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Palatability of Water-Soluble Extracts of Protein Sources and Replacement of Fishmeal by a Selected Mixture of Protein Sources for Juvenile Turbot (*Scophthalmus maximus*)

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Abstract Poor palatability is a limiting factor for replacing fishmeal with other protein sources in aquaculture. The water-soluble molecules with low molecular weights are the major determinants of the palatability of diets. The present study was conducted to investigate the palatability of water-soluble extracts from single protein source (single extract pellets) and the mixture of these extracts with different proportions (blended extract pellets) in juvenile turbot (*Scophthalmus maximus*). Then according to the palatability of blended extract pellets, an optimal mixture proportion was selected, and a new protein source made from raw protein materials with the selected proportion was formulated to replace fishmeal. Summarily, the palatability of single extract pellets for turbot was descendent from fishmeal to pet-food grade poultry by-product meal, wheat gluten meal, soybean meal, peanut meal, meat and bone meal, and corn gluten meal. Subsequently, according to the palatability of single extract pellets, 52 kinds of blended extract pellets were designed to test their palatability. The results showed that the pellets presented remarkably different palatability, and the optimal one was diet 52 (wheat gluten meal: pet-food grade poultry by-product meal: meat and bone meal: corn gluten meal = 1:6:1:2). The highest ingestion ratio (the number of pellets ingested/the number of pellets fed) was 0.73 ± 0.03, which was observed in Diet 52. Then five isonitrogenous (52% crude protein) and isocaloric ($20\,kJ\,g^{-1}$ gross energy) diets were formulated by replacing 0 (control), 35%, 50%, 65% and 80% of fishmeal with No.52 blending proportion. After a 10-weeks feeding trial, a consistent feed intake was found among all replacement treatments. Replacement level of fishmeal up to 35% did not significantly influence final body weight, specific growth rate, feed efficiency ratio, and protein efficiency ratio of turbot. Therefore, the water-soluble extracts of protein sources play an important role in improving the palatability of non-fishmeal protein sources in aquafeed.

Key words protein source; water-soluble extract; palatability; fishmeal replacement; turbot

1 Introduction

Fishmeal is a high quality protein source for aquaculture with balanced amino acid and favorable palatability (Gaylord and Rawles, 2005). In intensive farming, limited supply and high price of fishmeal have resulted in a high cost of production. Therefore, replacing fishmeal with other efficient protein sources is crucial for aquatic farming, especially for carnivorous fish such as turbot (*Scophthalmus maximus*) which requires 50%–65% protein in diet (Lee *et al*., 2003; Cho *et al*., 2005; Espe *et al*., 2012; Kroeckel *et al*., 2012). So far, it is difficult to replace fishmeal at a high proportion in carnivorous fish. Many limiting factors such as imbalanced amino acid, presence of anti-nutritional factors, reduced palatability, and increased fiber are parts of this challenge (Olli *et al*., 1994; Gómez-Requeni *et al*., 2004; Hansen *et al*., 2007). Among the limiting factors for high proportion of replacement fishmeal, poor palatability of non-fishmeal protein sources plays an important role in feed intake reduction and fish growth retardation (Arndt *et al*., 1999; Kissil *et al*., 2000; Bonaldo *et al*., 2011).

The continuous feeding behavior of fish is affected by physical factors of diet such as size, color, hardness, form of particle and so on. Meanwhile, the water-soluble components in the feed, with specific smell and taste, act as chemical stimulants to determine the palatability in a high degree (Xue *et al*., 2003). The olfactory and the taste bud of fish are sensitive to water-soluble molecules that are most of the available feeding stimulants for fish, including free amino acids, small peptides, and nucleotides (Gao and Li, 2004; Kousoulaki *et al*., 2009; Kader *et al*., 2010). These water-soluble nitrogen-components are more abundant in fishmeal than in plant protein sources

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(Aksnes, 2005). Therefore, poor palatability of non-fishmeal protein sources may be the result of low quantity of feeding stimulants (Deng *et al*., 2011).

