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RESEARCH PAPER

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Raw Fish-based Moist Pellet and Extruded Pellet on Growth, Levels and Expression of GH/IGF Axis, Intestinal Enzyme Activity and Gastrointestinal Morphology of Olive Flounder (*Paralichthys olivaceus):* An Actual Field Study

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Abstract

A six-month feeding trial was conducted to investigate the effect of moist pellet (MP) and commercial extruded pellet (EP) on the somatic growth, level and expression of GH/IGF axis, intestinal enzyme activity and gastrointestinal morphology of olive flounder (*Paralichthys olivaceus*) reared under actual farm conditions. MP was a raw fish-based diet composed of mackerel and cutlass fish while EP diet was a locally-produced commercial feed. Weight growth rate showed no significant difference ($P>0.05$), although EP-fed fish had a better somatic yield. Feed conversion ratio was better and condition factor has significantly improved when given EP diet ($P<0.05$). Plasma level of growth hormone and insulin-like growth factor 1 was comparably similar between diets, but EP-fed olive flounder had a higher hepatic IGF-1 expression ($P<0.05$). The activity of trypsin, chymotrypsin and lipase were insignificantly influenced by diets ($P>0.05$). The overall gastrointestinal morphology was in similitude regardless of diets, but EP- fed fish had a longer intestinal villi length and goblet cell count in the pyloric caeca ($P<0.05$). These dietary induced differences are discussed to have partly promoted better growth in EP diet. The findings suggest an equivalent, or better utilization of EP diet in the actual farm operation.

Introduction

The olive flounder (*Paralichthys olivaceus)* is one of the highly valued cultured species in East Asia, with most productions coming from South Korea and Japan (Bai & Lee, 2010). The United States as well considers farming of this flat-fish a promising marine aquaculture development (Stieglitz et al., 2021). This fish remains popular as aquaculture species due to its rapid growth capacity, immense aquaculture potential and widespread consumption (Geng et al., 2019). Production of olive flounder in South Korea outweighs other cultured species with a production of 45, 801 mt (out of 90, 545 mt) in 2022 (KOSIS, 2023). Farming technologies, including feeding management specific for olive flounder has been described in the plethora of published

works, nevertheless, lack of standardized feeding scheme is still apparent (Hamidoghli et al., 2020). In Korea and Japan, post-metamorphic juveniles were usually given pelleted diets and transitioned into raw fish and moist pellets few months after (Seikai et al., 2010). Moist pellet (MP) diet is basically raw fish, usually in frozen form, bound using a commercial binder and used as direct feed in aquaculture (Lee et al., 2016). Common fish includes mackerel, pacific sand lance, herring and sardines (Kim et al., 2009; Seikai et al., 2010). Extruded pellet (EP) for olive flounder, on the other hand, is a formulated diet specifically made to provide the necessary nutritional requirements of the animal. EP diets are usually formulated to contain fish meal as protein source, wheat flour as carbohydrate source and fish oil as lipid source. The ingredients are mixed and formed into an extruded pellet in an extruding machine or extruder.

Currently, there are still contradicting opinions on the efficacy and sustainability of MP and EP diets during the growing stage of olive flounder (Jang et al., 2022). Most of the local farmers prefer MP over EP due to better growth enhancement effect on the fish (Kim et al., 2014). However, the reliance on MP is discouraged due to its significant negative impacts on the environment and on the cultured animal such as water quality deterioration, increase incidence of fish disease, high feed conversion ratios and can put high pressure on the local fisheries to meet its demand (Seo et al., 2005; Murashita et al., 2021). EP, meanwhile, is advised due to its simple management, low water pollution, and easy observation of feeding activity (Cho & Cho, 2009).

Comparative studies on the effect of MP and EP diets on various aquaculture species has been reported with some contrasting results. For example, Kim et al. (2002) described that EP diet had a lower survival and comparable growth performance against MP fed rockfish (*Sebastes schlegeli*). In addition, the Bluefin tuna (*Thunnus orientalis*) juveniles had morphologically and physiologically adapted digestive system as a result of feeding EP, however, comparable difference to fish fed MP diet was also evident (Murashita et al., 2021). Significantly higher growth performance and improved hematological parameters and crude protein content of juvenile olive flounder fed EP diet over MP were similarly reported (Kim et al., 2014; Lee et al., 2016). In contrast, Jung et al. (2020) and Jang et al. (2022) demonstrated from olive flounder reared in Wando, southern region of South Korea that MP-fed fish had a significantly better growth rate over fish fed EP diet. These examples of contradicting results gave rise to the disputed consensus on the utilization of these diets for olive flounder that became a farm-specific approach. Nevertheless, we deemed that it was crucial to understand how these diets would impact the fish, not only on their morphological development but also on their underlying physiological functions, especially on the endocrinological control of growth and digestion capacity.

Diet type and nutrients therein correlates to the animal intrinsic growth rate, wherein high-quality nutritious diet promotes optimum somatic yield and overall health condition. One way to determine the effect of nutrition on the culture species is to measure its effect on growth associated hormones and factors. The GH-IGF axis is the main endocrinological growth regulator in vertebrates (including teleost) (Wood et al., 2005) and diet affects its production and efficiency (Triantaphyllopoulos et al., 2020). The GH produced in the anterior pituitary travels through the bloodstream, acts on its receptor in the liver, muscle and other organs and signals production of insulin growth-like factor 1 (IGF-1). Liver synthesizes around 70% of the total IGFs produced in the body. IGF-1 stimulates cell proliferation and differentiation of target organs after binding to its receptor (Wood et al., 2005). Previous reports showed that dietary source affects levels of growth associated hormones and factors in range of fish species (Picha et al., 2008; Hassaan et al., 2019; Triantaphyllopoulos et al., 2020).

