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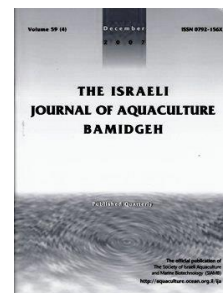
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Hydrolyzed Tuna Meat By-Product Supplement for Juvenile Red Sea Bream, *Pagrus major*, and its Effect on Growth, Enzyme Activity, Plasma Parameters, and Apparent Nutrient Digestibility

Roger Edward Mamauag^{1*}, Janice Alano Ragaza², Shunsuke Koshio^{3,4}, Manabu Ishikawa³, Saichiro Yokoyama³

¹*Southeast Asian Fisheries Development Center, Aquaculture Department, Tigbauan, Iloilo, Philippines, 5021*

²*Department of Biology, School of Science and Engineering, Ateneo de Manila University, Quezon City, Philippines*

³*The United Graduate School of Agricultural Sciences, Kagoshima University, Korimoto 1-21-24, Kagoshima, 890-0056, Japan*

⁴*Laboratory of Aquatic Animal Nutrition, Faculty of Fisheries, Kagoshima University, Shimoarata 4-50-20, Kagoshima, 890-0056, Japan*

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Abstract

A growth experiment was conducted on juvenile red sea bream, *Pagrus major* to investigate the effect of the inclusion in fish diets, of tuna meat by-product hydrolysate which was processed through enzymatic hydrolysis using a commercially available enzyme, derived from *Bacillus subtilis*. Six experimental diets were formulated in the experiment. Three diets contained 50, 150 and 250 g/kg of TPM-H (tuna meat by-product hydrolysate), and two diets with the unprocessed TPM (tuna meat by-product) at an inclusion level of 50 and 250 g/kg. A control diet was formulated without any addition of the test ingredients. Treatment diets were fed *ad libitum* to juvenile fish with an initial average body weight of 0.81 ± 0.13 g for 56 days. Results of the feeding trial suggest that the inclusion of TPM-H at 250 g/kg in fish diets improved body weight gain rate (3271.58%), feed intake (24.55 g/fish/56 days) and feed conversion efficiency (1.12) of the fish. Apparent nutrient digestibility of hydrolyzed tuna meat by-product improved compared to the unhydrolyzed ingredient. These results suggest that TPM processed as hydrolysates can be efficiently utilized by fish.

Introduction

Waste material from fish processing generally comprises 50% of the weight of fish materials processed (Shih et al., 2003; Kristinsson and Rasco, 2000). Fish processing by-products are mostly underutilized but are protein rich materials that can be utilized as ingredients in animal and fish diets. Japan is one of the major consumers of tuna in the world and only specific parts of tuna muscle are utilized for tuna flakes, sashimi, and other high value food products. The other parts such as dark meat, viscera, tail and head are considered by-products. Tuna meat by-product meal can be used as a fishmeal replacement by up to 50% inclusion without negatively affecting the growth of red sea bream (Uyan et al., 2006). The application of enzyme technology for protein recovery from fish processing by-products can produce a broad spectrum of food ingredients or industrial products for a wide range of applications (Tonheim et al., 2005). Hydrolysates produced by enzymatic activity contain a well-defined peptide and amino acid profile and are more likely to be absorbed by enterocytes compared to high-molecular weight macromolecules (Onal and Langdon, 2009). Hydrolysates are associated with small molecular weight compounds that serve as attractants for fish (Berge and Storebakken 1996; Aksnes et al., 2006; Mamauag et al., 2011), enhance immune response (Kotzamanis et al., 2007), and promote normal skeletal development (Zambonino-Infante et al., 1997). Numerous fish hydrolysate materials have been produced using different types of protease enzymes (pepsin, pancreatin, alcalase, ficin and papain) from plant, animal, and microbial origin (Nordgreen et al., 2009; Benjakul and Morrisey, 1997; Chalamaiah et al., 2010). The use of fish hydrolysates in fish diets improved fish performance in terms of body weight gain, feed efficiency, survival, and intestinal development at an inclusion level of 10-25% for Atlantic cod, *Gadus morhua* (Aksnes et al., 2006), Atlantic salmon, *Salmo salar* (Hevroy et al., 2005) and European sea bass, *Dicentrarchus labrax* (Cahu et al., 1999). Higher levels (>35%) of fish hydrolysate were not recommended as a protein source due to its negative effects on growth and feed efficiency.

