4.0°C for later use.

Fish husbandry, experimental design, and feeding trial

A total of 918 juvenile Asian seabass with an initial weight of 21.60 ± 0.13 g and body length of 11.5 ± 0.01 cm were obtained from a local farm and were acclimatized for 14 days, during which they were fed

Table 1
Growth performance, nutrient digestibility, and nutrient retention of juvenile Asian seabass fed different levels of feed additive supplemented diets for 70 days

Treatment	Control	SSF1	SSF2	MOS1	MOS2	SSF+MOS	P-Value
Growth Performance (%)							
IW	21.47 ± 0.62[a]	21.39 ± 0.06[a]	21.33 ± 0.06[a]	21.91 ± 0.14[a]	21.90 ± 0.38[a]	21.61 ± 0.46[a]	0.78
FW	46.28 ± 0.60[a]	49.96 ± 0.02[a]	49.68 ± 0.05[a]	51.82 ± 0.07	53.93 ± 0.88	54.92 ± 1.94	0.00
WG	24.80 ± 0.04[a]	28.57 ± 0.04	28.35 ± 0.02	29.91 ± 0.16	32.04 ± 1.07	33.31 ± 1.51	0.00
Fi	33.35 ± 0.54[a]	34.49 ± 2.69[a]	33.63 ± 2.15[a]	33.90 ± 2.08[a]	35.17 ± 2.13[a]	33.09 ± 0.32[a]	0.97
SGR	1.07 ± 0.02[a]	1.18 ± 0.00	1.17 ± 0.00	1.19 ± 0.01	1.25 ± 0.04	1.29 ± 0.02	0.00
FCR	1.35 ± 0.02[a]	1.21 ± 0.10[a]	1.18 ± 0.07[a]	1.13 ± 0.07[a]	1.11 ± 0.10[a]	1.00 ± 0.04	0.09
SR	70.00 ± 3.64[a]	77.78 ± 3.92[a]	76.47 ± 3.93[a]	70.94 ± 1.73[a]	70.94 ± 2.36[a]	75.82 ± 1.73[a]	0.71
Phosphorus (%), Initial							
Muscle	0.59 ± 0.00[a]	0.58 ± 0.00[a]	0.58 ± 0.00[a]	0.58 ± 0.00[a]	0.58 ± 0.00[a]	0.58 ± 0.00[a]	0.15
Feces	5.66 ± 0.01[a]	5.65 ± 0.00[a]	5.65 ± 0.00[a]	5.65 ± 0.01[a]	5.63 ± 0.01[a]	5.66 ± 0.00[a]	0.33
Phosphorus (%), Final							
Muscle	0.79 ± 0.01[a]	0.85 ± 0.01[a]	1.36 ± 0.04	1.66 ± 0.04	1.38 ± 0.00	1.27 ± 0.04	0.00
Feces	5.90 ± 0.01[a]	3.07 ± 0.32	4.02 ± 0.32	4.71 ± 0.17	4.39 ± 0.21	4.80 ± 0.16	0.00
Apparent digestibility coefficients (ADC), g kg^{-1}							
CP	0.64 ± 0.00[a]	0.68 ± 0.00	0.69 ± 0.00	0.65 ± 0.00	0.79 ± 0.00	0.69 ± 0.00	0.00
CL	0.73 ± 0.00[a]	0.84 ± 0.00	0.88 ± 0.00	0.85 ± 0.00	0.88 ± 0.00	0.89 ± 0.00	0.00
P	0.49 ± 0.00[a]	0.53 ± 0.00	0.53 ± 0.01	0.53 ± 0.01	0.55 ± 0.00	0.55 ± 0.00	0.00
Nutrient Retention (%)							
Protein	32.36 ± 0.13[a]	33.22 ± 0.13	33.59 ± 0.13	34.80 ± 0.13	35.04 ± 0.07	35.92 ± 0.04	0.00
Lipid	59.54 ± 0.20[a]	60.50 ± 0.20	60.72 ± 0.10	62.14 ± 0.07	62.75 ± 0.19	63.43 ± 0.28	0.00
Phosphorus	47.30 ± 0.14[a]	48.36 ± 0.24	48.55 ± 0.28	50.03 ± 0.05	50.21 ± 0.21	50.91 ± 0.17	0.00