Digestive enzymes play a crucial role in the growth and development of fish by facilitating the hydrolysis and assimilation of dietary nutrients. For instance, proteases such as trypsin and chymotrypsin are key enzymes responsible for protein digestion. Studies have shown that optimizing protease activity enhances protein utilization and growth in various fish species (Jiang et al., 2015). Lipases, another important class of digestive enzymes, aid in lipid digestion. Research has demonstrated that supplementing diets with exogenous lipases improves lipid utilization and growth performance in fish (Zheng et al., 2020). Furthermore, dietary type and ingredients are known to modulate changes in the morphology of digestion-related organs in fish, such as on intestinal villi and hepatic and stomach structural integrity (Tran-Ngoc et al., 2019; Jo et al., 2021; Seo et al., 2022).

Based on our literature search, the effect of MP and EP diets on the somatic growth, feed utilization, body composition, blood chemistry, immunity and intestinal microbiome in various fish species has been reported (Kim et al., 2002; Son et al., 2013; Lee et al., 2016; Jung et al., 2020; Jang et al., 2022). However, the effect on the level of growth associated hormone and factor and some aspects of digestive physiology have not been evaluated before. Hence, this study was conducted further to investigate the influencing effect of feeding MP and EP on growth hormone (GH), insulin-like growth factor 1 (IGF1), intestinal enzyme activity and gastrointestinal and hepatic morphology of sub-adult olive flounder (*P. olivaceus*) reared under actual aquaculture farm setting. This study would contribute to expanding knowledge on the use of these dietary strategies and support sustainable olive flounder aquaculture development in South Korea and other producing countries.

Materials & Methods

Experimental Feed, Animal and Feeding Trial

The MP and EP diets were designated as experimental treatment groups in this study. The MP feed used was a raw fish-based diet composed of mackerel and cutlass fish. The fish were chopped, mixed and bind together before storage in the refrigerator. The frozen MP was thawed before feeding to the fish. EP was a commercially available extruded diet formulated and manufactured by Suhyup Feed, South Korea. The formulation was not made available to the researchers as per company policy. The proximate composition of two diets is given in Table 1. Sub-adult fish (average weight of 254.24±1.26 g) coming from the same spawning cohort were used in this study. They were reared in a commercial aquaculture facility in Jinbo Fisheries, Jeju Island, South Korea for six months from April to October 2022. There were six rectangular concrete tanks (10 m × 10 m) designated in the facility for the feeding trial. These tanks correspond to two dietary treatments (EP and MP) replicated thrice. There were an approximate number of 2,300 fish per treatment (≈760 fish per tank) supplied with 9:1 or 8:2 mixture of natural seawater and groundwater in a flow-through water aquaculture system. Diets were hand-fed twice daily (6:30 AM and 4:00 PM) until satiety. The satiation level was determined based on the lack of feeding response. The temperature, salinity, pH, and dissolved oxygen levels, respectively, during feeding trial were measured once daily and were all within normal range for culture: 17.2±0.07°C, 32.3±3.45 psu, 7.81±0.13, and 11.4±0.29 mg/L.

Growth, Feed Utilization and Body Indices Measurement

Fish growth measurements were done at the commencement and termination of the feeding trial. Before stocking in tanks, fish were unfed for 24 h and 25 fish were randomly sampled from the cohort, individually measured. This signifies a common initial value for both treatments. At the end of the feeding trial, 25 fish each from both treatments were sampled after random collection from three replicates. The fish were anesthetized in 100 ppm 2-phenoxythenol and body weight (BW) was measured using a balance scale and total length (TL) using a ruler. The BW and TL were measured to assess growth rate and condition factor. The fish were then cut-open and the total viscera and liver alone were weighed to facilitate calculation of viscerosomatic index (VSI) and hepatosomatic index (HSI), respectively. Growth, feed utilization, survival and body indices calculation formulas are given, as follows:

Weight growth rate (WG), % = 100 × [final mean body weight (g) — initial mean body weight (g)/initial mean body weight (g)]

Specific growth rate (SGR), % = 100 × [(Ln final body weight (g) — Ln initial body weight (g)/days of culture]
Survival = 100 × (number of final alive fish / number of initial fish)

Hepatosomatic index (HSI), % = 100 × [liver weight (g) / body weight (g)]

Viscerosomatic index (VSI), % = 100 × [viscera weight (g) / body weight (g)]

Feed conversion ratio = 100 × (dry feed intake / body weight gain)

Condition factor (CF) = body weight (g) / [total body length (cm)] ^{3}

Sample Collection

All fish sampled (n=25) for somatic growth measurements were subjected to the following sample collection, but does not mean that all samples were subjected to analysis. Blood was collected from caudal peduncle using EDTA-treated syringe and plasma was separated after centrifugation (12,000 rpm for 10 mins at 4°C). Liver was divided into two portions-one for histological examination and another for hepatic IGF-1 mRNA analysis. The anterior intestine was excised for digestive enzyme analysis. The part of the liver and the remaining digestive organs were fixed in 10% buffered formalin. Other samples were snap-frozen in liquid nitrogen and stored at -80°C until analysis.

Table 1. Proximate composition of experimental diets fed to olive flounder (% dry weight)

	Experimental diets	
	Extruded pellet (EP)	Moist pellet (MP)
Moisture	4.82	66.80
Crude protein (% CP)	56.60	56.10
Crude lipid (% CL)	11.50	29.20
Crude ash (% CA)	12.90	8.68
Nitrogen Free Extract (% NFE)[1]	19.00	6.02
Gross Energy (kJ.g^{-1})[2]	5.09	6.18

[1]NFE = % CP + % CL + % CA

[2]Gross energy = (% CP × 5.65) + (% CL × 9.45) + (% NFE × 4.2) (Henken et al., 1986)

Biochemical Analysis

Plasma GH and IGF-1 concentration and intestinal enzyme activity were analyzed at the beginning and end of the feeding trial (n=8). For digestive enzyme analysis, intestine tissues were homogenized in 1.5 mL 1× phosphate buffered saline (PBS) and the supernatant were collected after centrifugation (5,000 rpm for 10 mins at 4°C). Plasma and tissue supernatant were utilized as samples in the enzyme-linked immunosorbent assay (ELISA) using commercially-available kits (GH - CSB-E12121Fh; IGF1 - CSB-E12122Fh supplied by Cusabio, TX, USA; trypsin - MBS017140; chymotrypsin - MBS1601668; lipase - MBS026917 supplied by MyBioSource, USA) and analysis followed manufacturer's instructions as stated in the manual. Absorbance was read at 450 nm wavelength in a microplate reader (Biochrome, UK) and concentration was calculated from the standard curve. The proximate composition analysis of the diets was done following standard procedures (AOAC, 2000).

