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Hydrolysates of whole forage-fish and Pacific krill are useful to reduce fish meal in practical diets for largemouth bass (*Micropterus salmoides*), and dietary fish hydrolysate suppresses expressions of intestinal oligopeptide transporter and taurine transporter genes

Yuexing Zhang [a], Yawei Zhang [a], Tianhong Ke [a], Bo Shi [a], Liying Huang [b,*], Zhiyong Dong [a,c], Mingyu Guo [a], Changarawe Samwel Mugeni [a], Aiyi Zhu [a], Linghua Wang [a,d], Jianbin Xu [d], Trond Storebakken [c]

[a] *Zhejiang Ocean University, Marine Science and Technology College, National Engineering Research Center for Marine Aquaculture, Zhoushan 316022, China*
[b] *Zhejiang Marine Fisheries Research Institute, Zhoushan, Zhejiang 316021, China*
[c] *Norwegian University of Life Science, Faculty of Bioscience, Department of Animal and Aquaculture Science, As NO-143, Norway*
[d] *Zhejiang Yifeng Marine Biological Products Co., Ltd., Yuhuan, Zhejiang 317600, China*

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ABSTRACT

Somatic and expressional effects of replacing dietary fishmeal (FM) with a 1:1:2 combination of soy protein concentrate, corn gluten meal, and hydrolysate of forage-fish (HWF) or Pacific krill (HPK) were investigated in juvenile largemouth bass for 66 days. The control diet (FMC) contained 320 g $kg^{-1}$of FM. Six extruded diets were produced with 3 rates of replacement at 25 %, 50 % and 75 % protein for each of the two hydrolysate combinations. Feed intake (FI) and weight gain (WGR) did not differ between fish fed HWF diets and FMC. Fish fed HPK50 and HPK75 had lower FI and WGR. FI linearly decreased with increasing HPK, while feed conversion ratio (FCR) increased. No significance was found in the FCR among groups fed FMC, HWF25, HWF50, and HPK25, whereas HWF75, HPK50, and HPK75 groups had significantly higher FCR. The apparent digestibility (AD) of crude protein (CP) increased with increasing HWF while HPK didn't cause significant change. AD of lipid and energy were not affected by diet, and AD of most amino acids increased proportionally with HWF. No proximate composition or retention of crude or digestible protein or energy other than whole-body ash revealed HWF-related differences. Whole-body CP, lipid, ash contents and CP retentions showed significant dose response in HPK fed fish. The condition factor decreased linearly with increasing HPK. Fish fed HPK50 diet upregulated peptide transporter 1 (*pept1*) expression in the foregut. Expressions of both taurine transporter (*taut*) and *pept1* were upregulated by increasing replacement from 0 % to 25 % HWF. Further increase in HWF replacement caused linear down regulation in expression of both transporters. In conclusion, HWF and HPK facilitated reduced use of fishmeal in practical diet for largemouth bass. Expressions of *pept1* and *taut* were dose-dependent in fish fed HWF. HPK just caused single diet upregulation.

1. Introduction

Fish meals (FM) are valuable feed ingredients for carnivorous farmed fish, both as attractant and a source of essential nutrients. The global production of FM has been relatively static, around five million tons, for a period of at least the last 20 years. In 2018 around 14 million tons of capture fish were used as raw material for producing marine feed ingredients (FAO 2020). No major increase in production of FM from whole-forage fish is expected. Instead, the contribution of by-products coming from wild caught fish or aquaculture processing are increasing and accounts for 27 % in 2018 based on the calculations of IFFO (https://www.iffo.com/product). Otherwise, products from pelagic crustaceans like Antarctic krill (*Euphausia superba*) (Storebakken, 1988) and Pacific krill (*E. pacifica*) are increasingly used especially in diets for juvenile high-value farmed fish species. As a tropical or subtropical marine crustacea, the Pacific krill is mainly available as by-catch of

* Corresponding author.
E-mail address: hlypcd@163.com (L. Huang).

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marine fisheries in China. Because of its limited annual availability with big fluctuations, it was historically discarded as a waste or being mixed in during FM processing. Being inspired by the advantages of using Antarctic krill products in aquafeed (Kaur et al., 2022; Kolkovski et al., 2000; Zhang et al., 2012a), the Pacific krill is currently gaining increased attention. This has in turn sparked the development of improved methods for catching and production of value-added products from Pacific krill, like low temperature-dried meals and krill hydrolysates.

Enzymatic hydrolysis of by-products, forage fish, and krill represents a valuable supplement to conventional FM production. Commonly used methods for production of enzymatic hydrolysates were recently reviewed by Siddik et al. (2021). Optimized hydrolysis liberates bioactive substances from fish protein, including peptides of varied molecular weights, free amino acids, and taurine (Tau). A number of liberated small and medium sized peptides may have anti hypersensitivity, antioxidative, antimicrobial, anti-inflammatory, and palatability enhancing properties (Siddik et al., 2021). The palatability enhancing ability of dietary hydrolysates is especially interesting because they facilitate replacement of high proportion of FM with plant protein concentrates, both in cold-water (Zhang et al., 2012a) and warmwater fish (Zhang et al., 2022).

Largemouth bass (LMB) (*Micropterus salmoides*) is the top species that contributed to the fastest increase in production of freshwater fish in China during the past three years. In 2020, the yield of LMB was up to 619,519 tons, a 29.7 % annual increase (China Fishery Statistical Yearbook, 2021). Rapid growth rate, strong disease-resistance, wide temperature adaptability (Hussein et al., 2020) and consumer preference in domestic market are key factors for this boost in production. Current commercial feed for LMB normally contains 45–50 % crude protein (CP) in dry matter (DM), with 30–40 % inclusion of animal proteins and FM. It is desirable to secure growth of LMB farming by reducing the high inclusion of FM in feed in order to reduce production cost, and the dependence of this limited feed ingredients.

Oligopeptide transporter 1 (PepT1) is a membrane bound transporter. It is mainly expressed in the brush border membrane of the proximal intestine (Ostaszewska et al., 2010) and takes a key role of assisting transmembrane transport of di- and tri- peptides (Spanier and Rohm, 2018; Wang et al., 2017), the main products of in vivo dietary protein digestion. Tau is conditionally essential for predatory fish (Salze and Davis, 2015; Sampath et al., 2020), and almost absent in plant protein ingredients. Thus, dietary supplementation of Tau must be carefully considered when high levels of plant materials were used to replace FM. The Tau transporter (Taut) is another membranous transporter that regulates transmembrane transport of Tau (Takeuchi et al., 2000; Tappaz, 2004). *Taut* transcription mainly occurs in the intestine and liver of fish, like turbot (*Psetta maxima*) and rainbow trout (*Oncorhynchus mykiss*) (Wang et al., 2016). The authors also reported that in vitro transcription of *taut* in turbot muscle was dose dependently down regulated in response to increasing concentration of Tau. Diet and osmotic pressure are key factors regulating the expression of *taut* (Zarate and Bradley, 2007) whereas diet and fasting are important regulators of *pept1* expression (Terova et al., 2009).

The first aim of the current experiment was to test the feasibility of replacing high-quality fishmeal with combinations of plant proteins and hydrolysates of whole forage-fish (HWF) or Pacific krill (HPK) on growth performance, nutrient digestibility and body composition of LMB. The second aim was to find out how dietary inclusion of combination with plant protein concentrates and either of the two hydrolysates affected the expression of oligopeptide transporter and Tau transporter genes, *pept1* and *taut*, in the intestine of LMB.