SSF1 – SSF 0.025%, SSF2 – SSF 0.05%, MOS1 – MOS 0.1%, MOS2 – MOS 0.2%, SSF +MOS – SSF 0.025% + MOS 0.05%, CP – crude protein, CL – crude lipid, P – phosphorus. IW – Initial Weight (g), FW – Final Weight (g), WG – Weight Gain (g), Fi – Feed Intake (g) Feed/fish, SGR – Specific Growth Rate (%) FCR – Feed Conversion Rate, SR (%) – Survival Rate, ADC – Apparent Digestibility Coefficient, and Nutrient retention (%). All results for Growth performance are depicted as mean ± standard error (SE). Means not labelled with the letter a are significantly different ($P < 0.05$) from the control level mean. As determined by one-way ANOVA analysis followed by grouping information using the Dunnett Method.

a control diet at the hatchery of Faculty of Fisheries and Food Sciences, University Malaysia Terengganu. Following acclimation, *L. calcarifer* juveniles were randomly divided into eighteen 120.0 L tanks (51 fish per tank) allocated in triplicate to six experimental treatments. This 70-day feeding trial, which consisted of one control and five experimental diet groups, was performed in a flow-through system with continuous aeration using a completely randomized design (Table 1). Water change occurred at a rate of 2 L min^{-1} of the tank volume to maintain optimum water quality during the feeding period. A LAQUA DO210 & PH210 multiprobe (HORIBA Advanced Techno Co., Ltd., Japan) and a UV Spectrophotometer UV-1800 (Shimadzu Corp., Japan) were used to estimate daily water quality parameters, which were recorded at the following average ranges: salinity 17.12 ± 0.09, water temperature 26.90 ± 0.00°C, approximate dissolved oxygen 5.14 ± 0.08 mg L^{-1}, ammonia nitrogen 0.01 ± 0.00 mg L^{-1}, pH range 8.49 ± 0.16, alkalinity 143.19 ± 5.97 mg L^{-1}, nitrite 0.01 ± 0.00 mg L^{-1}, and nitrate at 2.51 ± 0.83 mg L^{-1}. The fish were reared under similar natural photoperiod conditions (12 h light and 12 h darkness). The fish were fed with corresponding experimental diets at a rate of ± 3.0% of their body weight two times per day (08:00 and 16:00). Uneaten feed and metabolic wastes were collected by

siphoning twice daily and were immediately quantified for daily feed ratio adjustment. Throughout the feeding period, the mortality and total amount of feed consumed were recorded daily.

Growth performance, feed utilization, and survival indices measurement

Four fish per tank were sampled at random to record the initial weight (IW) and initial number (IN) of fish before the feeding trial, followed by subsequent weekly random sampling to estimate their growth parameters, feeding utilization indices, and survival rate. At the end of the feeding trial (70 days), the fish fasted for 24 H and were anaesthetized with MS-222 (100 mg L^{-1}) prior to final weight (FW) measurement. The final number of fish (FN) was also recorded. The fish growth (weight gain, WG), specific growth rate (SGR), feed conversion rate (FCR), survival rate (SR), apparent digestibility coefficient (ADC), and nutrient retention were assessed with the following equations (Magouz et al. 2021, Mallioris et al. 2022, Wang et al. 2022):

$$WG\ (\%) = [(\text{Final weight} - \text{Initial weight}) / \text{Initial weight}] \times 100 \quad (1)$$

$$SGR\ (\%) = [(\ln \text{Final weight} - \ln \text{Initial weight})/\text{day}] \times 100 \quad (2)$$

$$FCR\ (\%) = \text{Feed intake} / \text{Weight gain (g)} \quad (3)$$

$$SR = (\text{Final number of fish} / \text{Initial number of fish}) \times 100 \quad (4)$$

$$ADC = \text{Nutrients in feces} / \text{Nutrients in feed} \quad (5)$$

$$\text{Nutrient retention}\ (\%) = (\text{Nutrient gain} / \text{Nutrient intake}) \times 100 \quad (6)$$

Proximate Analysis of Fecal and Muscle Samples

Sample Collection

Feces samples were collected by siphoning the bottom of the tanks daily in the eighth week of the feeding trials. The fish were fasted for 24 h prior to body collection on day 70. Three fish were collected at random from each tank and anesthetized using 100 mg L^{-1} MS-222 solution. The feces and carcass samples were immediately stored at -80°C prior to freeze-drying in a Genesis SQ Super XL-70 (SP Scientific, USA). Muscle samples were extracted from freeze-dried carcasses. All freeze-dried samples were stored at -20°C prior to proximate composition analysis. Each sample was marked individually based on the numbering system of the tanks shown in Figure 1.

