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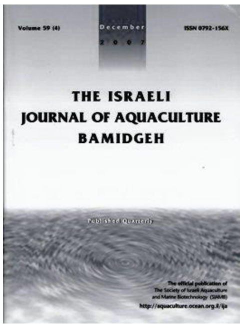

Apparent Digestibilities of Selected Feed Ingredients Fermented by Host Bacteria in Juvenile Turbot, *Scophthalmus maxima* L.

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Keywords: host bacteria; fermentation; feed ingredients; apparent digestibility; turbot

Abstract

Fermentation is considered a promising method to improve the limited utilization of plant protein sources. In the present study, *Shewanella* sp. MR-7, isolated from the intestine of juvenile turbot, was used to ferment selected plant sources. We investigated the apparent digestibility coefficients (ADCs) of three plant sources fermented and not fermented by *Shewanella* sp. MR-7 in diets of juvenile turbot: soybean meal (SBM), fermented soybean meal (FSBM), peanut meal (PM), fermented peanut meal (FPM), corn gluten meal (CGM) and fermented corn gluten meal (FCGM). The ADCs of dry matter (DM), crude protein (CP), gross energy (GE), and amino acids（AA）were analyzed. Our results showed that the ADC of the plant protein sources improved significantly with fermentation. Moreover, the FSBM showed the highest improvement for apparent amino acid digestibility while FPM showed the lowest compared with the unfermented plant protein source. In conclusion, our results indicated that *Shewanella* sp. MR-7 fermentation can greatly improve the ADCs of SBM, PM, and CGM in diets of turbot. This study provided valuable information for the use of host bacteria as zymocyte in aquaculture.

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Introduction

Fish meal (FM) is the most important feedstuff used as a protein source for its well-balanced amino acid composition, essential fatty acid content, palatability, and digestible energy. However, due to an increasing supply shortage and sharp price rises, numerous feed enterprises have focused on finding alternative plant protein sources to replace FM (Ergun et al., 2008). However, plant proteins may affect growth performance and nutrient utilization of fish due to the presence of indigestible carbohydrates, indigestible non-starch polysaccharides (NSP) and anti-nutritional factors (ANFs). In addition, some results have indicated that the poor performance resulting from plant proteins might be due in part to its low protein digestibility (Regost, 1999). Studies which demonstated that only 20%-50% FM could be successfully replaced suggest that FM replacement is still a drawback in the preparation of aquaculture feeds (Wei et al., 2015).

Fermentation is a traditional technique used to improve the digestibility of plant protein sources. After the fermentation period, the ANFs in plant proteins can be removed or inactivated (Egounlety & Aworh, 2003). Furthermore, protein macromolecules can be degraded into low molecular weight and water-soluble compounds (Hong & Kim, 2004). In recent studies, fermented plant proteins have been used as new protein sources to replace FM in the diets for Atlantic salmon (Refstie et al., 2005),Turbot (Zhou et al. 2016; Zhang et al., 2016), black sea bream (Azarm et al., 2014), Orange - spotted grouper (Shiu et al., 2015) and Japanese seabass (Liang et al., 2017). These studies suggest that fermented plant protein sources with improved nutritional values or digestibility could be used as high-quality alternative protein sources to replace FM in the diets of carnivorous fish.

Gut microbiota is considered a highly specialized organ of the host and has an irreplaceable role in host immune response and metabolism of fish (Pérez et al., 2010). There are many microorganisms with various functions in the digestive tract of aquatic animals. Several of them have been applied in aquaculture such as lactic acid bacteria (Balcázar et al., 2008) and *Shewanella* (Guzmán-Villanueva et al., 2014). Based on these scientific theories, there is an interesting research approach to use the intestinal bacteria of the host as zymophytes to ferment plant protein. Turbot, a carnivorous fish, is widely cultured around the world for its high economic value. A series of studies have reported that turbot require a high level of protein (about 500g/kg-600 g/kg) of the diet (Lee et al. 2003). Among the plant protein sources, soybean meal (SBM), peanut meal (PM) and corn gluten meal (CGM) have been considered desirable alternatives to FM and have been successfully used in the diets of shrimp (YE et al., 2011), snakehead (Hien, et al., 2015), red snapper (Wu et al., 2016) rainbow turbot (Ávila, et al., 2015; Gerile & Pirhonen, 2017), Japanese seabass (Wang, et al., 2017) and nile tilapia (Silva et al., 2017).