Most studies focused on the supplementation of seafood by-products, such as scallop meal (Kader *et al*., 2011), krill meal and squid meal (Kader *et al*., 2012), and fish protein hydrolysate (Zheng *et al*., 2013), to improve the palatability of plant protein based diets. However, few researcher has studied the palatability of water-soluble extracts from fishmeal and alternative protein sources. The present study was conducted to investigate the palatability of different pellets with single water-soluble extracts from fishmeal (FM), wheat gluten meal (WGM), pet-food grade poultry by-product meal (PPBM), meat and bone meal (MBM), soybean meal (SBM), peanut meal (PNM) and corn gluten meal (CGM). In addition, in consideration of the advantages of compound protein sources, such as balanced amino acid composition, less anti-nutrition factors, and a much more potential in replacing FM than a single non-fishmeal protein source, the palatability of a variety of blended pellets with different single extracts was investigated in this research.

2 Materials and Methods

2.1 Protein Sources

Brown fishmeal, wheat gluten meal, pet-food grade poultry by-product meal, soybean meal, peanut meal, corn gluten meal, and wheat flour were purchased from Qingdao Great Seven Biotechnology Company (Qingdao, Shandong, China). Meat and bone meal was purchased from Australia. The nutrition and amino acid composition of dietary ingredients are shown in Table 1.

Table 1 Nutrition and essential amino acid composition of dietary ingredients

	FM	WGM	PPBM	DMBM	SBM	PNM	CGM	WF
Nutrition (% dry matter)								
Moisture	7.20	10.48	8.09	6.56	12.16	11.18	8.43	13.58
Crude protein	73.28	82.10	71.99	51.96	59.58	55.11	65.72	15.85
Crude lipid	9.21	1.34	13.28	2.75	1.85	1.53	4.94	1.97
Ash	17.03	1.45	11.79	42.95	7.05	6.51	1.88	0.89
Gross energy (kJ g^{-1})	19.81	20.73	21.39	12.65	18.14	18.04	20.75	16.14
Essential amino acid (% dry matter)								
Arg	3.82	2.79	4.38	3.69	3.63	5.50	1.77	0.55
His	1.62	1.73	1.27	0.96	1.33	1.19	1.28	0.35
Ile	2.49	3.03	2.28	1.73	1.83	1.64	2.15	0.51
Leu	4.62	5.51	4.48	3.46	3.69	3.22	9.44	0.93
Lys	4.60	1.29	3.96	3.06	3.16	1.56	0.94	0.30
Met	1.75	1.37	1.31	0.85	0.68	0.80	1.52	0.24
Phe	2.41	3.98	2.54	1.97	2.47	2.46	3.28	0.72
Thr	2.50	2.07	2.31	2.01	2.05	1.41	2.03	0.40
Val	2.75	3.13	2.69	2.36	2.02	1.95	2.62	0.60

Notes: FM, fishmeal; WGM, wheat gluten meal; PPBM, pet-food grade poultry by-product meal; DMBM, defatted meat and bone meal; SBM, soybean meal; PNM, peanut meal; CGM, corn gluten meal; WF, wheat flour.

2.2 Water-Soluble Extract Pellets from Different Protein Sources

2.2.1 Single extract pellets

Water-soluble extracts were prepared by incubating 200 g protein source in one liter water. Three hours latter, the upper solution containing the water-soluble compounds was removed and centrifuged at 4000×*g* for 10 min. The water-soluble extracts were then obtained by lyophilizing the supernatants and stored at −20℃ for further experiment.

The extract pellets were prepared as described by Kasumyan and DÖving (2003). The pellet was prepared with 90% of wheat flour as the carrier and 10% of water-soluble extracts. The pellet with 100% of wheat flour was used as control. The extracts and wheat flour were thoroughly mixed, and then water was added to produce dough. The dough was made into strips using household noodle machine. After frozen for half an hour at −20℃, the strips were cut into pellets with the same size. The pH of the single extract pellets was determined with pH meter. These pellets were kept at −20℃ before use.

2.2.2 Blended extract pellets

The formulation of blended extract pellets was identical to that of the single extract pellets, including 90% of wheat flour and 10% of different proportions of water-soluble extracts. In consideration of the low extraction ratio, the proportion of WGM water-soluble extract was set at 1% in all blended extract pellets. In addition, according to the average amino acid composition of different protein sources (Table 1), amino acid levels in each combined proteins were restricted to Arg ≥3%, Lys ≥2%, and Met ≥1%. Therefore, the palatability experiment of blended extract pellets was carried out with 52 treatments (Table 3). The pellets were formulated in the same way as described above and then were stored at −20℃.