2. Materials and methods

2.1. Main ingredients

HWF and HPK were produced by Zhejiang Yifeng Marine Biological Products Co., Ltd. (Yuhuan, Zhejiang, China). Briefly, the processing procedures were as follows: Fresh whole forage-fish or Pacific krill were finely homogenized with addition of water. The homogenates were then intimately mixed with a commercial enzyme kit, mainly containing alcalase and neutrase from bacterial sources. Hydrolysis was carried out at pH ranging from 7.5 to 8.0 and temperature of 45–50 °C for about 2 h in a digestion vessel, with constant stirring. The sludges then were concentrated with evaporators under sub-atmospheric pressure to reduce moisture to less than 50 %. The concentrates were then rapidly heated to 105 °C and maintained for another 20 min for sterilization and prevention of further enzymatic hydrolysis, before packaging. The molecular weight distributions of the two hydrolysates are presented in Table 1. The HWF had high proportion of peptides with medium molecular weight, and low-weight peptides dominated in HPK. The chemical compositions of main protein-rich ingredients, including the two hydrolysates, FM, soy protein concentrate (SPC) and corn gluten meal (CGM) are presented in Table 2.

2.2. Experimental diets

Seven extruded, isonitrogenous, isoenergetic diets with approximately 50 % CP and 22 kJ g^{-1} gross energy (GE) were formulated as described in Table 3, in accordance with nutrient requirements of juvenile LMB (NRC, 2011). One diet was fish meal control (FMC) included a level of 320 g high-quality FM kg^{-1}, which is a commonly adapted level in practical feed for juvenile LMB in China currently. The other six experimental diets were produced with a 2:1:1 (DM) combination of HWF or HPK, SPC and CGM replacing 25, 50 or 75 % of FM. A 2 * 3 factorial experimental design was adopted with 2 hydrolysates and 3 replacement levels. Each diet was identified by source of hydrolysate (HWF or HPK) and replacement level (25, 50 or 75 %), and coded as HWF25/HPK25, HWF50/HPK50, HWF75/HPK75, respectively. L-Lys and DL-Met were supplemented to balance the dietary amino acid profile. The analyzed amino acid compositions of experimental diets were shown in Table 4. Yttrium oxide was used as inert marker for digestibility measurement (Austreng et al., 2000).

The diets were extruded at the Feed Technology Laboratory of the Sino-European Aquatic Nutrition and Feed Resource Institute, Zhejiang Ocean University (SEANUTR-ZJOU). All ingredients were ground through 180-μm mesh. Liquid raw materials were diluted with water and added by high pressure spraying. The moistened mash was heated up to 95–100 °C in 5 min by microwave preconditioning before extruding by a laboratory scale twin-screw extruder (Saibainuo, SYSLG30-IV, Jinan, China) with 3.0 mm die. The pellets were quickly dried to a moisture content of 7–9 %, followed by oil coating in a ZJB-40 vacuum coater (Zhucheng Xindeli Food Machinery Co., Ltd., Shandong,

Table 1
Molecular weight distribution of two hydrolysates obtained by enzymatic hydrolysis of whole forage-fish (HWF) and Pacific krill (HPK).

Range of molecular weights (Dalton), % of dry matter	Whole forage-fish hydrolysate (HWF)[a]	Pacific krill hydrolysate (HPK)[b]
<180	57.5	23.3
500–180	30.5	33.8
1000–500	8.4	20.4
2000–1000	2.7	13.1
3000–2000	0.5	3.9
5000–3000	0.2	2.8
10,000–5000	0.1	1.4
>10,000	0.1	1.5

[a] HWF, hydrolysate of whole forage-fish (approximately contributed by 50 % *Engraulis japonicus*, 30 % *Benthosema pterotum*, 15 % *Champsodon snyderi*, 4 % *Acropoma japonicum* and 1 % *Euphausia pacifica* in biomass).

[b] HPK, hydrolysate of Pacific krill (*Euphausia pacifica*); Both HWF and HPK were provided by Zhejiang Yifeng Marine Biological Products Co., Ltd. Yuhuan, Zhejiang, China

Table 2
Proximate and amino acid compositions of the protein-rich ingredients used in experimental diets (on dry matter basis).

Ingredients	FM[a]	HWF	HPK	SPC[b]	CGM[c]
Compositions, g (kg diet)$^{-1}$					
Dry matter	902	497	551	925	948
Crude protein	755	686	668	714	650
Lipid	88.5	63.3	42.3	0.50	12.2
Ash	163	224	236	56.7	9.04
Essential and semi-essential amino acids (EAA), g (100 g protein)$^{-1}$					
Arg	4.18	2.36	2.23	5.38	1.82
Cys	0.97	0.72	0.62	0.49	2.03
His	2.31	1.61	0.87	1.84	1.13
Ile	2.96	1.99	2.18	3.35	2.16
Leu	5.27	3.86	3.60	5.78	10.5
Lys	5.84	4.59	4.27	4.69	0.95
Met	2.04	1.36	1.68	0.70	1.50
Phe	2.90	1.72	2.01	3.74	3.91
Thr	3.19	2.13	2.12	2.93	2.13
Tyr	2.63	1.65	2.00	2.42	2.56
Val	3.53	2.55	2.55	3.43	1.82
Sum of EAA	35.8	24.6	24.1	34.8	28.7

[a] FM, Peruvian steam-dried whole fish meal produced from anchoveta (Engraulis ringens).
[b] SPC, soy protein concentrate, provided by Goldensea Grain and Oil Industry Co., Ltd, Wilmar, Qinhuangdao, China.
[c] CGM, corn gluten meal, provided by Lihua Starch Co., Ltd, Wilmar, Qinhuangdao, China.

China). The feeds were then sieved, damaged pellets were removed, and stored at −10 °C until fed to the fish.

Diet pellet physical qualities including pellet length and diameter, weight, durability, and hardness are showed in Table 5. Durability was measured prior to oil coating. Fifty coated pellets per diet were randomly selected, cross-sectional diameter and longitudinal section length were measured with an electronic vernier caliper of 0.01 mm accuracy. Another 3 * 1000 oil coated pellets were randomly sampled and batch-weighed to determine the average pellet weight. A ST-120B automatic feed hardness tester (Jinan Shengtai Instrument Co., Ltd., Jinan city, Shandong, China) was used to determine the hardness of feed pellet with 50 pellets per diet. The pellet durability index was measured by ST-136 feed durability tester (Jinan Shengtai Instrument Co., Ltd., Jinan, Shandong, China).

2.3. Feeding trial and experimental conditions

A batch of 2500 LMB fingerlings (~ 5.0 g fish^{-1}) were obtained from local hatchery (Hongli Aquaculture Co., Huzhou, Zhejiang), and acclimated with commercial feed in an indoor recirculated aquaculture system (RAS) for 6 weeks in the Fish Laboratory of SEANUTR-ZJOU. Before onset of the feeding trial, fish were starved for 48 h. A total of 1470 size graded LMB (21.8 ± 0.09 g) were randomly distributed into 21 1000-l cylindrical fiberglass tanks. Each diet was randomly assigned to triplicate tanks with 70 fish per tank. The fish were hand-fed 3 meals per day at 8:00, 14:00 and 20:00. After 40 min of each feeding, all uneaten pellets were immediately collected by siphoning, counted and quantified in weight as described by Zhang et al. (2012b). The daily feeding amount was set to tentatively 10 % in excess based on the average feed intake (FI) of the past 3 days. If the fish showed any signs of feeding at the end of each meal, more feed was fed until the fish were satiated. Each tank was supplied with recycled freshwater at a flow rate of 5–6 l min^{-1}. The rearing water was aerated 24 h day^{-1} and a photoperiod of 14 L:10D was adopted. The water temperature ranged from 21.4 to 24.8 °C, dissolved oxygen was above 6.0 mg l^{-1}, pH ranged 7.0–7.5, ammonia nitrogen and nitrite were below 0.2 and 0.1 mg l^{-1}, respectively, based on daily measurements. The feeding trial lasted for 66 days, and was followed by a 30-day digestibility trial with the same fish.