Shewanella sp. MR-7, isolated from the intestine of the juvenile turbot in our previous study, was used to ferment SBM, PM and CGM. As the new feed ingredients, the digestibility data are very important to evaluate their application for culture species. Hence, the present study was aimed to determine dry matter, crude protein, gross energy, and amino acids digestibility of *Shewanella* sp. MR-7 fermented SBM, PM, and CGM in diets for juvenile turbot and evaluate whether the fermentation process by *Shewanella* sp. MR-7 can improve the ADCs of the selected plant proteins. In addition, this study can provide new ideas to improve the quality of plant protein sources for further study.

Materials and methods

Diet preparation.

Regular soybean meal, peanut meal, and corn gluten meal were obtained from Qihao Biotech. Co., Ltd. (Shandong, China). *Shewanella* sp. MR-7 was obtained by a common skimmed milk transparent circle method from the intestinal mucosa of healthy turbot in our previous study. SBM, PM, CGM soaked with 100% distilled water mixed with 2.6‰ sea salt, 3.3‰ $(NH_4)_2SO_4$, 1.3‰ glucose were autoclaved at 100°C for 30 min in a steam tank (model HX14G-1, Shanghai, China) and then cooled to room temperature. Thereafter, the SBM, PM, CGM were inoculated with 10% of *Shewanella* sp. MR-7 (~10^{-9}colonyforming units (cfu)/mL) and fermented in an incubator at 25°C for 24 h. The resulting FSBM, FPM and FCGM were dried in an oven at 45°C until its moisture content was below 10%. The samples were collected to determine the nutritional profile (crude protein, crude lipid, moisture, ash, energy and amino acid).

The reference diet (RF) (Table 1) was formulated to satisfy the protein and lipid requirements of turbot (Lee, 2003). Six experimental diets composed of 70% reference diets and 30% of the test ingredients (on a dry weight basis) were prepared as described by Cho and Slinger (1982). Yttrium oxide (Y_2O_3, 0.1%) was used as an inert marker and incorporated into the reference and experimental diets.

Table 1. Reference and test diet formulations for digestibility coefficient determination.

Ingredients	*Reference diet (% Dry matter)*	*Test diet (% Dry matter)*
Fish meal [a]	60.00	42.00
Soybean meal [a]	5.00	3.50
Wheat meal [a]	22.95	16.04
Lecithin	2.00	1.40
Fish oil	4.50	3.15
Vitamin premix [b]	2.00	1.40
Mineral premix [c]	2.00	1.40
Choline chloride	0.30	0.21
Attractant [d]	0.50	0.35
Mold inhibitor	0.10	0.07
Antioxidant	0.05	0.035
$CaH_2(PO)_4$	0.50	0.35
Yttrium oxide [e]	0.10	0.10
Test ingredient	0.00	30.00

[a] Fish meal: crude protein 74.65%, crude lipid 8.5%; Soybean meal: crude protein 52.97%, crude lipid 2.12%; Wheat meal: crude protein 21.86%, crude lipid 1.55% .

[b] Supplied the following (mg/kg diet): retinyl acetate, 32; cholecalciferol, 5; tocopheryl acetate, 240; menadione sodium bisulphite, 10; ascorbic acid, 120; cyanocobalamin, 10; biotin, 60; choline dihydrogen citrate, 7 g; folie acid, 20; inositol, 800; niacin, 200; pantothenate, 60; pyridoxine HCL, 20; riboflavin, 45; thiamin HCL, 25; microcrystalline cellulose, 16 473.

[c] Supplied the following (mg/kg diet): $MgSO_4 \cdot 7H_2O$, 1200; $CuSO_4 \cdot 7H_2O$, 10; $FeSO_4 \cdot 7H_2O$, 80; $ZnSO_4 \cdot H_2O$, 50; $MnSO_4 \cdot H_2O$, 45; $CoCl_2$, 5; Na_2SeO_3, 20; Ca $(IO_3)_2$, 60; Zeolite powder, 18485.

[d] Supplied the following (% dry diet): betaine, 0.4; DMPT, 0.2; threonine, 0.2; glycine, 0.1; inosine-5-diphosphate trisodium salt, 0.1.

[e] Sigma-Aldrich, St. Louis, MO, USA

Proximate composition and amino acid composition of the test ingredients and diets are shown in table 2 and 3 respectively.