In the present study, tuna meat by-products were hydrolyzed using the enzyme Aroase AP-10 derived from *Bacillus subtilis* (from Yakult Pharmaceutical Ind. Co., Ltd. Tokyo, Japan). In the diet of the juvenile red sea bream the hydrolyzed product was evaluated as an ingredient in the diet of to determine its effect on feed efficiency through growth performance parameters, nutrient digestibility of the ingredient, and blood biochemical parameters of the fish.

Materials and Methods

Preparation of the tuna meat by-product hydrolysate. Tuna meat by-product meal (TMP, from Marusho Inc. Shizuoka, Japan) composed of dark meat and bones, was hydrolyzed using a commercially available exogenous enzyme, Aroase AP-10 derived from *Bacillus subtilis*. The TMP was dissolved in 65 g/l distilled water and stirred for 30 min. The pH of the solution was then adjusted to 7-8 with NaOH/1N HCl, after which the solution was heated at 50-60 °C for 2-3 h. The commercial enzyme was added at 0.3 g/l of the solution and heated for an additional 2 h. Enzyme inactivation in the solution was conducted by increasing the temperature to 95-100°C for 30 min. The mixture was then cooled to room temperature and centrifuged at 4500 x g at 4°C for 30 min. The soluble aqueous fraction was then decanted, pooled, and freeze dried to obtain a powder form of TMP protein hydrolysate (TMP-H).

Test Diets. A total of six experimental diets were formulated to contain 0 g/kg of TMP-H (control), 50 g/kg, 150 g/kg and 250 g/kg. Two diets were formulated to contain non hydrolyzed TMP at rates of 50 g/kg and 250 g/kg. All the dietary treatments were isonitrogenous and isolipidic containing 540 g/kg protein and a lipid content of 130 g/kg. Prior to mixing the ingredients, fish meal and TMP ingredients were milled and sieved in a 125µm mesh. The diets were prepared by thoroughly mixing all the dry ingredients in a food processor for 30 min. Blended lipid sources (pollack liver oil and soybean oil) were gradually added and mixed for 15 min. Carboxymethylcellulose (CMC) was carefully dissolved in 300 ml. of water, cooked in a water bath until it was completely dissolved and subsequently added to the mixture to serve as a binder. The dough was then pelletized to 1.2 to 2.2 mm diameter pellets, air dried for 10 min. and oven dried for 1 h

at 60°C. The diets were then stored in a cold room before beginning the experiment. Formulation of the experimental diets is shown in Table 1.

Table 1 Ingredients and proximate composition of diets (g/kg)

Ingredients	Control	TMP-H 5	TMP-H 15	TMP-H 25	TMP 5	TMP 25
Fish meal ^a	700	650	530	400	640	540
Tuna meat by-product ^b	-	-	-	-	50	150
TMP, hydrolysate ^c	-	50	150	250	-	-
Soybean oil ^d	40	40	40	40	40	40
Pollack liver oil ^e	40	40	60	80	40	40
Vitamin ^f	50	50	50	50	50	50
Mineral ^g	50	50	50	50	50	50
Cellulose	70	70	70	80	80	80
CMC	50	50	50	50	50	50
<i>Proximate composition</i>						
Protein	54.7	548.5	550.5	551.3	537.3	547.1
Lipid	13.5.2	132.3	131.8	134.8	133.2	132.9
Moisture	107.3	103.4	102.8	105.7	103.1	103.8
Ash	130.1	132.2	133.5	134.2	130.4	131.8

TMP-H 5 (tuna meat by-product hydrolysate 50 g/kg); TMP-H15 (150 g/kg); TMP-H25 (250 g/kg); TMP5 (tuna meat by-product 50 g/kg); TMP15 (150 g/kg).