2.4. Sampling and analyses

Before the feeding trial, 3 × 10 fish starved for 48 h were euthanized by an overdose of MS-222, and kept as initial samples at −20 °C prior to

Table 3
Formulation and proximate compositions of experimental diets (on dry matter basis).

Ingredients g kg^{-1}	Diets						
	FMC	HWF25	HWF50	HWF75	HPK25	HPK50	HPK75
HWF	0	44.0	88.0	132.0	0	0	0
HPK	0	0	0	0	45.0	90.0	135.0
FM	320.0	240.0	160.0	80.0	240.0	160.0	80.0
SPC	60.0	82.0	104.0	126.0	82.5	105.0	127.5
CGM	60.0	82.0	104.0	126.0	82.5	105.0	127.5
Canola meal	57.0	41.6	26.4	11.1	38.7	20.3	2.2
Fish oil	54.6	56.8	59.0	61.2	57.3	60.0	62.6
Soybean oil	54.6	56.8	59.0	61.2	57.3	60.0	62.6
L-Lysine	2.00	4.15	6.2	8.30	4.30	6.60	8.85
DL-Methionine	0.2	1.0	1.8	2.55	0.85	1.51	2.15
Constant ingredients[1, 2, 3, 4]	391.65	391.65	391.65	391.65	391.65	391.65	391.65
Analyzed content, kg^{-1}							
Dry matter, g	931	922	911	920	921	920	916
Crude protein, g	508	504	504	498	496	495	490
Lipid, g	150	154	152	154	157	147	154
Ash, g	88.2	84.7	81.2	75.4	83.4	80.4	76.4
Gross energy, MJ	21.9	22.1	22.1	22.1	22.1	22.1	22.2
Acid-soluble protein, g	55.8	69.5	83.5	100.3	68.8	85.3	102.3
Total free amino acids, g	7.81	12.06	17.03	22.88	13.11	20.79	26.51
Peptides, g	47.9	57.5	66.4	77.4	55.7	64.5	75.8
Taurine, g	3.75	4.41	5.50	6.37	4.93	6.05	7.05

[1]Constant ingredients, g (kg diet dry matter)$^{-1}$: Soybean meal, 160; Wheat gluten, 60; Wheat flour, 100; Tapioca flour, 40; Vitamin and mineral premix, 10; Soy lecithin, 10; Calcium phosphate ($Ca(H_2PO_4)_2$), 10; Mold inhibitor, 0.5; Antioxidants, 0.35; Choline chloride, 0.3; Yttrium oxide (Y_2O_3), 0.5. [2]Composition of vitamin and mineral premix (kg diet)$^{-1}$, Vitamins: A, 20 000 IU; D_3, 1000 IU; E, 200 IU; K_3, 100 mg; B_1, 34.66 mg; B_2, 37.34 mg; B_6, 28.58 mg; B_{12}, 0.40 mg; D-biotin, 1.14 mg; pantothenic acid, 124 mg; folic acid, 20 mg; nicotinamide, 206 mg; C, 200 mg. Minerals: $CuSO_4$ $5H_2O$, 10 mg; $FeSO_4$ H_2O, 300 mg; $ZnSO_4$ H_2O, 200 mg; $MnSO_4$ H_2O, 100 mg; KI (10 %), 80 mg; Na_2SeO_3, (10 % Se) 67 mg; $CoCl_2$ 6 H_2O (10 % Co), 5 mg; NaCl, 100 mg. The premix was provided by DSM Animal Nutrition & Health Greater China (Shanghai, China). [3]Soy lecithin, Youlin®, powder (> 95 %), Beijing Meiyas Phospholipid Technology Co., Ltd, Beijing, China. [4]Choline Chloride 50 % with silica Carrier, Shandong Aocter Group, Shandong, China.

Table 4
Analyzed amino acid compositions of experimental diets.[1]

Diets	FMC	HWF25	HWF50	HWF75	HPK25	HPK50	HPK75
Essential and semi essential amino acids (EAA), g (100 g protein)$^{-1}$							
Arg	5.39	5.16	5.07	4.88	5.22	4.94	4.92
Cys	1.07	1.07	1.11	1.18	1.11	1.20	0.84
His	2.58	2.45	2.42	2.37	2.46	2.31	2.17
Ile	4.04	3.98	4.02	4.01	4.06	3.97	3.97
Leu	8.00	8.24	8.63	8.97	8.31	8.46	8.88
Lys	6.05	5.94	5.97	6.22	6.10	6.12	6.14
Met	1.71	1.69	1.93	1.92	1.82	1.76	1.74
Phe	4.61	4.67	4.81	4.87	4.74	4.94	4.89
Thr	3.79	3.68	3.65	3.55	3.73	3.57	3.51
Tyr	3.25	3.26	3.36	3.39	3.33	3.33	3.57
Val	4.52	4.43	4.46	4.44	4.56	4.38	4.36
Sum of EAA	45.0	44.6	45.4	45.8	45.4	45.0	45.0
Nonessential amino acids (NEAA), g (100 g protein)$^{-1}$							
Ala	5.30	5.35	5.48	5.57	5.37	5.31	5.42
Asp	8.21	8.15	8.22	8.16	8.22	8.01	8.11
Glu	20.0	20.5	21.4	22.0	20.6	20.9	21.8
Gly	4.63	4.46	4.42	4.28	4.52	4.31	4.22
Pro	5.80	6.04	6.21	6.52	6.06	6.42	6.61
Ser	4.33	4.34	4.44	4.49	4.38	4.33	4.38
Sum of amino acids	93.3	93.4	95.6	96.8	94.6	94.2	95.5

[1] For diet codes see Table 3.

Table 5
Pellet physical qualities of experimental diets.

Physical quality	FMC	HWF25	HWF50	HWF75	HPK25	HPK50	HPK75
Length, mm	5.70	5.63	5.94	6.35	5.60	5.65	5.65
Diameter, mm	5.55	4.97	6.32	6.47	5.27	4.88	5.79
Pellet weight, g (1000 pellets)$^{-1}$	111	90.3	115	117	104	101	119
PDI[1],%	98.6	99.1	99.5	99.4	99.8	99.7	99.7
Hardness of pellets, N	40	46	72	88	60	82	119

For diet codes see Table 3.

[1]Pellet durability index, PDI (%) $= \frac{\text{weight of pellets after sifting (g)}}{\text{weight of pellets before sifting (g)}} \times 100$

whole-body analysis. At the end of the feeding trial, all fish in each tank were starved for 24 h, gently netted out, anaesthetized with MS-222 (90 mg l^{-1}), counted and weighed in batch after wipe-drying to quantify the growth and feed utilization. Seven fish were sampled and pooled for whole-body analysis and the other 10 fish were dissected and measured for morphologic indices individually. Three of the same 10 dissected fish were also sampled for molecular analysis. An approximately 5 mm intestinal-tissue sample was immediately taken from the uppermost central part of foregut and transferred to RNAlater (Omega Bio-Tek, Inc., USA) at 4 °C for 24 h and then transferred to −80 °C prior to RNA extraction.

The remaining live fish were kept in their respective tanks for digestibility assessment. The same feeding strategy and rearing conditions as in growth trial were adopted. The fish were anaesthetized by MS-222, and feces were obtained by stripping as described by Austreng (1978) on the day 7, 14, and 30 of the digestibility trial. The feces were pooled by tank, freeze dried, and stored at −20 °C prior to analysis. Analyses of molecular weight distribution of the two hydrolysates, proximate composition, amino acids and Y_2O_3 in feeds and feces, proximate analysis in fish, and water chemistry were conducted as described by Zhang et al. (2022). Tau concentrations in experimental diets were analyzed according to the method of GB/T 18246–2019 but with the amino acid analyzer (Hitachi, L-8900, Japan).