Table 2. Proximate composition and Amino acid (AA) composition of the experimental feeding ingredients (% Dry matter)

	SBM	FSBM	PM	FPM	CGM	FCGM
Proximate composition						
Crude protein (%)	52.97±0.56	54.21±0.67	53.68±0.81	55.39±0.55	65.54±0.33	66.33±0.47
Crude lipid (%)	2.12±0.2	2.11±0.21	1.81±0.09	1.79±0.33	4.25±0.16	4.14±0.05
Moisture (%)	11.39±0.02	11.35±0.13	7.09±0.25	7.11±0.06	4.92±0.11	4.87±0.17
Ash (%)	6.63±0.12	6.71±0.25	6.42±0.03	6.47±0.16	1.8±0.06	1.86±0.04
Energy(KJ/Kg)	20.04±0.72	21.23±0.55	20±1.05	21.01±0.67	22.73±1.12	23.04±2.11
Amino acid						
Arg	2.8	2.87	5.35	5.41	1.75	1.77
His	1.64	1.64	1.07	1.10	1.23	1.20
Phe	2.18	2.23	2.53	2.51	3.58	3.57
Lys	2.74	2.72	1.31	1.32	0.89	0.92
Val	2.11	2.12	1.83	1.88	2.21	2.21
Met	0.45	0.45	0.43	0.47	1.55	1.59
Ile	2.02	2.01	1.41	1.44	2.26	2.22
Leu	3.34	3.39	3.18	3.21	9.57	9.58
Thr	1.94	1.96	1.38	1.38	2.02	1.93
Ser	2.34	2.36	2.42	2.45	3.02	3.21
Glu	8.03	8.03	10.12	10.23	13.44	13.55
Gly	2.11	2.22	2.89	2.92	1.68	1.72
Ala	2.12	2.15	1.99	2.02	5.22	5.25
Cys	0.69	0.71	0.56	0.63	1.21	1.24
Tyr	1.31	1.33	1.84	2.01	2.98	3.02
Asp	5.26	5.15	5.54	5.64	3.61	3.70
Pro	2.21	2.27	1.84	1.88	5.21	5.20

[a] These protein sources were obtained from Great seven Bio-Tech (Qingdao, China)
Values are means ±standard error. (n=3) of three replicates and values within the plant proteins and fermented plant proteins with different letters are significantly different ($P<0.05$).

All ingredients were ground into fine powder and sieved through an 80 μm mesh. Ingredients of each of the diets were blended thoroughly first by hand and then mechanically. Lecithin was dissolved in oil and then mixed with all the ingredients. Finally, water was added into the mixture to produce a stiff dough which was pelleted with an experimental feed mill (F-26 (II), South China University of Technology, China) and dried for about 16 h in a ventilated oven at 45°C, and stored in a freezer at -20°C until use.

Table 3. Proximate composition and Amino acid (AA) composition of the experimental diets (% Dry matter)

	Reference diet	*SBM diet*	*FSBM diet*	*PM diet*	*FPM diet*	*CGM diet*	*FCGM diet*
Proximate composition							
Crude protein (%)	53.05	53.12	53.13	53.63	53.72	55.42	55.47
Crude lipid (%)	12.31	9.38	9.38	9.13	9.09	9.86	9.87
Ash (%)	9.53	8.68	8.66	8.88	8.85	7.65	7.62
Energy(KJ/Kg)	20.68	20.49	20.50	20.48	20.51	21.00	21.04
Amino acid							
Arg	2.65	2.87	2.85	3.34	3.29	2.32	2.29
His	1.47	1.46	1.44	1.34	1.33	1.32	1.32
Phe	2.10	2.10	2.24	2.25	2.30	2.37	2.36
Lys	3.38	3.43	3.46	2.66	2.74	2.50	2.49
Val	2.22	2.32	2.30	2.34	2.28	2.27	2.27
Met	1.18	0.93	0.93	1.02	1.00	1.21	1.20
Ile	1.89	1.94	1.97	1.79	1.85	1.86	1.90
Leu	3.49	3.58	3.50	3.65	3.73	4.97	4.98
Thr	2.00	2.04	2.02	1.74	1.63	1.86	19.0
Ser	2.03	2.05	2.01	2.24	2.11	2.29	2.19
Glu	7.80	8.53	8.55	8.76	8.72	9.16	9.08
Gly	2.81	2.73	2.67	2.71	2.77	2.26	2.34
Ala	2.96	2.74	2.75	2.97	2.82	3.33	3.40
Cys	0.62	0.65	0.65	0.60	0.66	0.81	0.74
Tyr	4.05	4.57	4.57	4.23	4.37	3.56	3.67
Asp	1.62	1.68	1.69	1.99	1.72	1.83	1.89
Pro	2.14	2.45	2.25	2.26	2.18	2.78	2.87