IGF-1 Gene Expression Analysis

The hepatic mRNA expression of IGF-1 was analyzed to determine changes in its expression before and after feeding trial (n=8). Briefly, total RNA was extracted using RNAiso (Takara, Japan) and quantitively checked using a spectrophotometer (Micro Digital, Korea). The optical density (OD) adsorption ratio (OD260/OD280) within 1.8 – 2.0 was used for subsequent cDNA synthesis. PrimeScript First Strand cDNA Synthesis Kit (Takara, Japan) were used to obtain cDNA, following manufacturer's instructions. Reverse transcriptase PCR (RT-PCR) was employed to obtain copies of the target gene, utilizing IGF-1 gene specific primer (Table 2). PCR product was electrophoresed in 2.0% (w/v) agarose gel and band was captured in an imaging system (Azure Biosystem C300 imaging system, Dublin, USA). Bands were quantified using densitometric method as demonstrated (Hwang et al., 2023) using Gene Tools software (Syngene, UK). Gene expression was calculated as relative against the housekeeping gene (18s ribosomal RNA).

Histological Analysis

Histological examination of the digestive organs (stomach, intestine and pyloric caeca) and liver to determine changes in the morphology, villus measurement and goblet cell quantification were done at the start of the study and after feeding trial (n=14). Generally, histological procedures herein are previously described in research works from our laboratory (Jo et al. 2021, Seo et al. 2022). Briefly, incised tissues were dehydrated in increasing alcohol concentrations; xylene cleaned and blocked in paraplast. Tissues were cut at 5 µm thickness in microtome (MicroTec, Germany), dried overnight and subjected to staining protocol. Hematoxylin (Dako, CA, USA) and eosin (Sigma, MO, USA) (H&E) and Alcian blue (EMD Millipore, MA, USA)/Period acid (Sigma Aldrich, MO, USA)-Schiff (Merck, Germany) (AB-PAS) procedures were employed to stain tissues. Ocular observation was done in light microscope (Olympus, Tokyo, Japan) and images were captured with the aid of imaging software (Mosaic 2.1, China). Intestinal villi height was determined following Seo et al. (2022) and goblet cell were quantified within the 1,000 μm^2 observable area.

Statistical Analysis

All quantitative data collected were analyzed using an independent sample T-test in SPSS ver. 27 (IBM, USA). Significance was set at 95% ($P<0.05$) confidence level. Data are presented as mean ± standard deviation (SD).

Results

Growth Performance, Feed Utilization and Body Indices

The growth performance (FBW, WGR, SGR, SR), body indices (HSI, VSI) and FCR values of olive flounder fed EP diet were better compared to MP-fed fish, however, statistically not significant (Table 3, $P>0.05$). Nonetheless, EP-fed fish had a significantly higher condition factor (Table 3, $P<0.05$).

Level of Plasma GH and IGF1 and Hepatic IGF1 Expression

The plasma level of GH and IGF1 were not significantly affected by dietary treatments (Figure 1, $P>0.05$). In contrast, the mRNA expression level of hepatic IGF-1 was considerably lower in the MP diet group (Figure 1, $P<0.05$).

Table 2. Oligonucleotide primer sequences used in this study

Gene		Sequence (5'-3')	Amplicon size (bp)	Accession no.
18s rRNA	Forward	GGTCTGTGATGCCCTTAGATGTC	107	EF126037.1
	Reverse	AGTGGGGTTCAGCGGGTTAC		
IGF-1	Forward	CGGCGCCTGGAGATGTACTG	144	AF016922.2
	Reverse	TGTCCTACGCTGTGCCT		

Table 3. Growth performance, feed utilization and body indices values of olive flounder fed EP and MP diets for six months.

Parameters	EP	MP
IBW (g)	237±7.3	
FBW (g)	593.40±67.46*	407.67±112.98
WGR (%)	143.15±13.89*	67.08±13.25
SGR (%/day)	0.54±0.03*	0.31±0.05
SR (%)	90.20±1.99	91.10±2.55
HSI (%)	1.90±0.18	1.82±0.08
VSI (%)	4.26±0.07	4.24±0.16
FCR	1.75±0.26	3.29±1.73
CF	1.13±0.03*	1.06±0.02

Values are presented as mean ± SD (n=25). Asterisk (*) indicates significant difference at P<0.05 and the lack thereof means otherwise. IBW, initial mean body weight; FBW, final mean body weight; WGR, weight growth rate; SGR, specific growth rate; SR, survival rate; HSI, hepatosomatic index; VSI, viscerosomatic index; FCR, feed conversion ratio; CF, condition factor.

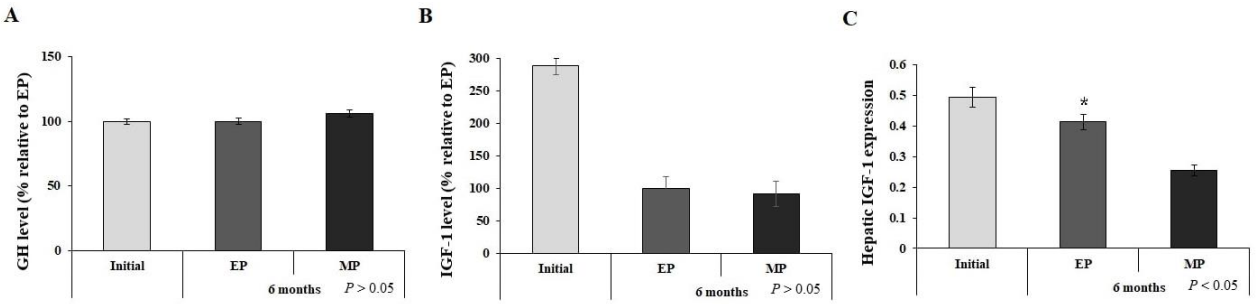

Figure 1. Growth-related factors of olive flounder fed EP and MP diets for 6 months (n = 8). (A) Plasma GH activity; (B) Plasma IGF-1 activity; (C) Hepatic IGF-1 mRNA expression. EP, extruded pellet; MP, moist pellet. Asterisk (*) indicates significant difference at P<0.05 and the lack thereof means otherwise

Digestive Enzyme Activity

There was no significant difference in the intestinal enzyme activity of trypsin, chymotrypsin, and lipase in olive flounder following feeding with EP and MP diets (Figure 2, P>0.05).