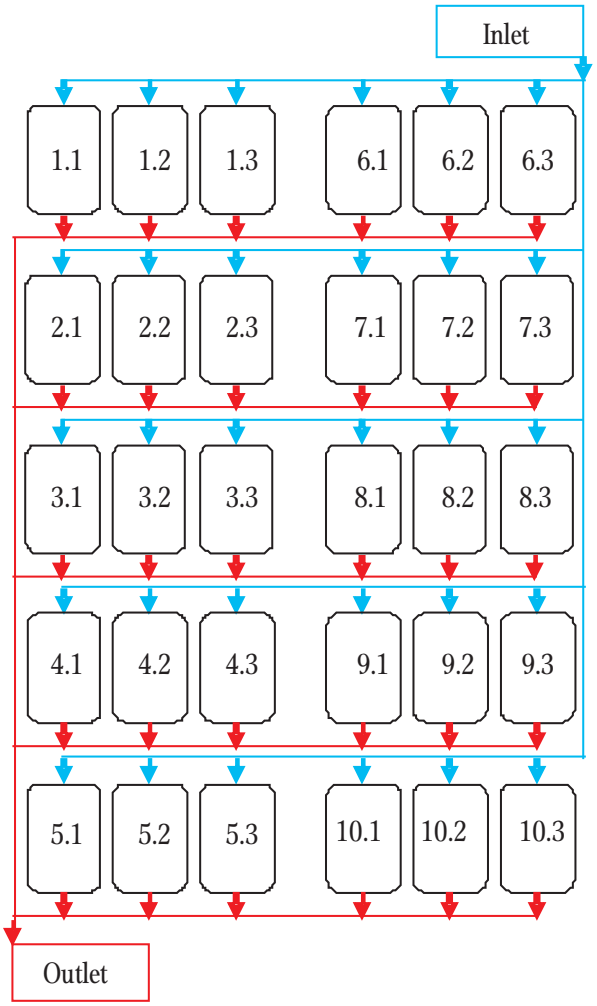

Figure 1. Experimental tank design and labels.

Proximate Composition Analysis of Feed, Muscle, and Feces

The Kjeldahl method was used to determine the crude protein in feed, muscle, and fecal samples. Crude lipid assays were performed with a Foss

apparatus (Labtec ST310) using petroleum ether as the lipid extractor. The moisture content was determined by oven-drying samples at 100°C for 6 h. Ash content was estimated with the dry ashing method in which samples were incinerated in a muffle furnace at 600°C for 3 h. Crude fiber contents were measured with a fiber analyzer (Ankom 200, USA), while phosphorus was analyzed with ICP-OES (Avio 200, USA).

Statistical Analysis

The experimental data was analyzed in descriptive analysis. One-way analysis of variance (ANOVA) followed by the Dunnett method was conducted for all datasets using the Minitab Express™ version 1.5.0 (Minitab Pty Ltd., Australia) with significant differences at $P < 0.05$. Pearson's correlation and principal component analysis (PCA) were performed with RStudio 2023.09.0 (Posit Software, USA) to describe the relationship between growth performance, nutrient digestibility, and nutrient retention. Data were expressed as means and standard errors.