2.5. Quantitative real-time PCR

The total RNA from 3 pooled fish per tank was extracted by E.Z.N.A.® Total RNA Kit II (Omega Bio-Tek Inc., USA), and cDNA library was structured by Prime Script™ RT reagent kit (Takara, Japan). Real-time PCR (RT-PCR) was carried out in 7500 Real Time PCR System (ABI, USA) with a 12.5 μl reaction system, whereby the process was operated following the manual of TB Green® Premix Ex Taq™ kit (Takara, Japan). The primer pairs were designed by primer premier 5.0 software, and specific details as shown in Table 6. RT-PCR program was as follows: 95 ℃ for 30 s, followed by 40 cycles of 95 ℃ for 5 s, 60 ℃ for 30 s, and finally 95 ℃ for 10 s. The data was calculated by $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.6. Calculation and statistical analysis

Parameters were calculated as follows:

Feed intake (FI, g DM fish^{-1}) = total feed intake per tank (g DM) (number of fish in tank)$^{-1}$;

Weight gain rate (WGR, %) = 100 × (final body weight (FBW) (g) - initial body weight (IBW) (g)) × (initial body weight (IBW))$^{-1}$ (g);

Feed conversion ratio (FCR, g DM intake (g gain)$^{-1}$) = FI (g) × (FBW (g) - IBW (g))$^{-1}$;

Protein retention efficiency (PRE, %) = 100 × (FBW (g) × CP in final fish (%) - IBW (g) × CP in initial fish (%)) × (FI (g) × CP in feed (%))$^{-1}$, CP means crude protein;

Energy retention efficiency (ERE, %) = 100 × (FBW (g) × GE in final fish (KJ g^{-1}) - IBW (g) × GE in initial fish (KJ g^{-1})) × (FI (g) × GE in feed (KJ g^{-1})) $^{-1}$, GE means gross energy;

Condition factor (CF, g/cm^3) = 100 × body weight (g) × (body length^3 (cm))$^{-1}$;

Hepatosomatic index (HSI, %) = 100 × liver weight (g) × (body weight (g)) $^{-1}$;

Viscera-somatic index (VSI, %) = 100 × visceral mass weight (g)

Table 6
Primer for real-time quantitative PCR1.

Genes	Primer sequence (5′ - 3′)	Size (bp)	TM (°C)	Accession no.
pept1	F: CCTATTTGCCTCGCTTTTGGTTGC	88	57	MZ773078
	R: CATTAACCTTCGCCGTGAATTGGG		57	
taut	F: TGTATCCGTCCGTTCTCAGGAAGG	113	59	MZ773077
	R: ATGGTGAGTCCCAGGAGATAGCTC		59	
β-actin	F: ATCGCCGCACTGGTTGTTGAC	336	56	XM_038695351.1
	R: CCTGTTGGCTTTGGGGTTC		53	

[1]*pept1*, oligopeptide transporter gene; *taut*, taurine transporter gene. The nucleotide sequence of *pept1* and *taut* was cloned and deposited to the NCBI (PRJNA658481).

× (body weight (g)) $^{-1}$;

Carcass-somatic index (CSI, %) = 100 × carcass weight (g) × (body weight (g)) $^{-1}$;

Apparent Digestibility of A* (AD, %) = 100 × (1-(Faecal A × Dietary Y_2O_3) × (Dietary A × Faecal Y_2O_3) $^{-1}$). A* indicates type of nutrient including cruder protein, total fat, gross energy or amino acids.

Details on the statistical analysis were similar to those reported by Zhang et al. (2022). Briefly, the results were analyzed by two-way ANOVA and regression, and differences were considered significant at $P < 0.05$. Linear or quadratic regressions and ANOVA were conducted. Quadratic regressions were only presented when the regression coefficient of the 2nd degree component was statistically significant. The statistical analyses were conducted using the SAS version 9.4 computer software (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Growth performance, feed utilization, and morphological indices

No mortality occurred, all the LMB took the feeds actively and grew well. The dietary inclusion of HWF combination had no significant effect both on FI and growth performance (FBW and WGR) (Table 7). However, higher inclusion of HWF combination (HWF75) had significantly increased FCR. Only lower inclusion of HPK combination (HPK25) in diet had no significant effect both on FI and growth. LMB fed diets with moderate and higher HPK inclusions had significantly lower FI and growth, and higher FCR than those fed FMC or HPK25 diets. Factorial analysis (Table 8) showed that diets containing HWF combination resulted in significantly lower FCR than those containing HPK combination. A replacement of 25 % resulted in significantly higher FI, FBW, and WGR than diets with 50 % and 75 % inclusions.

None of the morphologic indices of LMB fed diets with HWF combination significantly differed from that of LMB fed the FMC diet. Furthermore, no significant differences were found among LMB fed diets with HWF combination (Table 9). Similar results were also observed by morphometry in LMB fed diets with HPK, except an alleviation in CF in LMB fed FMC diet. The factorial analysis (Table 10) showed that LMB fed diets with HWF combination had significantly higher CSI and CF than the ones fed HPK diets. LMB fed diets with 25 % inclusion of combinations with hydrolysate had significantly higher CF than the ones fed diets with 50 % and 75 % inclusions.

Table 8
Factorial analysis of growth performance, feed intake and utilization of juvenile largemouth bass fed diets with HWF or HPK combinations.

Factors	FBW, g	FI, g DM fish^{-1}	WGR, %	FCR, g DM intake (g gain)$^{-1}$
Source of protein hydrolysates				
Forage fish (HWF)	94.7	62.7	335	0.86[b]
Pacific krill (HPK)	96.2	65.2	342	0.88[a]
Inclusion levels				
25 %	101[a]	68.0[a]	362[a]	0.86
50 %	93.4[b]	62.1[b]	329[b]	0.87
75 %	92.4[b]	61.7[b]	324[b]	0.87
Pooled S.E.M	2.98	2.81	14.4	0.01
Factorial ANOVA ($P > F$)				
Source of protein hydrolysates	0.45	0.21	0.48	<0.001
Inclusion levels	0.01	0.03	0.02	0.11
Interaction	0.03	0.04	0.04	<0.01

Values were presented as means and pooled S.E.M (n = 3), different superscript letters [a] and [b] indicate significant differences among treatments ($P < 0.05$). FBW, final body weight; WGR, weight gain rate; FI, feed intake; FCR, feed conversion ratio.

3.2. Apparent digestibilities of CP, lipid, gross energy and amino acids

Dietary inclusion of HWF combination caused significant increase of CP digestibility (Table 11), with LMB fed the HWF50 and HWF75 diets having significantly higher AD of CP than those fed FMC diet. No significant differences were seen in the AD of lipid or energy among dietary treatments. Different levels of HPK combination had no significant effect

Table 7
Growth performance, feed intake and utilization of juvenile largemouth bass fed diets with HWF or HPK combinations.

Items	Diet				P-value	Pooled S.E.M[1]	Regression	R^2
	FMC	HWF25	HWF50	HWF75				
Initial body weight (IBW), g	21.8	21.8	21.8	21.8	0.99	0.07	/	/
Final body weight (FBW), g	103	95.5	93.9	94.6	0.13	5.17	/	/
Feed intake (FI), g DM fish^{-1}	69.3	63.2	61.2	63.7	0.18	4.81	/	/
Weight gain rate (WGR), %	372	339	331	335	0.14	24.4	/	/
Feed conversion ratio (FCR), g DM intake (g gain)$^{-1}$	0.856[b]	0.857[b]	0.849[b]	0.873[a]	0.02	0.01	$9.15 * 10^{-6} x^2 - 5.01 * 10^{-4} x + 0.858$	0.52
	FMC	HPK25	HPK50	HPK75				
IBW, g	21.8	21.8	21.8	21.8	0.93	0.11	/	/
FBW, g	103[a]	105.5[a]	92.9[b]	90.2[b]	<0.001	3.10	$- 0.201 x + 105$	0.69
FI, g DM fish^{-1}	69.3[a]	72.9[a]	62.9[b]	59.8[b]	<0.001	2.93	$- 0.154 x + 72.0$	0.60
WGR, %	372[a]	385[a]	327[b]	314[b]	<0.001	15.6	$- 0.925 x + 384$	0.67
FCR, g DM intake (g gain)$^{-1}$	0.856[b]	0.870[ab]	0.884[a]	0.874[a]	0.03	0.010	$- 9.88* 10^{-6} x^2 + 1.02 * 10^{-3} x + 0.854$	0.63

For diet codes see Table 3. Values were presented as means and pooled S.E.M (n = 3), different superscript letters [a] and [b] indicate significant differences among treatments ($P < 0.05$).
[1] Pooled standard error of means.