Histological Observation, Intestinal Villi Measurement and Goblet Cell Count Quantification

The histological observation of the stomach of the olive flounder revealed a structural formation of serosa, muscularis, submucosa and mucosa. The muscularis is further divided into circular muscle and longitudinal muscle. The mucosal fold surrounding the lumen was observed to have developed and the mucosal layer including the gastric gland developed and increases throughout the feeding period. In addition, the longitudinal muscle was likewise observed to have a hypertrophic change (Figure 3). Nevertheless, discernible difference in the stomach structure after feeding dietary treatments seemed nil after ocular observations.

The intestine was separated into a muscular layer and a mucosal layer, and the mucosal folds of the olive flounder's intestines were enlarged or lengthened as the fish grew (Figure 4) regardless of diets. However, the villi (villi length) covering the entire mucosal layer of the intestine were more branched and the length were significantly increased when fed EP compared to when fed MP diet (Table 4).

The ocular observation of the hepatic tissues revealed an arranged array of hepatocytes with pancreatic trace along the organ. The hepatocyte morphology was normal and zymogen granules did not show abnormality in its structure. Steatosis and vacuolization were not found in the liver tissues. Generally, the structural morphology of the liver of olive flounder was observed to be similar in both dietary treatments (Figure 5). The intestinal mucosal epithelium included intermittently dispersed goblet cells with increase number as the fish grew and counts were no regards to diets (Figure 6, Table 5). Meanwhile, the number of goblet cells in the mucosal epithelium of the pyloric caeca had a similar distribution pattern expressed in the intestinal tissue, but a significant increase in number when fed EP compared to MP diet (Figure 7, Table 5).

Discussion

The rapid and continuous increase in the global production of aquaculture commodity is not without bottlenecks, one of which is the apparent effect of intensification on the environment. Main pollutants released by aquaculture industry are nitrogenous wastes and phosphates causing water quality deterioration, eutrophication and excessive algal

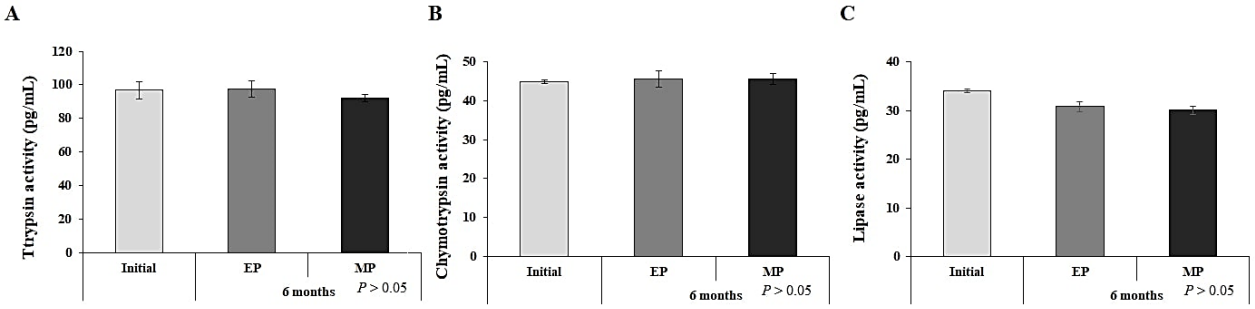

Figure 2. Digestive enzyme activity of olive flounder fed EP and MP diets for 6 months (n = 8). (A) Trypsin activity; (B) Chymotrypsin activity; (C) Lipase activity. EP, extruded pellet; MP, moist pellet. The lack of superscript letters indicates no significant difference between diets (P>0.05).