Fish and experimental conditions

Juvenile turbot (*Scophthalmus maxima*) (5.35±0.02 g) were obtained from a local hatchery farm and acclimated in the laboratory. After acclimation to the reference diet for 2 weeks, the fish were randomly distributed into 18 (three tanks to each diet) 200 L cylindrical fiberglass tanks with 40 fish per tank. Fish were fed to visual satiation twice daily (06:30 and 18:30) with the experimental diets. The feeding experiment lasted for 6 weeks. Seawater temperature and salinity were monitored daily. During the experimental period, the water temperature ranged from 18.2°C-19.8°C, salinity from 30.5‰ to 31.7‰, NH_4–N from 72-100 μg/L, NO_3–N from 92.6-120 μg/L, NO_2–N from 6.5-10.2 μg/L and dissolved oxygen was kept at approximately 7 mg/L.

Fecal collection

Fecal collection was conducted according to Wei et al., 2015. Fecal samples were collected from each tank 5 hours after feeding. Diets were fed twice daily (06:30 and 18:30) to apparent satiation for 7 days prior to fecal collection. Manual stripping of fish was accomplished by gently applying pressure to the lower abdominal region of turbot, under anesthesia, into a plastic weighing pan. Care was taken to exclude urinary excretion from the collection. After stripping, fish were given a salt bath (15–20 ppm) for 10-15 min to reduce handling stress before returning to culture tanks. Five-day interval between fecal stripping was followed to keep stress level of the fish at a minimum. During the entire period, the process was repeated seven times to obtain triplicate fecal samples per feed diet and for the calculation of ACDs. Fecal samples for a given tank were dried overnight at 45^{o}C, pooled and stored at -20^{o}C until analyses.

Chemical analysis

Dry matter and ash analysis of ingredients, diets and feces were performed according to standard methods (AOAC, 1995). Yttrium content of diets and feces were determined by inductively coupled plasma original emission spectrometer (ICP-OES) [IRIS Advantage (HR), Thermo Jarrell Ash, Woburn, USA]. Crude protein was determined by the Kjeldahl method after acid digestion using a Kjeldahl System (1030-Auto-analyzer, Tecator, Sweden). Amino acids in ingredients, diets, and feces were analyzed with an amino acid analyzer (Biochrom 30, GE) following acid hydrolysis (AOAC 1995). Total energy was determined in the reference diet by adiabatic bomb calorimetry (Parr1281; Parr Instrument Company Inc., Moline, IL, USA).

Digestibility determinations and statistical analyses

The ADCs of the diets were derived from the equation:

$$ADC\ (\%) = 100\ [1- (M_i / M_f)\ (C_f / C_i)]$$

Where C_i and C_f are the concentrations (%DM) of nutrients in the diet and feces, respectively, and M_i and M_f are the concentrations (%DM) of markers in the diet and feces, respectively. The ADC of a nutrient in an ingredient (ADC_{Ingr}) added to the reference diet was calculated by difference, assuming no associative effects between the added ingredient and the reference diet. The apparent digestibility of the test feed ingredient used the nutrient contribution of the test ingredient rather than its weight contribution (Forster, 1999).

$$ADC_{ingr}\ (\%) = (ADC_{com} - (ADC_{Ref}\ (1 - SR_{Nut}))) / SR_{Nut}$$

Where ANC_{Com} is the ADC (%) of the nutrient in the combined diet, ADC_{Ref} is the ADC (%) of the nutrient in the reference diet, and SR_{Nut} is the substitution rate (as decimal) for the nutrient in question. Calculation of SR_{Nut} is as follows:

$$SR_{Nut} = (N_{Test}\ SR_{Wt}) / ((N_{Test}\ SR_{Wt}) + (N_{Ref}\ (1 - SR_{Wt})))$$

where N_{Test} is the concentration (%) of the nutrient in the test ingredient, N_{Ref} is the concentration (%) of the nutrient in the reference diet, and SR_{Wt} is the substitution rate of the ingredient in the reference diet on a weight basis (in decimal : 0.3).