2.3 Experimental Diets in Feeding Trial

The blend water-soluble extract pellets from WGM:

PPBM: MBM: CGM = 1:6:1:2 showed the highest ingestion ratio (RI, the number of pellets ingested/the number of pellets fed) in turbot. So the raw protein materials were blended with a similar proportion to formulate a new protein source to replace FM. Five isonitrogenous (52% crude protein) and isocaloric (20 kJ g^{-1} gross energy) diets were designed by replacing 0 (control, FM0), 35% (FM35), 50% (FM50), 65% (FM65), 80% (FM80) of FM with the formulated new protein source. Crystalline L-histidine, L-lysine, and DL-methionine were supplemented to the experimental diets to match the essential amino acid profile of the control diet. The formulation and proximate chemical composition of diets are presented in Table 2.

Table 2 Formulation and proximate chemical composition of the feeding diets

Ingredients (% dry matter)	Treatments				
	FM0	FM35	FM50	FM65	FM80
Fishmeal	62.00	40.30	31.00	21.70	12.40
Wheat gluten meal	0.00	2.30	3.30	4.30	5.30
Pet-food grade poultry by-product meal	0.00	13.80	19.80	25.80	31.80
Defatted meat and bone meal	0.00	2.30	3.30	4.30	5.30
Corn gluten meal	0.00	4.60	6.60	8.60	10.60
Wheat flour	28.00	25.28	25.04	23.01	21.06
His	0.00	0.07	0.09	0.12	0.15
Lys	0.00	0.32	0.45	0.58	0.71
Met	0.00	0.08	0.12	0.15	0.19
Fish oil	1.50	3.31	4.08	4.86	5.64
Palm oil	3.75	2.89	1.47	1.20	1.00
Microcrystalline cellulose	0.00	0.00	0.00	0.63	1.10
Soybean lecithin	2.00	2.00	2.00	2.00	2.00
Choline chloride	0.25	0.25	0.25	0.25	0.25
Attractant	0.50	0.50	0.50	0.50	0.50
Vitamin premix	0.50	0.50	0.50	0.50	0.50
Mineral premix	1.00	1.00	1.00	1.00	1.00
$Ca(H_2PO_4)_2$	0.30	0.30	0.30	0.30	0.30
Ethoxyquin	0.05	0.05	0.05	0.05	0.05
Calcium propionate	0.05	0.05	0.05	0.05	0.05
Yttrium trioxide	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00
Proximate composition(% dry matter)					
Moisture	3.35	3.23	2.98	3.54	3.78
Crude protein	52.37	51.79	51.93	51.15	51.24
Crude lipid	12.56	14.07	13.76	14.77	15.52
Ash	11.40	11.47	10.66	10.63	9.94
Gross energy (kJ g^{-1})	19.36	19.69	19.59	19.66	19.77

Notes: Attractant, betaine: dimethyl-propiothetin: glycine: alanine: 5-phosphate inosine = 4:2:2:1:1. Vitamin premix (mg kg^{-1} diet): retinal palmitate, 32; cholecalciferol, 5; DL-α-tocopherol acetate, 240; menadione, 10; thiamin-HCl, 25; riboflavin, 45; pyridoxine-HCl, 20; cyanocobalamin, 10; D-calcium pantothenate, 60; amine nicotinic acid, 200; folic acid, 20; biotin, 60; mesoinositol, 800; ascorbyl polyphosphate (contained 35% ascorbic acid), 2000; microcrystalline cellulose, 1473. Mineral premix (mg kg^{-1} diet): $MgSO_4·7H_2O$, 1200; $CuSO_4·5H_2O$, 10; $FeSO_4·H_2O$, 80; $ZnSO_4·H_2O$, 50; $MnSO_4·H_2O$, 45; $CoCl_2·6H_2O$ (1%), 50; Na_2SeO_3 (1%), 20; Calcium iodine, 60; zoelite, 8485.