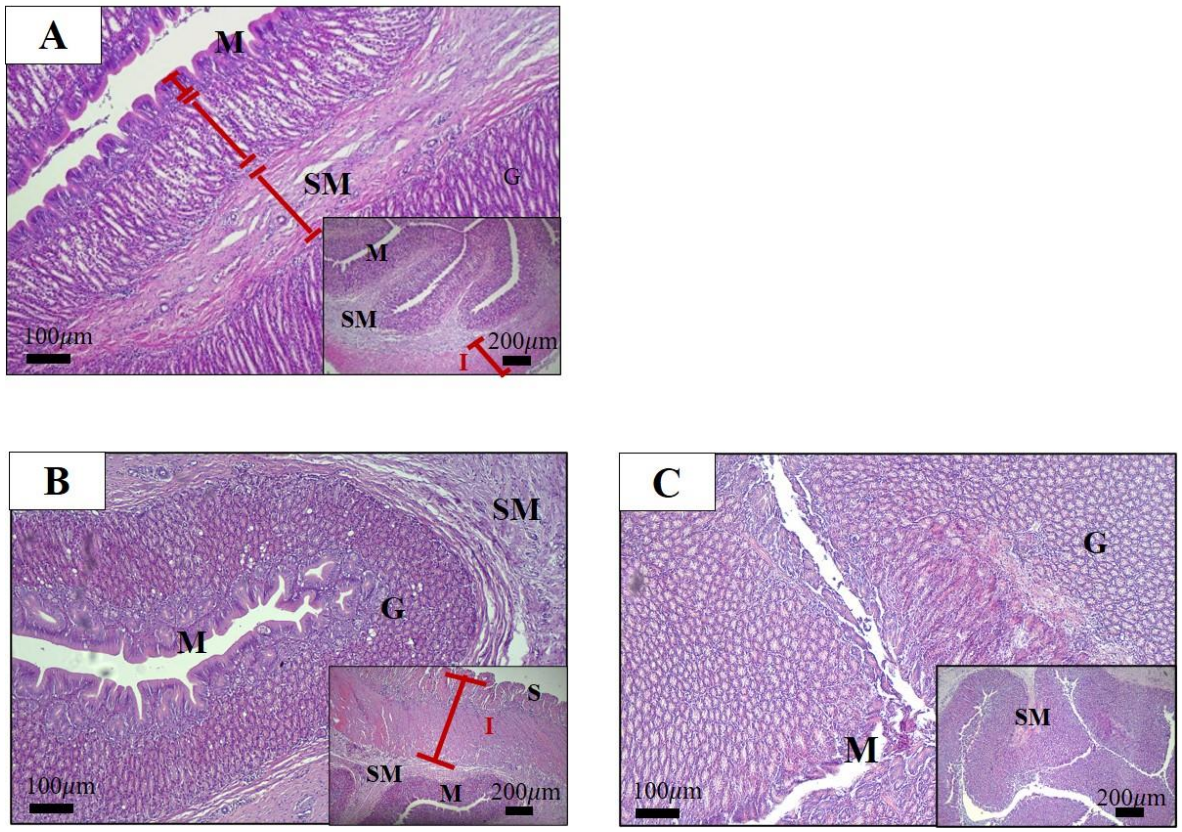

Figure 3. Stomach histological structure of the olive flounder fed EP and MP diets for six months stained with hematoxylin and eosin (H&E). (A) Initial, before the experiment, (B) Fed extruded pellet after six months; (C) Fed moist pellet after six months. S, serosa; LM, longitudinal muscle; CM, circular muscle; I, internal muscular layer; M, mucosa; SM, submucosa; G, gastric glands. Main image magnification, 100 × (scale bar = 100 µm); inset image magnification, 40 × (scale bar = 200 µm).

blooms (Chatla et al., 2020). These wastes are mainly coming from feces and feed wastage (uneaten or excessive amount of feed). Reduction of these wastes is one of the challenges in aquaculture with decades of continuous research highlighting valuable discoveries especially on feed formulation and digestibility (Bureau & Hua, 2010). Thus, selection of best possible feed is essential on aquaculture production without or least possible effect on the surrounding waters.

The EP and MP diets are both used in Korean fish aquaculture, with varying growth promoting influence. In the current study, EP promoted better, although not significant, growth performance of olive flounder. In rockfish, Kim et al. (2002) and Son et al. (2013) showed that EP can be utilize as feed without significant changes in the growth and flesh quality (body composition, textural properties and sensory scores). Kim et al. (2009) similarly described comparable growth of olive flounder fed EP and MP diets. However, other reports showed otherwise (Jung et al., 2020; Jang et al., 2022) and variation can stem from utilization of different raw materials and/or the quality thereof. The FCR value of olive founder is better when fed EP, which signifies better feed utilization and less feed wastage. This also

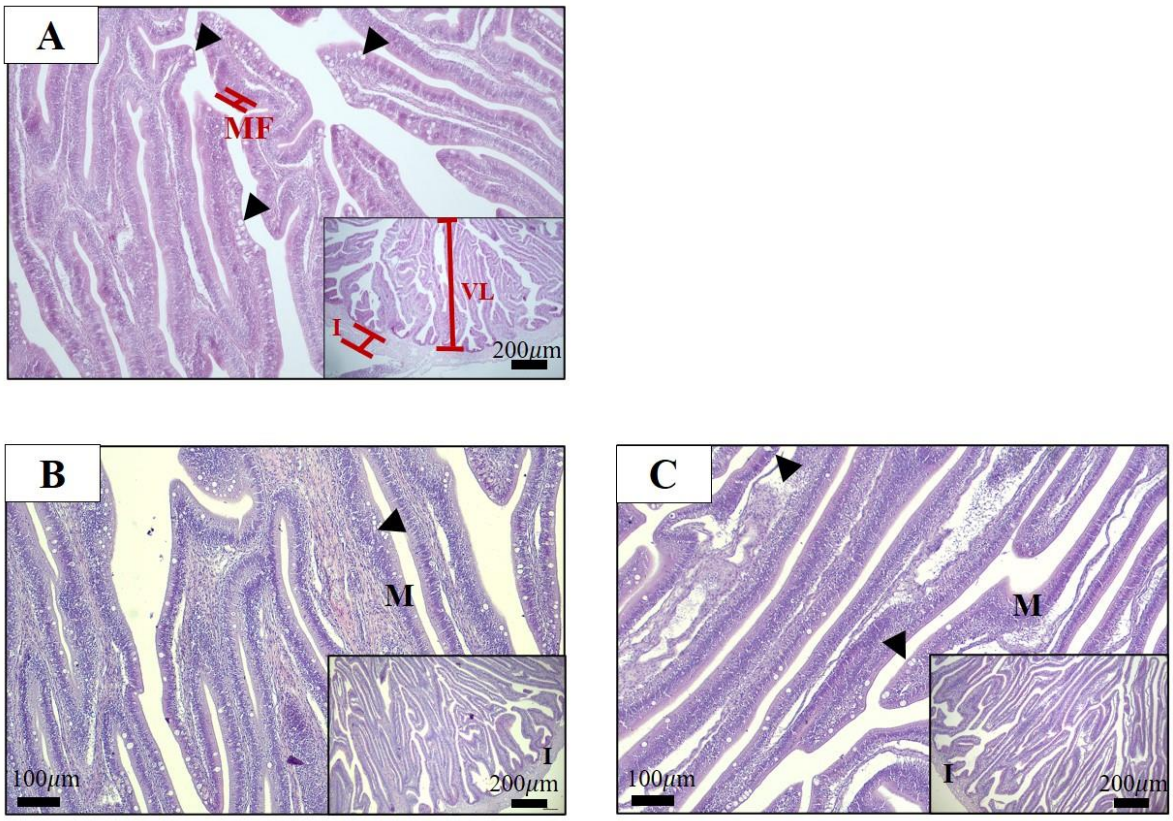

Figure 4. Intestine histological structure of the olive flounder fed EP and MP diets for six months stained with hematoxylin and eosin (H&E). (A) Initial, before the experiment, (B) Fed extruded pellet after six months; (C) Fed moist pellet after six months. MF, mucosal fold; I, internal muscular layer; M, mucosa. The black arrowhead shows a goblet cell. Main image magnification, 100 × (scale bar = 100 μm); inset image magnification, 40× (scale bar = 200 μm).