Table 9
Morphologic indices[1] of largemouth bass fed diet with HWF or HPK combinations.

Items	Diet				P-value	Pooled S.E.M.	Regression	R^2
	FMC	HWF25	HWF50	HWF75				
HSI, %	1.61	1.65	1.67	1.76	0.34	0.11	/	/
VSI, %	9.95	9.92	10.0	10.5	0.32	0.42	/	/
CSI, %	88.4	88.2	88.6	88.0	0.54	0.61	/	/
CF, g $(cm)^{-3}$	3.04	2.94	2.75	2.77	0.08	0.15	/	/
	FMC	HPK25	HPK50	HPK75				
HSI, %	1.61	1.81	1.79	1.72	0.22	0.13	/	/
VSI, %	9.95	10.1	10.4	10.4	0.15	0.26	/	/
CSI, %	88.4	87.9	87.9	87.8	0.37	0.50	/	/
CF, g $(cm)^{-3}$	3.04[a]	2.78[b]	2.67[b]	2.60[b]	0.02	0.16	$-5.69 * 10^{-3} x + 2.99$	0.63

For diet codes see Table 3. [1]Values were presented as means and pooled S.E.M (n = 3), different superscript letters [a] and [b] indicate significant differences among treatments ($P < 0.05$); HSI, hepato-somatic index; VSI, visceral-somatic index; CSI, carcass-somatic index; CF, condition factor.

Table 10
Factorial analysis of morphologic indices[1] of juvenile largemouth bass fed diets with HWF or HPK combinations.

Factors	HSI, %	VSI, %	CSI, %	CF, g $(cm)^{-3}$
Source of protein hydrolysates				
Forage fish (HWF)	1.69	10.1	88.3[a]	2.82[a]
Pacific krill (HPK)	1.77	10.3	87.8[b]	2.69[b]
Inclusion levels				
25 %	1.73	10.0	88.0	2.86[a]
50 %	1.73	10.2	88.3	2.71[b]
75 %	1.74	10.4	87.9	2.69[b]
Pooled S.E.M	0.06	0.14	0.17	0.04
Factorial ANOVA ($P > F$)				
Source of protein hydrolysates	0.17	0.32	0.02	< 0.01
Inclusion levels	0.98	0.14	0.23	0.01
Interaction	0.35	0.46	0.46	0.64

[1]Values were presented as means and pooled S.E.M (n = 3), different superscript letters [a] and [b] indicate significant differences among treatments ($P < 0.05$). HSI, hepato-somatic index; VSI, visceral-somatic index; CSI, carcass-somatic index; CF, condition factor.

on AD of CP, lipid or energy. Factorial analysis revealed that only AD of CP was higher in LMB fed HWF diets than that of LMB fed HPK diets. No significant difference on AD of lipid or energy were found in LMB fed diets with both hydrolysates (Table 12). Neither the AD of CP, lipid, nor energy was significantly affected by inclusion level of HWF or HPK combination in the diets.

Dietary inclusion of HWF combination significantly improved amino acid digestibility, that the AD of Arg, His, Ile, Leu, Lys, Phe, Tyr, Val, essential and semi essential amino acids in total (EAA), and total amino acids (TAA) were significantly higher in LMB fed diets with 50 % and 75 % inclusion of HWF combination than that in LMB fed FMC diet (Table 13). The AD of those amino acids increased linearly with gradual inclusion of HWF combination in diet. No significant differences in AD of amino acids were found in LMB fed HPK diets. Factorial analysis showed that HWF diets resulted in higher AD of Arg, His, Ile, Leu, Lys, Met, Phe, Tyr, Val, EAA, and TAA than HPK diets (Table 14). Moreover, diets with 75 % inclusion of combinations with hydrolysate resulted in significantly higher AD of Leu, Phe and Tyr than diets with 25 % inclusion.

3.3. Whole-body compositions and retentions of protein and energy

Whole-body ash was the only component that significantly affected by dietary inclusion of HWF combination (Table 15). The ash content was significantly lower in LMB fed HWF75 diet than that of LMB fed FMC diet and diets with lower inclusions of HWF combination. Retentions of both crude and digestible protein, and gross and digestible energy were not significantly affected by dietary inclusion of HWF combination either. The LMB fed HPK diets had a linearly negative dose response in whole-body ash content. Whole-body protein and lipid contents were slightly elevated in LMB fed HPK25 diet, while protein retentions were high in LMB fed HPK25 and HPK 75 diets. The factorial analysis revealed that only whole-body ash content was significantly differed among all three inclusion levels of the hydrolysate combinations (Table 16).

3.4. Gene expression

Regulation of expression of both taut (Fig. 1) and pept1 (Fig. 2) in the foregut of LMB fed HWF diets were varied in a similar pattern. Both expressions were significantly up-regulated in response to increasing dietary inclusion of HWF combination from 0 in FMC diet to 25 % replacement in HWF25 diet. Further increase of HWF combination from 25 % to 75 % caused a linear reduction in the gene expressions of both transporters. The regression of analyzed dietary Tau level on taut expression (Fig. 3A) was explained by a 2^{nd} degree regression with a maximum about 5 g Tau per kg feed. Simultaneously, the regression of dietary peptides level on pept1 expression (Fig. 3B) indicated a maximum about 60 g peptides per kg feed. The only significant response to dietary HPK was the up-regulated expression of pept1 in the LMB fed HPK50.

Table 11
Apparent digestibilities (AD) of crude protein, lipid, and gross energy of juvenile largemouth bass fed diets with HWF or HPK combinations.

Apparent digestibility, %	Diet				P-value	Pooled S.E.M.	Regression	R^2
	FMC	HWF25	HWF50	HWF75				
AD of crude protein	84.4[b]	86.0[ab]	87.1[a]	87.8[a]	0.03	1.26	0.0446x + 84.7	0.65
AD of lipid	92.5	93.9	93.2	93.4	1.14	0.76	/	/
AD of gross energy	79.4	81.1	82.1	82.2	1.12	1.59	/	/
	FMC	HPK25	HPK50	HPK75				
AD of crude protein	84.4	85.5	85.6	86.3	0.35	1.38	/	/
AD of lipid	92.5	94.1	92.9	93.5	0.37	1.31	/	/
AD of gross energy	79.4	81.6	80.1	80.2	0.34	1.61	/	/

For diet codes see Table 3. Values were presented as means and pooled S.E.M (n = 3), different superscript letters [a] and [b] indicate significant differences among treatments ($P < 0.05$).

Table 12
Factorial analysis of apparent digestibilities (AD) of crude protein, lipid, and gross energy of juvenile largemouth bass fed diets with HWF or HPK combinations.