The experimental ingredients were ground into fine power through 246-μm meshes. Soybean lecithin and palm oil were blended into fish oil. Then all the ingredients were thoroughly mixed with the oil, and water was added to produce stiff dough. The dough was extruded using a twin-screw extruder (F26 (II), South China University of Technology, China) to made into pellets (3 mm in diameter) and then dried for 12 h in a ventilated oven at 45℃, stored at −20℃ until use.

2.4 Fish and Experimental Procedure

2.4.1 Palatability evaluation of single and blended water-soluble extract pellets

Turbot were obtained from Haiyang Fish Farm (Haiyang, Shandong, China). Fish were fed with commercial diet for one week to acclimate to the system before the trial. Turbot (initial body weight 10.85 ± 0.01 g) were randomly allocated to polycarbonate tanks (total volume 216 L) 20 each. Before the experiment, turbot were starved for 24 h. During the trial, turbot were fed with pellets containing different extracts. Each pellet was randomly allocated in triplicate tanks. Turbot each tank were fed with 80 pellets, and the uneaten were removed and counted 1 h later. The ingestion ratio was used to evaluate the palatability. After feeding, air pump was reopened and water was changed. Filtered seawater was continuously pumped from the coast adjacent to each tank at a rate of approximately 1.5 L min^{-1}. All experimental tanks were continuously aerated seawatered. During the experimental period, temperature varied between 19 and 22℃, salinity between 30 and 33, and pH between 7.5 and 8.0.

2.4.2 Feeding trial

The preparatory work of feeding trial was identical to what we described in 2.4.1. At the start of the experiment, turbot were fasted for 24 h and then randomly distributed to 15 tanks, 30 each (initial weight 8.63 g ± 0.01 g). Turbot were hand-fed to apparent satiation twice a day at 07:00 and 18:00 for 10 weeks. Water system and experimental condition were identical to what we described above. At the end of the feeding trial, turbot were starved for 24 h before sampling. Then the total number and body weight of turbot per tank were recorded. The ingredients and diets were analyzed, which included the moisture, crude protein, crude lipid and ash using the standard methods (AOAC, 1999). Briefly, samples were dried to a constant weight at 105℃ to determine the dry content; crude protein was determined by measuring nitrogen (N × 6.25) with Kjeldahl method (Kjeltec TM 8400, FOSS, Tecator, Sweden); crude lipid was determined by measuring ether extraction with Soxhlet method (B-801, Switzerland); and ash was determined by combusting the samples at 550℃ for 5 h.

2.5 Calculation and Statistical Analysis

The variables were calculated by following methods:

$$RI = \frac{\text{The number of pellets ingested}}{\text{The number of pellets fed}},$$

$$SGR\,(\%) = 100\times\frac{\text{Ln (Final body weight)} - \text{Ln (Initial body weight)}}{\text{Duration}},$$

$$FI\,(\%\,\text{d}^{-1}) = 100\times\frac{\text{Total amount of feed consumed (g)}}{\text{(Initial body weight + Final body weight)/2/Duration}},$$

$$FER = \frac{\text{Wet weight gain (g)}}{\text{Total amount of feed consumed (g)}},$$

$$PER\,(\%) = 100\times\frac{\text{Wet weight gain (g)}}{\text{Protein intake (g)}},$$

$$SR\,(\%) = 100\times\frac{\text{Final fish number}}{\text{Initial fish number}},$$

where *RI* is the mean ratio of ingestion, *SGR* is specific growth rate, *FI* is feed intake, *FER* is feed efficiency ratio, *PER* is protein efficiency ratio, and *SR* is survival rate.

All data were subjected to one-way analysis of variance (ANOVA) using the SPSS 17.0 for Windows. In cases where data were not homoscedastic, data transformation with logarithms was used to meet ANOVA criteria. Difference among the mean values was tested by the Tukey's multiple-range test. The level of the significance chosen was at $P<0.05$ and the results were presented as means ± standard error (n=3).