Statistical analysis

Statistical evaluation of the data was conducted using the computer software application SPSS18.0 for Windows. All data in this study are presented as means ± standard error of three replicates and analyzed by one-way ANOVA to test the effects of experimental treatments. Differences among means were considered significant at $P \leq 0.05$. Tukey's test was subsequently used to identify the significant differences among the treatment mean values.

Results

The effect of *Shewanella* sp. MR-7 fermentation on nutritive value and amino acid composition of soybean meal (SBM), peanut meal (PM) and corn gluten meal (CGM) are presented in Table 2. The results showed that the nutritive value and amino acid composition of the three plant protein sources did not differ significantly after the *Shewanella* sp. MR-7 fermentations. The proximate composition and amino acid composition of the test diet formulations are presented in Table 3. ADCs for dry matter, crude protein, and energy of the test ingredients in juvenile turbot are summarized in Table 4.

Table 4. ADCs (%) of dry matter, crude protein, gross energy of feedstuffs for turbot

Ingredients	*Apparent digestibility coefficients（ADCs %）*		
	Dry matter	*Protein*	*Energy*
SBM	35.03±1.82[a]	65.44±1.07[a]	46.33±1.07[a]
FSBM	40.15±2.02[b]	69.09±2.02[b]	50.5±2.02[b]
PM	32.08±1.12[a]	70.67±1.33[a]	49.56±0.67[a]
FPM	34.54±1.07[b]	72.5±1.01[b]	52.83±0.42[b]
CGM	26.63±2.02[a]	46.80±0.67[a]	35.25±1.07[a]
FCGM	29.19±0.33[b]	49.50±1.11[b]	38.88±2.33[b]

Values are means ±standard error. (n=3) of three replicates and values within the plant proteins and fermented plant proteins with different letters are significantly different ($P<0.05$).

The results suggest that the fermented process of *Shewanella* sp. MR-7 improved the digestibility of SBM, PM, and CGM for turbot. Compared with the unfermented plant proteins, the apparent digestibility coefficients all significantly improved ($P < 0.05$): apparent dry matter digestibility of SBM (35.03% to 40.15%), PM (32.08% to 35.94%), CGM (26.63% to 32.19%); apparent crude protein digestibility of SBM (65.44% to 69.09%), PM (70.67% to 73.5%), CGM (46.8% to 51.5%); apparent energy digestibility of SBM (46.33% to 50.5%), PM (49.56% to 52.83%), CGM (35.25% to 40.88%).

The results of apparent amino acid availability coefficients of the experimental diets are presented in Table 5. The fermented plant proteins showed better ADC of amino acid than the unfermented ones. Compared with the unfermented sources, 8 kinds of amino acids (Histidine, Lysine, Methionine, Isoleucine, Leucine, Serine, Proline and Alanine) in FSBM were significantly improved, while 3 kinds of amino acids (Argnine, Isoleucine and Serine) and 6 kinds of amino acids (Argnine, Histidine, Isoleucine, Leucine, Serine and) in FPM and FCGM respectively were significantly improved.

Table 5. Apparent amino acid availability (%) of the ingredients for turbot

AA	*SBM*	*FSBM*	*PM*	*FPM*	*CGM*	*FCGM*
Arg	80.37±0.96	81.05±0.67	76.32±1.67[a]	80.44±1.67[b]	39.87±1.01[a]	44.01±1.39[b]
His	71.46±0.15[a]	74.41±2.15[b]	56.24±0.56	58.28±1.27	53.22±1.39[a]	56.21±1.09[b]
Phe	74.80±0.67	75.89±0.35	82.45±1.16	84.10±0.83	46.55±2.27	49.90±1.67
Lys	75.05±1.29[a]	79.16±0.62[b]	56.16±2.25	60.16±2.00	60.21±3.05	64.16±1.12
Val	66.79±0.94	68.32±1.78	89.95±2.24	91.86±3.48	34.67±2.10	35.17±3.30
Met	63.45±3.26[a]	69.89±1.67[b]	99.54±3.76	104.05±4.26	34.05±2.13	37.05±1.23
Ile	66.70±1.24[a]	70.05±0.76[b]	90.82±1.35[a]	94.26±1.37[b]	33.83±0.63[a]	36.83±1.02[b]
Leu	78.33±0.67[a]	80.62±1.12[b]	101.33±1.03	104.46±2.03	52.30±2.12[a]	57.67±3.01[b]
Thr	60.12±0.25	62.89±0.94	58.42±4.85	62.42±4.67	26.17±2.04	29.20±1.33
Ser	64.95±3.26[a]	69.67±0.16[b]	62.65±1.45[a]	66.45±1.67[b]	40.35±1.67[a]	45.22±0.47[b]
Glu	69.44±2.39	72.36±1.63	79.56±1.34	82.45±1.42	43.44±3.12	46.19±2.36
Gly	63.46±2.19	64.65±0.36	45.33±2.15	48.67±1.90	40.69±2.35	43.07±2.55
Ala	70.33±0.78[a]	75.29±1.67[b]	94.35±2.69	96.84±1.99	44.19±3.06	48.89±1.60
Cys	98.25±3.30	106.67±0.30	93.62±3.56	97.81±1.36	58.14±2.35	60.41±0.76
Tyr	78.60±1.23	80.82±3.23	82.56±4.14	86.31±1.52	44.67±3.32	48.68±2.32
Asp	56.46±2.17	60.35±2.07	57.62±2.05	60.29±1.46	37.62±0.57[a]	42.51±0.43[b]
Pro	68.66±1.14[a]	72.16±1.12 [b]	80.23±1.99	83.78±1.15	44.20±2.67	46.35±1.07