^a Nippon Suisan Co. Ltd., Tokyo Japan

^b Marusho Inc., Shimizu, Shizuoka, Japan.

^{c,d} Rikken vitamin, Tokyo, Japan

^{e,f,g} Similar to as reported by Kader et al. (2010)

Feeding trial. The growth experiment was conducted at the Kamoike Marine Laboratory, Faculty of Fisheries, Kagoshima University, Japan for 56 days. Juvenile red sea bream (average initial body weight of 0.82 ± 0.15 g) were stocked in 100 l tank (80 l water volume) 15 fish/tank, in triplicate. All the fish were fed the designated diets twice daily (8:00 and 16:00 h), *ad libitum*. Uneaten feed was collected, freeze dried, and weighed for corrected feed intake calculation. Periodic sampling was carried out every two weeks to monitor growth and survival in the tanks. The seawater flow to the tanks was 1.4 l/min and a photoperiod of 12 h light:12 h dark was maintained throughout the experiment. Water temperature was 26.0 ± 1.3 °C, pH 7.7 ± 0.3, and salinity 32.9 ± 0.8 g/l.

Initial samples from the stock tank of 20 fish were taken for body chemical composition and amino acid analysis. In order to minimize error in proteolytic enzyme activity analysis and body weight data, fish were starved for 24 h before terminal sampling. All fish were anaesthetized with chilled water to ensure ease of handling during blood collection. Total number of fish and individual body weight of fish in each tank were recorded. Three fish from each replicate tank were randomly chosen and stored at -20°C for body chemical composition and amino acid analysis. Fish were dissected for liver and digestive tract samples, individually weighed, and stored at -80°C for further analysis. Blood was collected with heparinized syringes from the caudal vein of the fish in each replicate tank. The blood was then pooled, and kept in a cooler for analysis.

Proximate analysis and body chemical composition. The test diets and fish samples (initial and final) in each dietary treatment were analyzed for moisture with the Sartorius MA 35 moisture analyzer (Goettingen, Germany), and Eyela FDU 1100 (Rikakikai, Co. Ltd., Tokyo, Japan) freeze drier, respectively. Ash and crude protein were analyzed with Tecator Kjeltac Systems, (Hillerød, Denmark) and total lipids were determined with the Soxhlet method using standard AOAC methods (AOAC 1995).

Amino acid analysis. Amino acid, diets, and fish samples were analyzed with high performance liquid chromatography (HPLC, Shimadzu Corp., Kyoto, Japan) according to Teshima et al., (1986). About 2 mg of each dry sample was weighed and hydrolyzed with 4 N-methanesulfonic acid for 22 h at 110 °C for total amino acids analysis. The pH of the hydrolysate was adjusted to pH 2.2, passed through a 45 µm syringe filter (Ministart RC 15, Sartorius Stedim Biotech, Germany) and stored at 4°C for HPLC injection. A known amount of norleucine (0.06 mg) was used as an internal standard. To quantify free amino acid, a 40 mg sample was mixed with 100 µl norleucine (as internal standard 0.6 mg), 900µl cold distilled water, and 2.5 ml of cold 10% trichloroacetic acid (TCA). It was then homogenized; samples were centrifuged at 3000 x g for 15 min at 4°C and washed with

diethyl ether to remove TCA from the homogenate. The pH of the homogenate was then adjusted to 2.2 and diluted to 5 ml with sodium citrate, filtered, and stored at 4°C ready for HPLC injection.

Blood plasma biochemical parameters. Plasma chemical parameters such as hemoglobin, blood urea nitrogen (BUN), glutamyl oxaloacetic transaminase (GOT), glutamic pyruvate transaminase (GPT), total cholesterol (TCH), and total bilirubin (TBB) were measured spectrophotometrically with an automated analyzer (SPOTCHEM EZ Model SP4430i Arkray, Inc., Kyoto, Japan). Biological antioxidant potential (BAP) and reactive oxygen metabolites (d-ROMs) were also measured spectrophotometrically from blood plasma with an automated analyzer FRAS4 (Diacron International s.r.l., Grosseto, Italy) following Morganti et al. (2002). Plasma samples were obtained by centrifugation at 3000 x g for 15 min using a high speed refrigerated microcentrifuge (MX 160, Tomy Tech. Inc., USA).