3 Results

3.1 The Palatability of Single Extract Pellets

The water-soluble extract accounted for 7.75%±0.03%, 0.62%±0.06%, 7.01%±0.02%, 5.63%±0.08%, 5.07%±0.02%, 3.18%±0.12%, and 4.59%±0.04% in weight, respectively, for FM, WGM, PPBM, SBM, PNM, CGM and MBM. According to previous studies (Lamb and Finger, 1995; Aihara *et al*., 2008), the palatability results (Fig.1) were valued with the criteria of *RI*. The extract from FM, WGM and PPBM showed better palatability, and their *RI*s were nearly one. SBM, PNM and MBM extracts showed medial palatability, while CGM extract was the least attractive. Overall, the palatability of water-soluble extracts from rich to poor was FM, PPBM, WGM, SBM, PNM, MBM and CGM. The pH of pellets were approximately 4.78–6.60, with the minimum of CGM pellets.

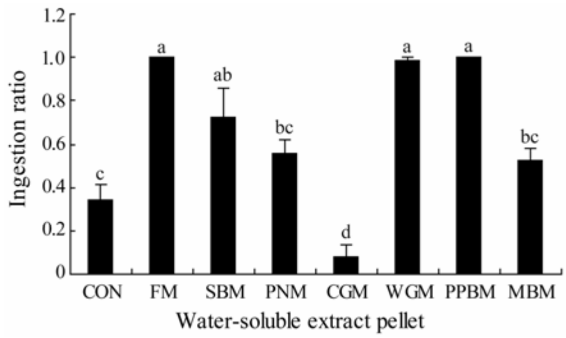

Fig.1 The ingestion ratio of single water-soluble extract pellets for turbot. The same letters above the bars denote no significant difference among treatments at $P>0.05$. CON, the control, the pellet with 100% of wheat flour; FM, fishmeal; SBM, soybean meal; PNM, peanut meal; CGM, corn gluten meal; WGM, wheat gluten meal; PPBM, pet-food grade poultry by-product meal; MBM, meat and bone meal.

3.2 The Palatability of Blended Extract Pellets

To meet the requirement of balanced amino acid profile, pellets of 52 kinds of combined extracts were evaluated. The palatability of the blended extract pellets are shown in Table 3 with the top ten appealing and repellent blend marked with upward arrow and downward arrow, respectively. Different combinations had remarkably different palatability, and their *RI*s tested ranged from 0.22±0.04 to 0.73±0.03. The pellet made from water-soluble extracts (1:6:1:2) of WGM, PPBM, MBM, CGM showed the highest *RI*, 0.73±0.03.

Table 3 The ingestion ratio of blended water-soluble extract pellets to turbot

Treatments	WGM	PPBM	MBM	SBM	PNM	CGM	*RI*	Treatments	WGM	PPBM	MBM	SBM	PNM	CGM	*RI*
1	1	1	2	2	2	2	0.53±0.01	27↑	1	2	4	0	0	3	0.67±0.05
2	1	1	3	3	0	2	0.51±0.01	28	1	3	1	3	0	2	0.58±0.01
3	1	1	3	2	1	2	0.57±0.02	29	1	3	1	2	1	2	0.62±0.01
4	1	1	3	1	2	2	0.42±0.05	30↑	1	3	1	1	2	2	0.71±0.03
5	1	1	4	2	0	2	0.47±0.04	31	1	3	2	2	0	2	0.66±0.06
6	1	1	4	1	1	2	0.48±0.04	32	1	3	2	1	1	2	0.54±0.04
7↑	1	1	2	3	0	3	0.67±0.04	33↑	1	3	3	1	0	2	0.70±0.06
8	1	1	2	2	1	3	0.44±0.03	34↓	1	3	3	0	1	2	0.30±0.03
9↑	1	1	3	2	0	3	0.72±0.02	35↓	1	3	1	2	0	3	0.26±0.04
10↑	1	1	3	1	1	3	0.67±0.03	36	1	3	1	1	1	3	0.57±0.03
11↓	1	1	4	1	0	3	0.31±0.03	37	1	3	2	1	0	3	0.36±0.03
12	1	1	4	0	1	3	0.43±0.03	38	1	3	2	0	1	3	0.56±0.07
13↓	1	1	4	0	0	4	0.29±0.04	39	1	3	3	0	0	3	0.61±0.02
14	1	2	1	4	0	2	0.60±0.04	40	1	4	1	2	0	2	0.48±0.05
15	1	2	1	3	1	2	0.56±0.03	41	1	4	1	1	1	2	0.53±0.05

(to be continued)

(continued)