Values are means ± standard error. (n=3) of three replicates and values within plant proteins and fermented plant proteins with different letters are significantly different ($P<0.05$).

Discussion

To date, species of Shewanella sp., isolated from GI, have been applied as probiotics to improve abalone growth, gut environment, and disease prevention (Jiang et al., 2013; Guzmán-Villanueva et al., 2014). This is the first time that the value potential nutritional benefit of *Shewanella* sp. fermented protein resources in fish diets was evaluated. The present study suggested that fermented process of *Shewanella* sp. MR-7 improved the apparent digestibility coefficients (ADCs) of soybean meal (SBM), peanut meal (PM), and corn gluten meal (CGM). A previous study also found that the dry matter and crude protein digestibility of SBM in diet for turbot could be improved through the processing of fermentation, which is in agreement with the present study of (Wang, 2016). Moreover, previous studies indicated that the large molecular weight globulins of non–fermented PM and CGM could be hydrolyzed to smaller components by fermentation (Neumann et al., 1984; Beuchat et al., 1975).

The ADCs values of *Shewanella* sp. MR-7 fermented SBM were lower than those registered by Wang (2016) for fermented SBM fed to turbot and Azarm & Lee (2014) fed to black sea bream. This might be due to the different fecal stripping methods. The importance of digestibility coefficients availability has been well acknowledged for ingredient evaluation and selection in aquaculture feeds (Glencross et al., 2005). It is suggested that high levels of fecal carbohydrates from plant proteins can decrease fecal composition and increase the dissolution of the fecal matter when excreted into the water thereby effectively reducing the fecal nutrients collected, and consequently enhancing the digestibility value. A similar phenomenon was observed and therefore the fecal stripping method was chosen for ADCs determination in present study. The ADC values of nutrients could be species-specific, could be affected by the size of the fish, and the extent of hydrolysis of protein by fermentation with different zymocytes.

Amino acid (AA) availability is an important indicator to reflect the protein ADC which can mainly reflect the digestibility of plant protein sources for carnivorous fish. It was reported that fishmeal had the highest AA availability as compared to SBM, PM and CGM and that different amino acid availability and imbalanced amino acid profiles both contributed to the low performance of other than fishmeal proteins (Wei et al., 2015). In the present study, the ADC values of 8 amino acids in FSBM improved significantly, while ADC values of 3 and 6 kinds of amino acids in FPM and FCGM respectively were improved significantly. The AA digestibility of the FSBM, FPM and FCGM diets in the present study had better performance in turbot diets which suggests that *Shewanella* sp. MR-7 fermentation enhances the digestibility of plant proteins.

In conclusion, the present study showed that ADCs of dry matter, crude protein, energy, and amino acid in the test ingredients for turbot were improved by *Shewanella* sp. MR-7 fermentation. Different zymocyte may have diverse effects on proteolysis. Although no previous studies have evaluated the fermentation function of *Shewanella* sp. MR-7, our data indicated that *Shewanella* sp. MR-7 is a promising zymocyte that can improve the utilization of plant proteins. Therefore, this study might be helpful in developing cost effective and sustainable dietary formulations for turbot, meanwhile, it can provide new ideas to improve the quality of plant protein sources for further studies.

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