Table 4. Intestinal villi length and goblet cell count in the intestine and pyloric caeca of olive flounder fed EP and MP diets for 6 months.

		Diet			
		EP		MP	
		Initial	Final	Initial	Final
Intestine	Villi length (μm)	1244.40±64.74	1570.27±93.42*	1244.40±64.74	1372.42±156.89
	Goblet cell count†	124±25	114±13*	124±25	95±3
Pyloric caeca	Goblet cell count†	69±5	265±29	69±5	211±21

Values are shown as mean ± SD (n = 14). Dagger (†) indicates goblet cell count (cell/1,000 μm²) rounded up to whole numbers. Asterisk (*) indicates significant differences at P<0.05 and the lack thereof means otherwise.

signifies less financial losses as EP is better translated into body mass. MP fed fish had a poor FCR (188% higher than EP) which can be a source of significant pollution in the surrounding waters and high economic losses. The body indices (VCI, HSI and CF) are known as indicators of overall health. In contrast to VCI and HSI which were statistically similar, condition factor in EP-fed fish was higher than in MP-fed fish. According to Nehemia et al. (2012) an increase in condition factor equates to general improvement in overall wellbeing or condition. Previous report showed that dietary ingredients can significantly influence the CF of cultured rockfish (Kim & Cho 2019). Comprehensively, EP diet can support growth comparable to MP with better feed utilization, low wastage and overall condition of olive flounder.

Plasma GH and IGFs and the hepatic expression of IGFs are usually employed to assess fish growth after feeding stimulation or feeding trial (Fuentes et al., 2013; Park et al., 2019; Moon et al., 2023). GH stimulates IGF-1 secretion in the liver, and hormonal synthesis may be slowed by starvation, lack of growth hormone receptors or difference in nutritional source or ingredients (Pierce et al., 2005; Triantaphyllopoulos et al., 2019). For instance, dietary protease altered gene expression of GH and IGF-I of Nile tilapia (*Oreochromis niloticus*) (Hassaan et al., 2019), different diets containing various lipid: crude protein (LCP) ratios influence plasma GH and IGF-1 concentrations in Arctic charr (*Salvelinus alpinus*) (Cameron et al., 2007) and low-fish meal diets negatively affects GH and IGFs in olive flounder (Seo et

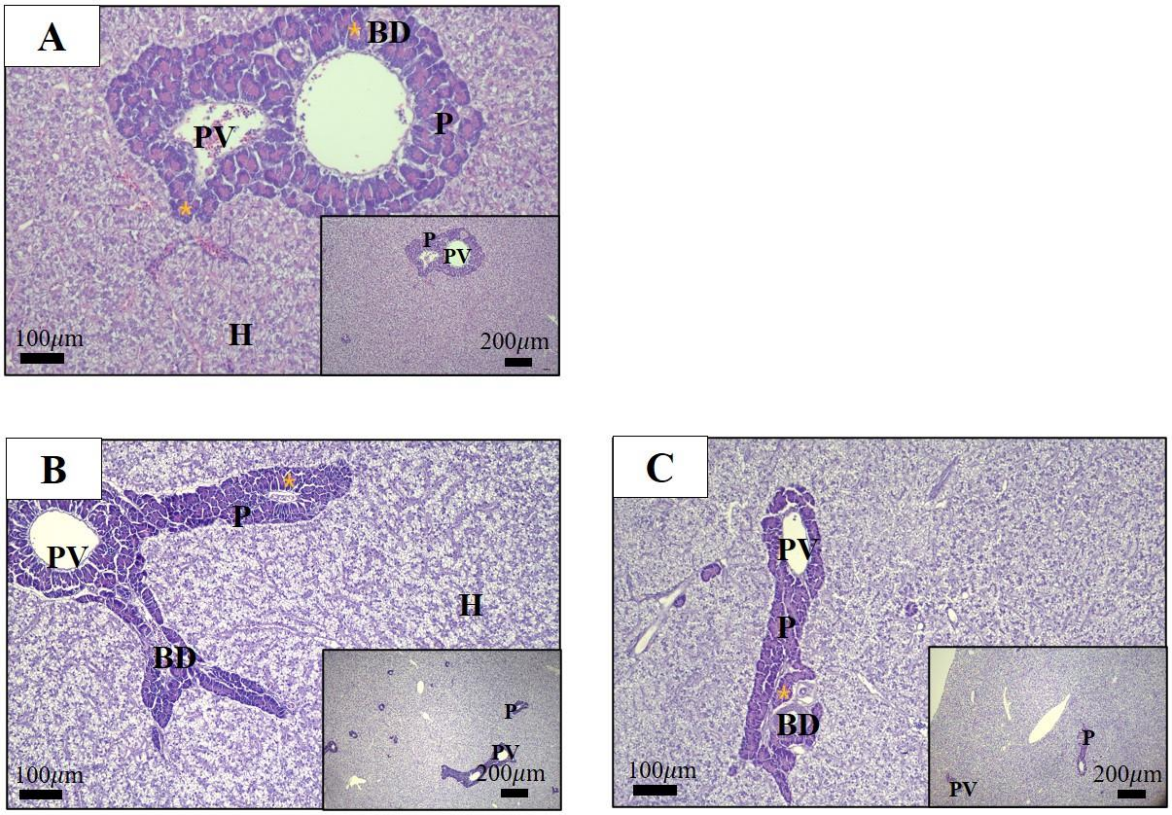

Figure 5. Hepatic histological structure of the olive flounder fed EP and MP diets for six months stained with hematoxylin and eosin (H&E). (A) Initial, before the experiment, (B) Fed extruded pellet after six months; (C) Fed moist pellet after six months. PV, hepatocytes portal vein; H, hepatocytes; P, pancreas; BD, bile duct. Yellow asterisk (*) show zymogen granules. Main image magnification, 100 × (scale bar = 100 µm); inset image magnification, 40 × (scale bar = 200 µm).