Treatments	WGM	PPBM	MBM	SBM	PNM	CGM	*RI*	Treatments	WGM	PPBM	MBM	SBM	PNM	CGM	*RI*
16	1	2	2	3	0	2	0.49±0.03	42↑	1	4	1	0	2	2	0.68±0.01
17↑	1	2	2	2	1	2	0.69±0.08	43	1	4	2	1	0	2	0.36±0.04
18	1	2	2	1	2	2	0.56±0.03	44	1	4	2	0	1	2	0.38±0.03
19	1	2	3	1	1	2	0.58±0.06	45	1	4	3	0	0	2	0.41±0.03
20↑	1	2	4	1	0	2	0.72±0.03	46	1	4	1	1	0	3	0.50±0.04
21↓	1	2	4	0	1	2	0.33±0.02	47↓	1	4	1	0	1	3	0.30±0.02
22	1	2	1	3	0	3	0.59±0.04	48	1	4	2	0	0	3	0.56±0.01
23↓	1	2	2	2	0	3	0.26±0.04	49	1	5	1	1	0	2	0.34±0.01
24↓	1	2	2	1	1	3	0.31±0.07	50	1	5	1	0	1	2	0.57±0.04
25↓	1	2	3	1	0	3	0.22±0.04	51↓	1	5	2	0	0	2	0.33±0.03
26	1	2	3	0	1	3	0.65±0.04	52↑	1	6	1	0	0	2	0.73±0.03

Notes: *RI*, the ingestion ratio = the number of pellets ingested/the number of pellets fed; ↑, Treatments with rich palatability; ↓, Treatments with poor palatability. The water-soluble extracts from proteins: WGM, wheat gluten meal; PPBM, pet-food grade poultry by-product meal; MBM, meat and bone meal; SBM, soybean meal; PNM, peanut meal; CGM, corn gluten meal.

3.3 Growth Performance

The replacement results indicated that the survival rate (*SR*) and feed intake (*FI*) showed no significant difference ($P>0.05$) among all dietary treatments. With the increase of replacement level, the final body weight (*FBW*) and specific growth rate (*SGR*) decreased. However, compared with the control (FM0), fish fed with the diet FM35 showed no significant difference ($P>0.05$) in *FBW*, *SGR*, feeding efficiency ratio (*FER*), and protein efficiency ratio (*PER*) (Table 4).

Table 4 Effect of replacement of fishmeal by blended proteins on growth parameters of turbot

	Treatments				
	FM0	FM35	FM50	FM65	FM80
IBW (g)	8.61±0.01	8.62±0.01	8.64±0.01	8.65±0.01	8.64±0.01
FBW (g)	36.71±1.49^{a}	32.14±1.47^{ab}	28.18±1.53^{b}	29.13±0.31^{b}	27.33±1.23^{b}
SGR (%)	2.45±0.07^{a}	2.23±0.08^{ab}	2.00±0.09^{b}	2.06±0.02^{b}	1.95±0.08^{b}
FI (%d^{-1})	2.12±0.04	2.07±0.03	2.06±0.06	2.03±0.02	2.06±0.04
FER	0.99±0.04^{a}	0.94±0.02^{ab}	0.87±0.01^{b}	0.90±0.01^{ab}	0.88±0.03^{b}
PER	1.87±0.05^{a}	1.82±0.03^{a}	1.67±0.02^{b}	1.84±0.01^{a}	1.61±0.03^{b}
SR (%)	88.89±4.84	88.89±2.94	87.78±2.94	86.67±5.09	83.33±5.09

Notes: Values in the same row with the same superscripts have no significant difference ($P>0.05$). *IBW*, initial body weight; *FBW*, final body weight; *SGR*, specific growth rate; *FI*, feed intake; *FER*, feed efficiency ratio; *PER*, protein efficiency ratio; *SR*, survival rate.