Results

Table 1 summarizes the effects of singular or combined supplementation of feed additives (Solid-state fermented *Aspergillus niger* [SSF] and Mannan-rich oligosaccharides [MOS]) at different doses on the growth performance, feed utilization, and survival parameters of juvenile Asian seabass. Generally, our findings indicate that the substantial differences among treatments for most growth performance and feed utilization parameters were significant. The individual initial weights (IW) of the fish in the experiment ranged from 20.71 g to 21.91 g. The final weight (FW) increased 1.2 and 1.5 fold for all treatments. Of all the treatments, combined dietary supplementation of SSF 0.025%+MOS 0.05% of juveniles of Asian seabass showed the highest SGR at 1.29%, followed by MOS 0.2% at 1.25% and SSF 0.025% at 1.18%, surpassing control treatment at 1.07% that were significantly lower in comparison to control. Notably, the SSF 0.025%+MOS 0.05% treatment demonstrated the most efficient FCR (1.00) that was statistically significant ($P < 0.05$) compared to the control treatment (FCR = 1.35). MOS 0.2% (FCR =1.11) and SSF 0.05% (FCR = 1.18) displayed better FCR when compared to the control treatment. However, SR was observed to be above 70% for all treatments with no significant differences ($P>0.05$). Fish fed SSF 0.025% had the highest SR of 77.78 compared to SSF 0.025%+MOS 0.05% (75.82). Similarly, feed intake (Fi) also showed no statistical differences among treatments ($P > 0.05$). The apparent digestibility coefficient (ADC), which represents the efficiency of nutrient absorption, was significantly different for protein digestibility in all the experimental treatments compared to the control ($P < 0.05$). MOS 0.2% exhibited the highest ADC for CP at 0.79 ± 0.00, outperforming the control treatment (0.64; $P < 0.05$), followed by combined SSF 0.025%+MOS 0.05% (0.69), and SSF 0.05% (0.69). With regards to lipid digestibility, SSF 0.025%+MOS 0.05% was highest (0.89), followed by MOS 0.2% (0.88), and SSF 0.05% (0.88), and all three were significantly greater than the control treatment (0.73; $P < 0.05$). For phosphorus ADC, all treatments were significantly higher ($P < 0.05$) than the control, the highest was MOS 0.2% (0.55) followed by SSF 0.025%+MOS 0.05% (0.55) and SSF 0.025% (0.53). Nutrient retention results for protein, lipids, and phosphorus were also statistically greater ($P < 0.05$) for all treatments compared to the control. The nutrient retention of protein, lipids, and phosphorus for the combined feed additives of SSF 0.025% + MOS 0.05% were 68.80%, 88.80% and 54.50%, respectively. MOS 0.2% showed retention levels of protein at 35.04%, lipids – 62.75%), and phosphorus – 50.21%, while the retention levels of the same nutrients for SSF 0.05% were 33.59%, 60.72%, and 48.55%, respectively. Figure 2 (A) shows that SGR is the key parameter of interest. SGR displayed a strong negative correlation with FCR (-0.78). Generally, SGR demonstrated a notable positive relationship with the ADC of the nutrients phosphorus (0.83), lipids (0.82), and protein (0.68).