Non-specific protease activity. Liver and digestive tract samples of fish were washed with distilled water and blotted dry using filter paper. Then 50 mM Tris HCl Buffer solution (pH 7.6) was added at a ratio of 1:4 w/v, homogenized, and samples were centrifuged at 10,000 x g, 4°C for 30 min. The supernatant was then removed and stored at -80°C for protease and protein content assay.

The protease enzyme activity of liver and digestive tract was determined using the f Cupp-Enyard (2008) method with casein as substrate (0.65% w/v casein solution, 50 mM potassium phosphate buffer). In a 15 ml vial, 5 ml of 0.65% casein solution was equilibrated in a water bath at 37°C for 5 min. One ml of the prepared sample was then added to the vial and incubated at 37°C for exactly 10 min. The protease activity and consequential liberation of tyrosine during this incubation time was measured. TCA (5 ml) was then added to stop the reaction. The samples were incubated for 30 min at 37°C after which the solution was filtered with a 0.45 µm filter. Sodium carbonate and Folin's reagent was added to the sample which was again incubated for 30 min at 37°C. After the 30 min incubation, 2 ml of the solution was filtered into the cuvettes and measured in a spectrophotometer at a wavelength of 660 nm.

The calculation to determine the enzyme activity in units per ml was:

$$\text{Units/mg} = (\mu\text{mole tyrosine equivalents released}) \times 11 / (1) \times (10) \times (2)$$

where

11 = total volume (in milliliters) of assay

10 = time of assay (in minutes) as per unit definition

1 = volume of enzyme (in milliliters) of enzyme used

2 = volume (in milliliters) used in colorimetric determination.

Diet digestibility and ingredients. The reference diet formulation used in the digestibility experiment was similar to the control diet used in the growth experiment with a slight modification. The test diet was prepared to contain 70% of the reference diet mixture and 30% of the test ingredient (TMP and TMP-H) following the method of Cho et al. (1982). All diets contained 1 % Cr₂O₃ as an inert indicator. Red sea bream (21 g) from a similar batch of fish used in the growth experiment were randomly stocked in tanks at a stocking density of 12 fish/tank. Water quality parameters were similar to the growth experiment. The fish were acclimated to the control diet for 4 days after which they were fed the reference and test diets twice daily (0700 and 1500) for 20 days. Three days after the initial feeding, feces and uneaten feeds were gently siphoned out 3h after every feeding and rinsed with distilled water. Feces was collected over 21 days, then freeze-dried and stored for proximate analysis for (a) digestibility coefficient of the nutrient in the diet and (b) apparent digestibility coefficient (ADC) of the nutrients in the ingredients which were calculated with the formula described by Cho et al. (1982).

$$(a) \text{ ADC} = 1 - (F / D \times D_i / F_i)$$

D = % nutrient in the diet

F = % nutrient feces

D_i = Cr₂O₃ Diet

F_i = Cr₂O₃ Feed

$$(b) ADC_i = ADC_t + ((1-S) D_r / sD_i) (ADC_t - ADC_r)$$

ADC_t = test diet

ADC_r = reference diet

D_r = % nutrient in reference diet

D_i = % nutrient of the test ingredient

S = proportion of test ingredient in test diet (0.3)

1-s = proportion of reference diet in test diet.

Statistical analysis. Three replicates were assigned to each dietary treatment, using a completely randomized design. Data were tested in Super ANOVA (Abacus Concepts, Berkley, CA, USA) or Sigma Stat 3.5 (Systat Software Inc. San Jose, CA, USA). Differences between diets were tested by one-way ANOVA on tank means and Tukey-Kramer test if means are significant. Relative data limited to 0-100% were arcsine transformed prior to testing. Differences were considered significant at $P < 0.05$.

Results

The proximate composition of the dietary treatments is shown in Table 2.