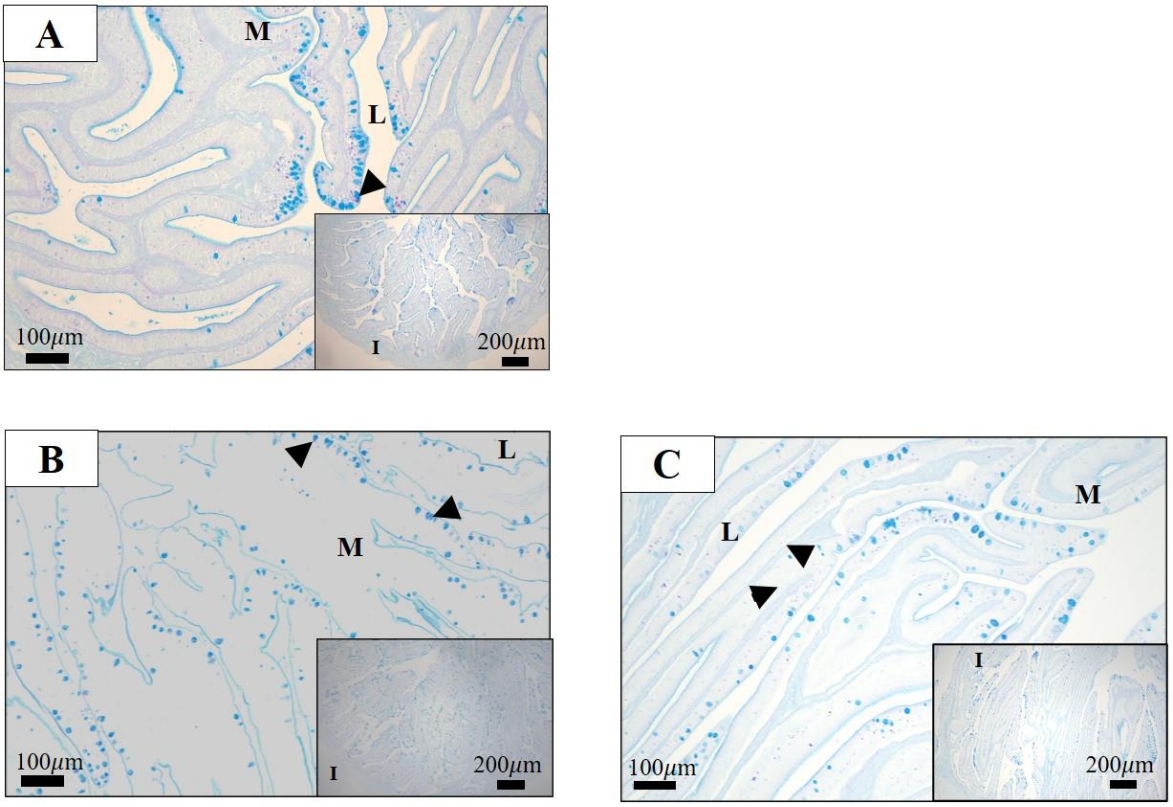

Figure 6. Intestine histological structure of the olive flounder fed EP and MP diets for six months stained with Alcian Blue/Periodic Acid-Schiff's (AB-PAS). (A) Initial, before the experiment, (B) Fed extruded pellet after six months; (C) Fed moist pellet after six months. I, internal muscular layer; L, lumen; M, mucosa. The black arrowhead shows goblet cell. Main image magnification, 100 × (scale bar = 100 µm); inset image magnification, 40 × (scale bar = 200 µm).

Table 5. Goblet cell count in the intestine and pyloric caeca of the olive flounder fed EP and MP diets for six months.

Goblet cell count (cell/1,000 µm²)	Rearing period	Diet	
		EP	MP
Intestine	Initial	124±25	
	Final	113±39*	94±19
Pyloric caeca	Initial	69±5	
	Final	254±40	213±51

Values are shown as mean ± SD (n = 14) rounded up to whole numbers. Asterisk (*) indicates significant differences at P<0.05 and the lack thereof means otherwise.