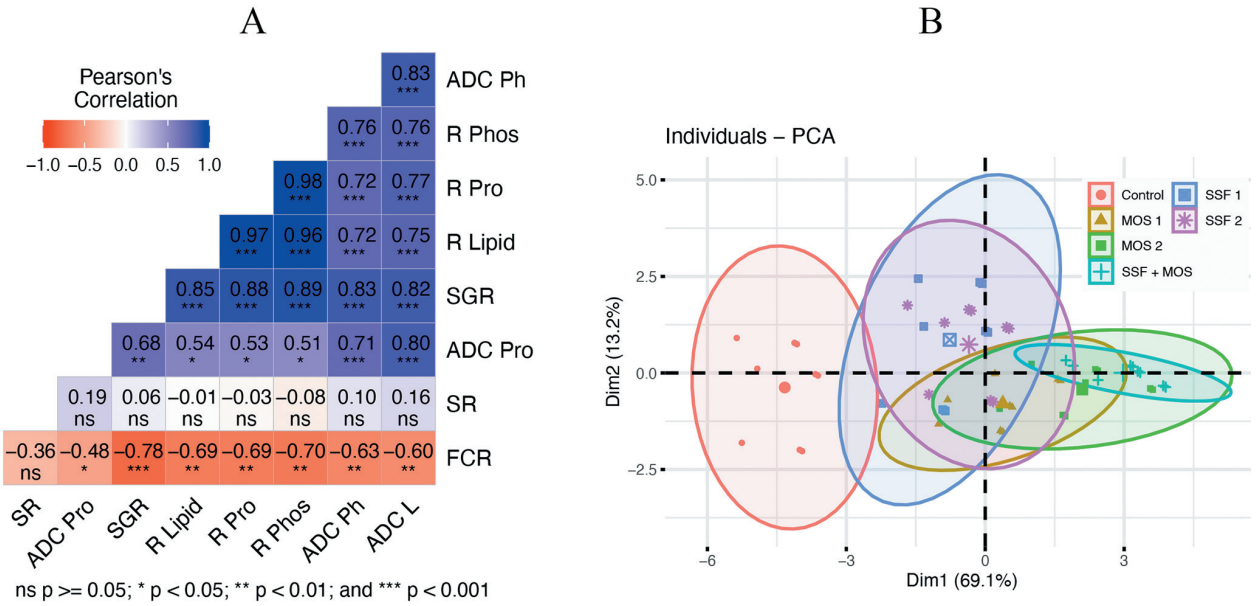

Figure 2. Pearson's correlation coefficients (A) and scores plot (B) of PCA analysis among ADC Phosphorus, ADC Protein, ADC Lipid, Retention Protein, Retention Phosphorus, Retention Lipid, SGR, SR, and FCR for different levels of feed additive supplemented diets for 70 days. *** Correlation is significant at P < 0 .001, ** Correlation is significant at P < 0.01 and * correlation is significant at P < 0.05.

Likewise, retention efficiencies (R) exhibited very high correlations with SGR for phosphorus (0.89), protein (0.88), and lipids (0.85). It was also apparent that R showed significant medium to high correlations with the ADC values of the nutrients phosphorus (0.76), lipids (0.75), and protein (0.53). The PCA results revealed that all treatment groups, except SSF1, exhibited discernible clustering patterns that differed notably from the control group (Figure 2B). SSF1 demonstrated a unique clustering pattern and displayed higher similarity to the control group compared to the other treatment groups with regard to the parameters assessed (growth performance, nutrient digestibility, and nutrient retention). Based on PCA analysis, it was observed that similar feed additive treatment groups were clustered together (SSF1 vs SSF2 and MOS1 vs MOS2), while the combined feed additives (SSF+MOS) exhibited distinctive effects on the parameters assessed compared to the other treatment groups.

Discussion

In the present study, juvenile Asian seabass were observed to exhibit greater growth, lower feed conversion ratio (FCR), and higher nutrient digestibility and retention when fed with MOS at concentrations of 0.1% and 0.2%. These findings align with previous studies conducted on rainbow trout (*Oncorhynchus mykiss* (Walbaum)) fed with MOS 0.2% (Mínguez et al. 2016), milkfish (*Chanos chanos* (Fabricius)) fed with MOS 0.3% (Harikrishnan et al. 2023), and Atlantic salmon (*Salmo salar* L.) fed with MOS concentrations of 0.5% and 1.0% (Kazlauskaite et al. 2022). MOS has been documented to improve functional gut health by increasing digestive enzyme activity to boost nutrient digestibility and the absorption of lipids, proteins, and phosphorus for better growth performance with a lower FCR (Flores-Kossack et al. 2020, El-Saadony et al. 2021, Xue et al. 2022, Sudewi et al. 2023). Electron microscopy and transmission electron microscopy showed that gilthead seabream (*Sparus aurata* L.) supplemented with MOS 0.2% had a greater capacity for nutrient capture