Table 2 Composition of reference and test diets used in the *in vivo* digestibility (g/kg)

Ingredients	Reference	Test _{TMP}	Test _{TMP-H}
Fish meal	700	490	490
Tuna meat by-product		300	
TMP, hydrolysate			300
Soybean oil	40	28	28
Pollack liver oil	40	28	28
Vitamin	50	35	35
Mineral	50	35	35
Cellulose	60	39	39
CMC	50	35	35
Cr ₂ O ₃	10	10	10

Total essential amino acid and proximate composition of the TMP and TMP-H ingredients indicated similar amino acid profiles, however, protein content increased and lipid content decreased after processing the TMP (Table 3).

Table 3 Amino acid (g/100g dry diet) and proximate composition of TMP and TMP-H ingredients

Amino acid	TMP	TMP-H
EAA		
Methionine	1.3	1.1
Threonine	2.9	2.1
Valine	2.5	2.0
Isoleucine	2.8	2.8
Leucine	5.4	4.5
Phenylalanine	2.5	2.6
Histidine	4.8	4.8
Lysine	4.5	4.6
Tryptophan	0.1	0.1
Arginine	4.5	4.3
Proximate composition (g/kg)		
Protein	721.2	850
Lipid	134.2	11
Ash	92.1	84.1

As summarized in Table 4, the results indicated a significantly ($P < 0.05$) improved weight gain in fish fed the diets supplemented with TMP-H. The TMP-H25 diet exhibited the highest body weight gain by as much as 3271 % in comparison with the rest of the dietary treatments. The body weight of fish fed TMP5 and TMP25 were not significantly ($P > 0.05$) different from those fed the control diet. Feed intake (FI) significantly ($P < 0.05$) improved when fish were fed the diets with TMP-H, with fish fed the TMP-H25 diets showing the highest feed intake. Similarly, feed conversion efficiency (FCE) was significantly improved ($P < 0.05$) in the dietary treatments supplemented with TMP-H regardless of the level of inclusion in comparison to the control diet. However, FI and FCE of fish fed the diets with TMP was not significantly different ($P > 0.05$) with that of the control diet group. After 56 days, survival rates were greater than 90% and were not significantly different among the dietary treatments. Proximate (moisture, crude protein, crude fat and ash) and total amino acid analysis compositions of fish did not exhibit significant ($P > 0.05$) differences in all groups. Moreover, hepatosomatic index did not differ significantly ($P > 0.05$) among fish fed with the experimental diets.

Table 4 Performance and whole body proximate composition (%) of red sea bream juvenile¹

	<i>Initial</i>	<i>Control</i>	<i>TMP-H 5</i>	<i>TMP-H 15</i>	<i>TMP-H 25</i>	<i>TMP 5</i>	<i>TMP 25</i>
Initial body weight (g)		0.82±0.0	0.81±0.1	0.82±0.01	0.82±0.02	0.81±0.0	0.81±0.0
Final body weight (g)		22.6±0.6 ^a	24.9±0.3 ^b	25.3±0.4 ^b	28.5±0.3 ^c	22.7±0.4 ^a	22±0.3 ^a
Survival (%)		93.3±0.0	93.3±0.0	93.3±0.0	95.6±0.0	93.3±0.0	95.6±0.0
Feed intake ²		21.7±0.3 ^a	22±0.6 ^a	22.4±0.5 ^a	24.5±0.3 ^b	21.9±0.3 ^a	21.3±0.3 ^a
FCE ³		1.0±0.0 ^a	1.1±0.0 ^b	1.1±0.1 ^b	1.1±0.0 ^b	0.99±0.0 ^a	0.99±0.0 ^a
HSI ⁴		1.1±0.0	1.1±0.0	1.1±0.0	1.11±0.0	1.08±0.0	1.07±0.0
Protein	9.6±0.2	11.2±0.4	11.2±0.4	11.5±0.3	112.3±0.2	111.3±0.2	111.2±0.5
Fat	1.8±0.8	2.9±0.9	2.8±0.2	2.8±0.9	2.8±0.5	2.7±0.8	2.8±0.4
Ash	2.9±0.3	3.3±0.0	3.3±0.7	3.3±0.0	3.3±0.5	3.3±0.1	3.4±0.1
Moisture	80.2±0.3	81.0±0.3	80.4±0.7	80.1±0.6	80.3±0.7	80.5±0.6	80.2±0.2

¹Each value is the mean ± SEM of data from triplicate groups. Within a row, means with the same letters are not significantly different (P>0.05).