4 Discussion

The present study showed that the palatability of single water-soluble extracts from rich to poor were FM, PPBM, WGM, SBM, PNM, MBM and CGM in turbot. The *RI*s of FM, PPBM and WGM were nearly one, suggesting that the water-soluble extracts from these protein sources have much better palatability to turbot. Yun *et al*. (2014) pointed out that FM is a high quality protein source for aquaculture. It was characterized by high protein content, favorable amino acid profile, no anti-nutritional factor, and reasonable palatability. Studies (Rawles *et al*., 2006; Saadiah *et al*., 2011; Zhou *et al*., 2011; Xue *et al*., 2012) of PPBM showed that high quality PPBM is rich in protein and low in ash, which may lead to the good palatable effect. On the other hand, studies (Francis *et al*., 2001; Hasimoglu *et al*., 2007) of SBM have indicated that anti-nutritional factors, such as trypsin inhibitor, polysaccharides, tannin, saponin and phytic acid, may affect its palatability to turbot. The diet with 0.5% phytic acid reduced the feed efficiency ratio of rainbow trout (*Salmo gairdneri*) (Spinelli *et al*., 1983). Unbalanced amino acid composition may be another reason for poor palatability of water-soluble extracts from other protein sources. Liu *et al*. (2012) pointed out that PNM is rich in arginine and short in lysine, and CGM has a very low level of lysine (Regost *et al*., 1999). The poor palatability of PNM and CGM could be explained by the unbalanced amino acid composition. In this study, the pH of CGM extract pellet was 4.78, lower than that of other pellets. It has been demonstrated that carnivorous fish much likely prefer alkaline and neutral substances (Johnsen and Adams, 1986; Reig *et al*., 2003). The acidity of CGM might also negatively affect its palatability (Masumoto *et al*., 1996). Therefore, the poorest palatability of CGM extract pellet in this study could be caused by different reasons.

Compared to a single non-fishmeal protein source, compound protein sources are more balance in nutritional composition and have less anti-nutritional factors (Fournier *et al*., 2004; Kader *et al*., 2010; Kader and Koshio, 2012). As a result, they are widely used in aquaculture. Therefore, the current study also studied the palatability of pellets produced with blended water-soluble extracts. Different extract combinations showed significant difference in *RI* which changed from 0.22±0.04 to 0.73±0.03. In the present study, turbot displayed feeding preference to the mixture of water-soluble extracts (1:6:1:2) from WGM, PPBM, MBM, CGM with the highest *RI* (0.73±0.03). This result could be considered as a result of the high content of PPBM in the mixture, which improved the amino acid balance and minimized the negative factors.

It can be deduced that there may be some palatability correlations between extracts and raw protein materials.

Therefore, the present study was carried out to investigate the substitution effect of a new protein source with blended raw protein materials for FM. The new protein source was formulated with WGM, PPBM, MBM and CGM at a ratio of 1:6:1:2 based on the palatability result of blended water-soluble extract pellets. In the present study, *FI* of turbot showed no significant difference among all dietary treatments. However, it was reported that the combination of WGM, CGM and lupine reduced the *FI* in turbot, even with supplemental attractants (Fournier *et al*., 2004). Our unpublished results also showed that 45% replacement of FM by SBM, CGM and MBM reduced the *FI* for turbot in the existence of experimental attractants. In our study, *FI* showed no significant difference when up to 80% of FM was replaced. Thus it could be concluded that, compared with the supplemental attractants, the blended protein sources contributed dominantly to palatability improvement. So the result in this study may be helpful for breaking through the major challenge of the poor palatability when replacing FM by other protein sources, even in the presence of supplemental attractants. In addition, the combination could successfully replace 35% FM with no significant influence on growth parameters such as *FBW* and *SGR*. However, studies of Black Sea turbot (*Scophthalmus maeoticus*) (Ergun *et al*., 2008) and Senegalese sole (*Solea senegalensis*) (Cabral *et al*., 2011) showed that SBM and the combination of peas, SBM, WGM and CGM significantly decreased *FBW* of the experimental fish at a 35% replacement level. The difference could be considered as a result of the rich palatability of the compound protein sources used in this study.

In summary, the water-soluble extracts of FM, PPBM and WGM are better in palatability to turbot, and the evaluation of the palatability of water-soluble extracts of protein sources can be a good guidance for predicting the palatability of non-fishmeal protein sources. However, due to the low extraction ratio, the raw protein sources instead of water-soluble extracts were used to replace FM. To confirm the importance of water-soluble extracts from protein, further research will be conducted to study the determinant component in the water-soluble extracts, which will be applicable in improving the palatability of non-fishmeal protein sources.

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