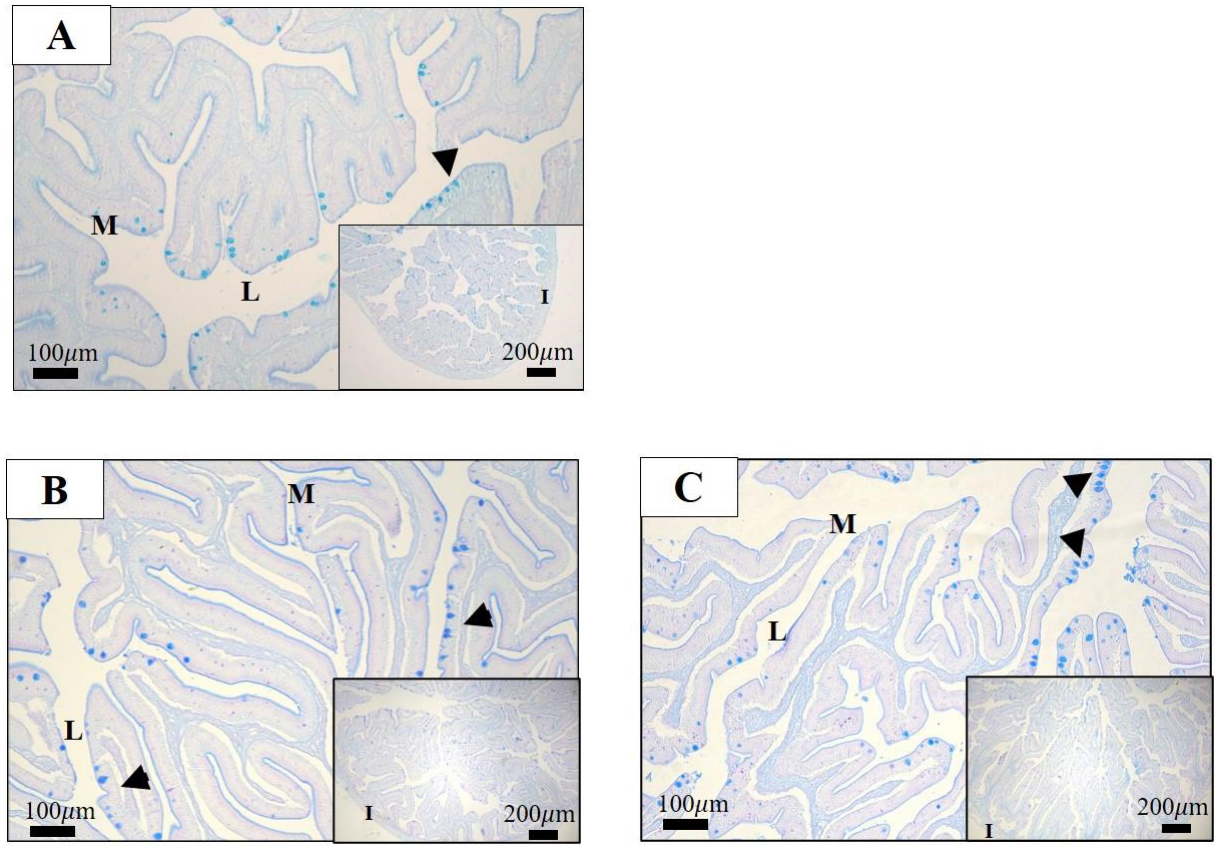

Figure 7. Pyloric caeca histological structure of the of the olive flounder fed EP and MP diets for six months stained with Alcian Blue/Periodic Acid-Schiff's (AB-PAS). (A) Initial, before the experiment, (B) Fed extruded pellet after six months; (C) Fed moist pellet after six months. I, internal muscular layer; L, lumen; M, mucosa. The black arrowhead shows goblet cell. Main image magnification, 100 × (scale bar = 100 µm); inset image magnification, 40 × (scale bar = 200 µm).

al., 2022). In the current study, the concentration of plasma GH and IGF-1 were not affected by both diets. However, hepatic IGF-1 expression was higher in EP-fed olive flounder suggesting nutritional influence on transcriptomic enhancement of signal transduction in GH receptor in the liver. However, since GH concentration was similar, we hypothesized that circulating IGFs produce inhibitory negative feedback on the brain as proposed (Benedito-Palos et al., 2007).

Due to the importance of proper digestibility of nutrient sources, various investigations on the digestive physiology of fish species have been undertaken and showed that fish feed had a direct impact on it (Gilannejad et al., 2021). Proteins and lipids in the feed are hydrolyzed by proteinases (trypsin and chymotrypsin) and lipases to produce a variety of nutrients which are subsequently absorbed by the body. The activity of digestive enzymes secreted from the intestine is associated with growth, and digestive enzymes can influence physiological aspects of digestion (Muhlia-Almazán et al., 2003). In the current study, experimental diets did not significantly influence digestive enzyme activity of olive flounder. This result corroborates with the work of Murashita et al. (2021) wherein digestive enzyme activity of bluefin tuna was comparable in both raw fish feed and commercial diet.

The efficacy of digestion is highly related to structural organization of the digestion-related organs.

For example, gastric gland development in the stomach, density of mucosal fold in the intestine, undegenerated hepatocytes and distributions of goblet cells influence digestive capacity of the animal (Escaffre et al., 2007; Back et al., 2020; Jo et al., 2021). The overall morphology of stomach, intestine and liver were similarly observed without signs of degeneration or abnormal structural formation. These means that both diets induce normal digestion processes. Furthermore, the intestinal villus is directly correlated with increase nutrient intake (Kumari et al., 2013) and is affected by diet or the ingredients in the diet (He et al., 2020; Zhang et al., 2020). In this study, villus height was significantly longer in EP-fed fish which correlates to better nutrient absorption. The goblet cell in the intestine and pyloric caeca function of protecting the mucosal epithelium by secreting mucus and facilitating the transit of excreta into the digestive system. Additionally, it is crucial for the lubrication of enzyme cofactors, protein polymers, water-soluble nutrients, absorption, and transport (Hur et al., 2016). The increase number of goblet cells in the intestine and pyloric caeca in EP-fed olive flounder suggests a healthier mucosal surface and higher absorption of digestible substances.

Conclusion

The EP-fed olive flounder had a comparable growth and better feed utilization whilst maintaining a better health condition against the MP-fed fish. EP diet can induce higher transcriptomic regulation of IGF-1 and improve the digestive function. Thus, utilization of the commercially available extruded pellet in olive flounder aquaculture is an appropriate feed based on its dietary influence on the somatic growth and some aspects of the digestive physiology of the fish. In a nutshell, we conclude that EP-diet can be utilized as an environment-friendly feed option for olive flounder without negatively affecting the fish growth and basic physiological functions.

Ethical Statement

The guidelines of the Korean Association for Laboratory Animals were followed during the conduct of the current study (approval no. 18-0680).

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Author Contribution

CYJ: methodology, formal analysis and investigation, writing – original draft preparation; BSS: methodology, formal analysis and investigation; JC: formal analysis and investigation, Writing - review and editing; JHL: methodology, formal analysis and investigation; SJP: methodology, formal analysis and investigation; HH: formal analysis and investigation; JSM: formal analysis and Writing - review and editing; SHL: methodology, conceptualization; SWH: methodology, conceptualization; KJL: methodology, data analysis; JWS: methodology; JSK: methodology; funding acquisition; YHC: conceptualization, supervision, writing - review and editing. All authors contributed to the article and approved the submitted final version.

Conflict of Interest

The authors declare that they have no competing interests with regards to the current work.

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