Factors	AD of crude protein, %	AD of lipid, %	AD of gross energy, %
Source of protein hydrolysates			
Forage fish (HWF)	87.0[a]	93.5	81.8
Pacific krill (HPK)	85.8[b]	93.5	80.7
Inclusion levels			
25 %	85.8	94.0	81.4
50 %	86.3	93.1	81.1
75 %	87.0	93.5	81.2
Pooled S.E.M	0.67	0.76	0.84
Factorial ANOVA ($P > F$)			
Source of protein hydrolysates	0.02	0.97	0.07
Inclusion levels	0.11	0.37	0.95
Interaction	0.62	0.91	0.14

Values are presented as means and pooled S.E.M (n = 3), different superscript letters [a] and [b] indicate significant differences among treatments ($P < 0.05$)

4. Discussion

The current experiment revealed palatability enhancing effects of hydrolysates of whole-forage fish and Pacific krill in feed for LMB. The marine hydrolysates were included in extruded diets feed at low concentration, in combination with plant protein concentrates at high inclusion levels. This palatability enhancing and the corresponding growth stimulation effects depend on several factors such as raw material freshness, peptide source, concentration, size, and composition. Typically, moderate amounts of short chain peptides seem to be most efficient in stimulating FI of farmed carnivorous fish. In the current study, the feeding and growth performance of LMB fed HWF combination was not impaired. This is in keeping with our previous findings in juvenile hybrid grouper (*E. fuscoguttatus*♀ × *E. lanceolatus*♂) (Zhang et al., 2022), that diets with combination of SPC, CGM, and hydrolyzed stickwater from tuna processing by-product had improved fish growth and feed conversions. Li et al. (2021) also found that moderate amounts of dietary protein hydrolysate combined with plant protein can partially replace FM and effectively improve growth rates and feed conversion efficiency. The current results with LMB also support previous findings that a small amount of marine protein hydrolysate in low fishmeal aquafeed can help increased FI as fishmeal-rich feeds. Dietary marine hydrolysates also facilitate utilization efficiency of plant protein, more efficient feed conversion and improved growth performance of cultured carnivorous fish species (Khosravi et al., 2015a; Refstie et al., 2004).

Claimed advantages of marine crustacean hydrolysates rely on not only their high content of soluble proteins, peptides and free amino acids, but also their effectiveness as feeding attractants (Kousoulaki et al., 2018). For example, studies done on several different species of fish (Aksnes et al., 2006a, 2006b; Khosravi et al., 2015b; Swanepoel and Goosen, 2018; Xu et al., 2016) show that supplementing the feed with a small amount of krill hydrolysate can increase the quality of the feed. The most relevant features of krill hydrolysates are peptides (White et al., 2016) and free amino acids (Shamushaki et al., 2007), which both can stimulate the appetite and increase FI. Krill hydrolysate may also contain additional flavour components that can further improve the attractiveness (Choi et al., 2020; Derby et al., 2016). In the current study, the LMB fed HPK25, performed at the same level as the ones fed the FMC diet. Similar findings were reported by Dai et al. (2020) that supplementing shrimp hydrolysate to a basal diet significantly improved FI and protein digestibility of LMB. But these desired results may not materialize if the hydrolysate exceed proper amount. High dietary levels of Pacific krill hydrolysate caused reduced FI. This, in term resulted in low final body weight and increased feed conversion ratio. Kim et al. (2003) pointed out that hydrolysis of proteins may result in the formation and release of bitter peptides that can negatively affect FI. Another reason behind might also be that the contents of dietary plant proteins, especially SPC and CGM, increased proportionally with that of hydrolysates. These protein sources may have counteracted the desired effect

Table 13
Apparent digestibilities (AD) of essential and semi-essential (EAA), and total amino acids (TAA) of juvenile largemouth bass fed diets with HWF or HPK combinations.

AD of AA, %	Diet				P-value	Pooled	Regression	R^2
	FMC olol	HWF2555	HWF500	HWF75		S.E.M.		
Arg	92.1[b]	92.7[ab]	93.5[a]	93.7[a]	0.04	0.68	0.0221 x + 92.2	0.61
Cys	82.2	81.3	81.1	85.5	0.18	2.79	/	/
His	87.0[b]	87.5[ab]	89.2[a]	89.4[a]	0.04	1.17	0.0358 x + 86.9	0.56
Ile	86.8[b]	88.0[ab]	89.5[a]	89.6[a]	0.02	1.10	0.0393 x + 87.0	0.63
Leu	90.1[b]	91.3[ab]	92.5[a]	92.7[a]	0.01	0.87	0.0353 x + 90.3	0.68
Lys	85.8[b]	86.0[b]	88.3[a]	88.6[a]	0.03	1.32	0.0433 x + 85.6	0.58
Met	84.6	85.0	86.7	87.0	0.17	1.61	/	/
Phe	89.3[c]	90.7[b]	92.1[a]	92.5[a]	<0.01	0.84	0.0442 x + 89.5	0.78
Thr	84.8	84.9	85.8	86.0	0.52	1.40	/	/
Tyr	88.2[b]	89.7[ab]	91.2[a]	91.3[a]	< 0.01	0.95	0.0427 x + 88.5	0.70
Val	86.6[b]	87.6[ab]	89.2[a]	89.3[a]	0.03	1.16	0.0386 x + 86.7	0.60
EAA	87.9[b]	88.7[ab]	90.1[a]	90.4[a]	0.03	1.04	0.0359 x + 87.9	0.63
TAA	88.0[b]	88.8[ab]	90.0[a]	90.4[a]	0.04	1.09	0.0337 x + 88.1	0.58
	FMC	HPK25	HPK50	HPK75				
Arg	92.1	92.7	92.3	92.7	0.66	0.80	/	/
Cys	82.2	83.3	83.2	79.1	0.35	3.46	/	/
His	87.0	87.8	87.7	87.8	0.76	1.26	/	/
Ile	86.8	87.8	87.6	87.9	0.64	1.34	/	/
Leu	90.1	91.0	91.2	92.1	0.13	1.01	/	/
Lys	85.8	87.1	86.1	86.2	0.65	1.45	/	/
Met	84.6	84.9	82.3	80.7	0.07	2.10	/	/
Phe	89.3	90.4	90.9	91.5	0.07	0.99	/	/
Thr	84.8	85.5	83.7	84.1	0.48	1.65	/	/
Tyr	88.2	89.3	89.5	90.5	0.09	1.06	/	/
Val	86.6	87.6	87.2	87.8	0.66	1.37	/	/
EAA	87.9	88.8	88.4	88.9	0.64	1.17	/	/
TAA	88.0	89.0	88.6	89.2	0.54	1.17	/	/

For diet codes see Table 3. Values were presented as means and pooled S.E.M (n = 3), different superscript letters [a], [b], and [c] indicate significant differences among treatments ($P < 0.05$).

Table 14
Factorial analysis of apparent digestibilities of essential and semi essential (EAA) and total amino acids (TAA) of juvenile largemouth bass fed diets with HWF or HPK combinations.

Factors	Arg	Cys	His	Ile	Leu	Lys	Met	Phe	Thr	Tyr	Val	EAA	TAA
Source of protein hydrolysates													
Forage fish (HWF)	93.3[a]	82.6	88.7[a]	89.1[a]	92.2[a]	87.7[a]	86.2[a]	91.8[a]	85.6	90.7[a]	88.7[a]	89.7[a]	89.7[a]
Pacific krill (HPK)	92.6[b]	81.9	87.7[b]	87.8[b]	91.5[b]	86.5[b]	82.6[b]	90.9[b]	84.4	89.8[b]	87.5[b]	88.7[b]	88.9[b]
Inclusion levels													
25 %	92.7	82.3	87.6	87.9	91.2[b]	86.5	85.0	90.6[b]	85.2	89.5[b]	87.6	88.8	88.9
50 %	92.9	82.1	88.4	88.6	91.9[ab]	87.2	84.5	91.5[a]	84.7	90.4[ab]	88.2	89.3	89.3
75 %	93.2	82.3	88.6	88.8	92.4[a]	87.4	83.8	92.0[a]	85.1	90.9[a]	88.6	89.6	89.8
Pooled S.E.M	0.41	2.11	0.61	0.63	0.47	0.70	1.09	0.46	0.84	0.55	0.68	0.57	0.58
Factorial ANOVA ($P > F$)													
Source of protein hydrolysates	0.02	0.61	0.04	0.01	0.04	0.03	< 0.001	0.01	0.06	0.02	0.03	0.02	0.05
Inclusion levels	0.42	0.99	0.16	0.27	0.03	0.33	0.48	< 0.01	0.83	0.03	0.26	0.21	0.21
Interaction	0.19	0.048	0.16	0.23	0.46	0.02	0.01	0.47	0.13	0.34	0.22	0.15	0.20

Values were presented as means and pooled S.E.M (n = 3), different superscript letters [a] and [b] indicate significant differences among treatments ($P < 0.05$).