thanks to an increase in microvilli density and length in both the anterior and posterior gut regions (Dimitroglou et al. 2010). Similar results have been observed on the gut absorptive surface of seabass and cobia larvae, where MOS dramatically increased the number of acid mucins per unit area and absorptive cell microvilli in the anterior and posterior intestinal regions (Salze et al. 2008, Torrecillas et al. 2011, Xia et al. 2018). Among all the treatments, significant growth improvement with the highest nutrient utilization was noted in the juvenile Asian seabass fed SSF 0.025% + MOS 0.05%, which can likely be attributed to multiple beneficial functions of each of the feed additives. The literature indicates that MOS acts as a prebiotic that stimulates the growth and colonization activity of beneficial bacteria in fish guts (El-Saadony et al. 2021 Flores-Kossack et al. 2020, Sudewi et al. 2023, Xue et al. 2022), whereas *Aspergillus niger*, through solid-state fermentation (SSF), produces a range of enzymes that break down complex carbohydrates, proteins, and fibers of aquafeed into simpler, more digestible forms. Combining SSF and MOS in diets may amplify the benefits of each feed additive, resulting in a synergistic enhancement of their beneficial functions. The SSF fermentation process facilitates the microbe-assisted transformation of raw materials, including those with anti-nutritional properties, into a more digestible, bioavailable form. This process results in the enhanced nutritional value and overall quality of the feed (Chilakamarry et al. 2022, Ferreira et al. 2022, Ibarruri et al. 2021, Wang et al. 2023). SSF could generate a variety of hydrolytic enzymes capable of degrading fibers and antinutrient components, notably from plant-based sources, into higher quality essential amino acids such as methionine and lysine in aquafeed (Jannathulla and Dayal 2022). These SSF enzymes have also been proven to provide a cost-effective and sustainable supply of enzyme-rich feed for growth and health improvement in a number of aquaculture species (Li et al. 2021), including juvenile Nile tilapia (*Oreochromis niloticus* (L.)) (Bowyer et al. 2020) and rainbow trout (Davies et al. 2021). The dietary supplementation of 0.025% SSF and 0.05% MOS may be an effective nutritional strategy to support the performance of juvenile Asian seabass given the greatly improved growth metrics and positive synergistic effects of these two feed additives in this trial. The significantly greater growth of juvenile Asian seabass fed the combined feed additives of SSF 0.025% + MOS 0.05% in the present study was also reflected by their higher nutrient retention levels for protein, lipids, and phosphorus. The retention of these nutrients has been documented as an important promoter of fish growth, critically supporting various physiological functions in fish including energy metabolism, tissue growth, and reproduction (Li et al. 2009, Radhakrishnan et al. 2020). Notably, phosphorus is an essential mineral for skeletal development and energy metabolism (Sugiura et al. 2004, Fontagné et al. 2009, Schamber et al. 2014), whereas dietary protein is essential for muscle development and maintenance (Vareltzis 2000, Nemova et al. 2021), and lipids provide a concentrated energy source necessary for the development and maintenance of cell membranes (Watanabe 1982, Izquierdo et al. 2000). Consistent with our nutrient retention results, rainbow trout, Atlantic cod (*Gadus morhua* L.), and Atlantic salmon fed diets with higher phosphorus, protein, and lipid levels, respectively, showed positive correlations between growth and nutrient retention (Lellis et al. 2004, Hansen et al. 2007, Hixson et al. 2017). While a positive correlation between SGR and ADC of each nutrient was observed in the present study, a negative correlation was revealed between FCR and SGR. These correlation patterns have also been documented in seabass and Siberian sturgeon (*Acipenser baerii* Brandt) and indicate that feed additives could enhance nutrient absorption and ingestion through increased metabolic processes and efficient feed conversion to biomass, resulting in higher growth rates (Besson et al. 2020, Sayed Hassani et al. 2020). Additionally, a negative association was found between FCR and ADC, indicating that fish with higher ADC values utilized nutrients more effectively, enhancing growth efficiency (Al-Noor et al. 2023). Furthermore, we hypothesize that the significant inverse relationship between FCR and nutrient retention (NR) observed in the present study suggests that feed

additives could increase nutrient assimilation and decrease requisite feed quantities, which Elvy et al. (2023) report in their empirical findings on freshwater Chinook salmon (*Oncorhynchus tshawytscha* (Walbaum)).

Conclusion

The present study revealed that incorporating a dietary supplementation of SSF 0.025% + MOS 0.05% supported greater growth performance, feed utilization, and nutrient retention in juvenile Asian seabass. Further studies that explore the gene expression profiles of fish subjected to this additive combination (SSF+MOS) would provide insights into the cellular and molecular interactions of these feed additives.

Acknowledgements. This research was supported and funded by Alltech Biotechnology Malaysia Sdn Bhd (Grant vote: 53453). This research was conducted at the Institute of Climate Adaptation and Marine Biotechnology (ICAMB) and the Faculty of Fisheries and Food Science, University Malaysia Terengganu. We would like to thank the science officers in these institutions for their technical assistance.

Authors contribution. G.P.F., L.L.W., and N.M.N. conceptualized and designed the study. G.P.F. and L.L.W. conducted the experiments and data collection. G.P.F., P.L., N.M.N., and L.L.W. performed the data analysis. G.P.F. and L.L.W. drafted the manuscript. P.L., H.W., G.L., N.M.N., Y.Y.S., and MDD provided critical revisions and feedback on the manuscript. All authors contributed to the final manuscript and approved the submitted version.

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