Table 15,
Whole-body proximate compositions and retentions of protein and energy of juvenile largemouth bass fed diets with HWF or HPK combinations.

Items, %	Diet				P-value	Pooled	Regression	R^2
	FMC	HWF25	HWF50	HWF75		S.E.M.		
Moisture	66.6	66.6	66.6	66.6	0.99	0.28	/	/
Crude protein	16.7	16.9	16.9	17.0	0.46	0.21	/	/
Lipid	12.7	12.7	12.8	13.3	0.07	0.33	/	/
Ash	3.36[a]	3.38[a]	3.33[a]	3.24[b]	< 0.01	0.04	$-4.46 * 10^{-5} x^2 + 1.64 * 10^{-3} x + 3.37$	0.76
PRE	38.8	39.6	40.2	39.5	0.27	0.87	/	/
PRE based on digestibility	46.0	46.1	46.1	45.0	0.67	1.39	/	/
ERE	49.7	48.9	49.9	49.2	0.72	1.36	/	/
ERE based on digestibility	62.6	60.3	60.8	59.9	0.54	2.73	/	/
	FMC	HPK25	HPK50	HPK75				
Moisture	66.6	66.3	66.8	66.8	0.08	0.26	/	/
Crude protein	16.7[b]	17.0[a]	16.7[b]	16.8[b]	0.01	0.11	/	
Lipid	12.7[b]	13.4[a]	12.8[b]	12.9[b]	0.01	0.24	/	
Ash	3.36[a]	3.35[a]	3.29[ab]	3.23[b]	0.01	0.05	$-1.86 * 10^{-3} x + 3.38$	0.67
PRE	38.8[b]	39.9[a]	38.6[b]	39.8[a]	0.02	0.53	/	
PRE based on digestibility	46.0	46.7	45.1	46.1	0.44	1.34	/	/
ERE	49.7	49.5	47.7	48.6	0.12	1.12	/	/
ERE based on digestibility	62.6	60.7	59.5	60.6	0.28	2.09	/	/

For diet codes see Table 3. Values were presented as means and pooled S.E.M (n = 3), different superscript letters [a] and [b] indicate significant differences among treatments ($P < 0.05$); PRE, protein retention efficiency; ERE, energy retention efficiency.

Table 16
Factorial analysis of whole-body proximate compositions and retentions of protein and energy of juvenile largemouth bass fed diets with HWF or HPK combinations.

Factors	Moisture, %	Crude protein, %	Lipid, %	Ash, %	PRE, %	PRE based on digestibility, %	ERE, %	ERE based on digestibility, %
Source of protein hydrolysates								
Forage fish (HWF)	66.6	16.9	13.0	3.32	39.8	45.7	49.3	60.3
Pacific krill (HPK)	66.6	16.9	13.1	3.29	39.4	46.0	48.6	60.3
Inclusion levels								
25 %	66.4	17.0	13.1	3.37[a]	39.8	46.4	49.2	60.5
50 %	66.7	16.8	12.8	3.31[b]	39.4	45.6	48.8	60.2
75 %	66.7	16.9	13.1	3.24[c]	39.7	45.6	48.9	60.2
Pooled S.E.M	0.19	0.11	0.20	0.03	0.45	0.74	0.73	1.21
Factorial ANOVA ($P > F$)								
Source of protein hydrolysates	0.86	0.29	0.43	0.22	0.29	0.64	0.16	0.94
Inclusion levels	0.21	0.36	0.15	<0.001	0.54	0.35	0.79	0.94
Interaction	0.20	0.21	0.02	0.81	0.04	0.22	0.10	0.59

Values were presented as means and pooled S.E.M (n = 3), different superscript letters [a], [b], and [c] indicate significant differences among treatments ($P < 0.05$). PRE, protein retention efficiency; ERE, energy retention efficiency.

of the hydrolysates. This is in keeping with the findings of Chen et al. (2019) and Zhang et al. (2019) that plant protein ingredients may weaken the feeding attractiveness and palatability. Otherwise, the relatively higher hardness of HPK50 and HPK75 may also had the negative impact on the intake. Effective utilization of feed requires both the optimal composition of feeds and optimal physical quality of the feed pellets. Previous finding in salmonids shown that the physical properties of feed may affect FI, possibly by affecting the fish gastric evacuation rate (Aas et al., 2020, 2017; Oehme et al., 2014). Both physical properties of individual feed ingredients and process parameters adopted during feed production influence the physical properties of the feed pellet, such as hardness, durability and water stability (Draganovic et al., 2011; Kraugerud and Svihus, 2011; Kraugerud et al., 2011; Samuelsen et al., 2013, 2014; Sørensen, 2012). Especially the cohesive

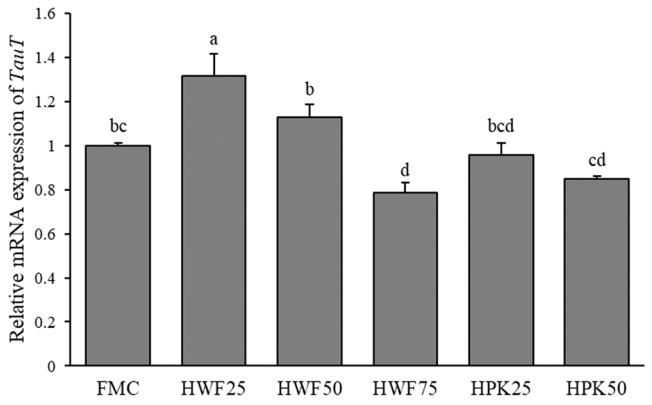

Fig. 1. Expression of taurine transporter gene (*taut*) in foregut of juvenile largemouth bass fed diets with gradual replacement of fish meal with 25, 50 or 75 % of a combination of soy protein concentrate (SPC), corn gluten meal (CGM), and hydrolysate of whole forage-fish (HWF), or 25 or 50 % combinations of SPC, CGM, and hydrolysate of Pacific krill (HPK). Expression values are normalized by *β-actin*-expressed transcripts. Columns represent means with bars indicating standard error of means (n = 3). Different superscript letters [a, b, c,] and [d] indicate significant differences among mean values. Values for HPK75 are not reported due to error in analysis.

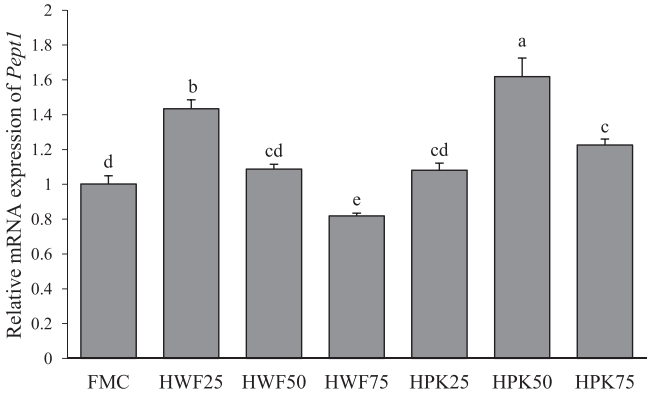

Fig. 2. Expression of the oligopeptide transporter gene (*pept1*) in foregut of juvenile largemouth bass fed diets with gradual replacement of fish meal with 25, 50 or 75 % of a combination of SPC, CGM, and HWF or HPK. Expression values are normalized by *β-actin*-expressed transcripts. Columns represent means with bars indicating standard error of means (n = 3). Different superscript letters [a, b, c, d,] and [e] indicate significant differences among mean values.

and visco-elastic properties of individual ingredients, may be important for the binding capacity but increased the hardness of the feed pellet as well (Storebakken et al., 2015; Zhang et al., 2012c). Oehme et al. (2014) observed that soaking feed in water increased FI and explained this finding as an outcome of decreased pellet hardness. The resulted decreased pellet hardness by soaking could be the causes of increased intake of soaked feed. Similarly, Aas et al. (2017) ascribed higher capacity to digest feed and faster gastrointestinal rate of passage in fish that have been fed pellets softened by soaking compared to fish fed dry extruded pellets.

Generally, protein and amino acids from hydrolysate are more completely digested than fed intact proteins (Webb, 1990). This was also found in the current experiment, where the digestibility of CP and most of the essential amino acids increased linearly in response to the level of HWF. The hydrolysis of the whole forage fish and Pacific krill also resulted in increased concentration of soluble protein, peptides, and free amino acids, agreeing with the findings of Petrova et al. (2021). Di- and tri-peptides and free amino acids are main absorption forms of protein in the intestine. Yuan et al. (2019) indicated that a moderate level of dietary protein hydrolysate could improve amino acid metabolism, while Srichanun et al. (2014) suggested that adding protein hydrolysate can stimulate protease activity and increase digestibility of protein. However, increased water solubility of dietary amino acids in peptides and free amino acids may also have resulted in over-estimation of digestibility due to higher leakage from the feed into the water (Harnedy and FitzGerald, 2012; Rocha Camargo et al., 2021). Experience with extruded diets, however, indicates that the overall rate of nutrient leaking is low during that short time elapsed from feeding until uneaten feed is flushed out of the rearing tank (Shomorin et al., 2019). On the other hand, the observed increase in AD of CP and amino acids did not result in improved growth performance. Thus, it may be that improved dietary amino acid uptake increased catabolism rather than protein accretion in the LMB. The completely different performance in digestibility of protein and essential amino acids observed in LMB fed diets with HWF and HKP, may partly explained by the different nutritive properties of these two hydrolysates. Such as the presence of chitin in HPK, which may hinder the digestion and slow down the subsequent absorption of nutrients. The anti-nutritional effect of chitin may reduce the intestinal absorption efficiency of small peptides and free amino acids. Research conducted by Köprücü and Özdemir (2005) confirmed that chitin could inhibit the digestion and absorption of nutrients in tilapia. Leduc et al. (2018) identified a bioactive peptide, KNPEQ in shrimp protein hydrolysates, which stimulated intestinal movement and accelerated the transport of feed in the intestine of European sea bass. This may also be the reason that led to protein and essential amino acids from HWF to be more efficiently digested than HPK and FMC, hence

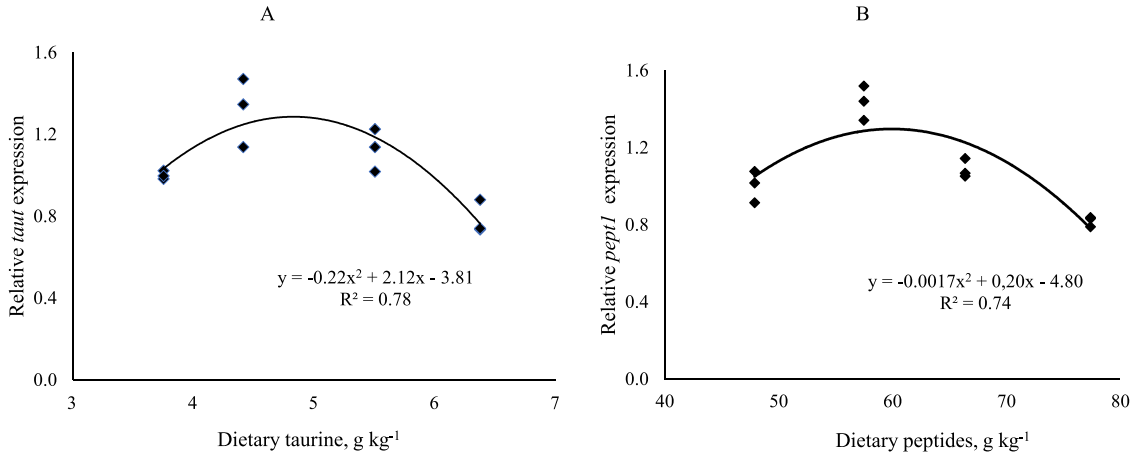

Fig. 3. Expression of taurine transporter gene (*taut*) (A) and oligopeptide transporter 1gene (*pept1*) (B) in foregut of largemouth bass in response to the increasing dietary taurine and peptides concentrations, respectively, when fed FMC and HWF diets.

promoted efficient feed utilization.

Diets containing HWF and HPK were expressed fundamentally different in expressions of the two intestinal key transporter genes, *taut* and *pept1*, in LMB. Proximate composition and gross energy of the feeds were almost identical and should not have major differences in their effects on *taut* and *pept1* expression (Liu et al., 2014). However, some other factors may have caused the differences in expressions of *taut* and *pept1*. Such as the extremely different peptide profiles apart from proximate composition of these two hydrolysates, that HPK contained less peptides sized under 0.18 kD, majority of peptides sized in the range of 0.5–20 kD; The different amino acid composition, that HPK had slightly more free amino acids, less acid soluble protein, and more Tau than HWF; The difference in FI and feed physical properties, that LMB fed diets with HWF had lower FI than that fed HWF diets and HPK diets were harder than the HWF diets at each inclusion level. It was not our intension to identify the specific components in the HWF and HPK that caused these differences. Components of HPK not analyzed in this experiment, such as chitin (Yu et al., 2020) and fluoride (Landymore et al., 2019), may also have contributed to the differences in response to diets with whole-fish and krill hydrolysate. In the current study, an up-regulation of expression both taut and pept1 when dietary HWF was low was observed. However, a dose dependent down regulation was found in response to abundance of dietary HWF. This is in keeping with previous findings (Cai et al., 2015; Ostaszewska et al., 2010; Wei et al., 2021). The regression analyses of dietary Tau on expression of *taut* and dietary peptide on expression for *pept1* confirm that these two important intestinal transporters were regulated in response to dietary dose. This was in contrast to the response to dietary HPK, where significant regulation of mRNA for *pept1* was seen for one dietary treatment only.

5. Conclusion

The current study demonstrated the potentials of both marine hydrolysates from whole forage-fish and Pacific krill as high-quality protein sources for LMB practical feed. The HWF performed better both in feed conversion and CP digestibility than HPK. The combination of HWF with SPC and CGM by the mixing ratio of 2:1:1 could effectively decrease the inclusion of high-quality FM from 320 g kg^{-1} to 80 g kg^{-1} in extruded practical feed for juvenile LMB without impairing FI, growth, feed utilization, whole-body compositions excluding ash, retentions of protein and energy, nutrient digestibility. The HPK combination could successfully decrease the FM level to 240 g kg^{-1} under similar conditions. Both type and dosing of two marine hydrolysates in diet affected the expression of -foregut *taut* and *pept1*, but in different patterns.

Data availability statement

The data that support the findings of this study and not presented in the figures and tables are available from the corresponding author on reasonable request.

Ethics statement

largemouth bass (*Micropterus salmoides*) is a commercially farmed species in China not the protected species by Chinese law. During the feeding period and sampling procedures, the experimental fish were maintained in compliance with the Laboratory Animal Welfare Guidelines of China (Decree No. 2 of Ministry of Science and Technology, issued in 1988).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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