²g dry diet / fish / 56 days

³Feed conversion efficiency = body weight gain / dry feed intake

⁴Hepatosomatic index = (100 x liver weight) / fish weight

Juvenile red sea bream proteolytic enzyme activity from liver and digestive tract indicated that dietary treatment with TMP-H and TMP did not influence (P>0.05) the digestive enzyme capacity of the fish. Blood plasma indicators for liver integrity (TBB, GOT, GPT and BUN) as well as oxidative stress indicators (BAP and d-ROM) did not differ significantly (P>0.05) in all the dietary treatments (Table 5).

Table 5 Blood parameters in red sea bream juvenile¹

	<i>Control</i>	<i>TMP-H 5</i>	<i>TMP-H 15</i>	<i>TMP-H 25</i>	<i>TMP 5</i>	<i>TMP 25</i>
Hb (g/dl)	6.3±0.1	6.6±0.1	6.2±0.1	6.5±0.1	6.4±0.1	6.3±0.0
TBB (g/dl)	0.4±0.3	0.42±0.1	0.41±0.3	0.40±0.2	0.42±0.3	0.4±0.1
GOT (IU/l)	48.3±0.6	47.6±0.2	47.1±0.3	47.2±0.5	47.5±0.6	47.6±0.20
GPT (IU/l)	24.4±0.4	25.2±0.4	24.8±0.3	24.1±0.2	24.7±0.4	24.5±0.5
BUN (mg/dl)	4.3±0.4	4.3±0.3	4.3±0.1	4.24±0.5	4.3±0.4	4.2±0.1
TCH (mg/dl)	327.4±0.7	328.4±0.9	320.8±1.3	320.5±0.6	330.4±0.8	30.6±0.7
BAP (U Carr)	2106±134	2110±89	2107±77	2118±101	2113±114	2109±109
d-ROM (μmol/l)	124±8.2	119±13.2	121±9.4	118±7.4	123±8.9	121±6.3

¹Each value is the mean ± S.E.M. of data from triplicate groups. Within a row, means with the same letters are not significantly different (P>0.05).

²Hb: hemoglobin, TBB: total bilirubin, GOT: glutamyl oxaloacetic transaminase, GPT: glutamic pyruvate transaminase, BUN: blood urea nitrogen, TCH: total cholesterol, BAP; biological antioxidant potential, d-ROM; reactive oxygen metabolites.

Protein and dry matter apparent digestibility coefficient (ADC %) of the reference diet, test diets (TMP and TMP-H) and ingredients (TMP and TMP-H) are presented in Table 6.

Table 6 Percent apparent nutrient digestibility of the reference diet, test diet and test ingredients¹

Ingredient	Test diet		Ingredient	
	Reference	TMP	TMP-H	TMP
ADC _p ²	97.1±0.4	95.3±0.1 ^a	97.8±0.8 ^b	92.6±0.3 ^a
ADC _{DM} ³	83.4±0.2	84.2±0.5 ^a	85.5±0.4 ^b	85.3±0.3 ^a

¹Each value is the mean ± SEM of data from triplicate groups. Within a row, means with the same letters are not significantly different (P>0.05).

²Apparent digestibility coefficient of protein.

³Apparent digestibility coefficient of dry matter.

The results indicated a higher protein digestibility (P<0.05) of the TMP-H ingredient of 98.58% vs. 92.64% (TMP). Similarly, dry matter digestibility was significantly (P<0.05) higher for the TMP-H ingredient (88.34%).

Discussion

The present experiment was conducted to evaluate hydrolyzed TMP as an ingredient for juvenile red sea bream diets. Protein digesting enzyme (subtilisin, a serine endopeptidase) derived from *Bacillus spp.* has been widely used in numerous studies to prepare functional feed protein hydrolysate due to its superior protein recovery, low lipid content, and excellent functional properties (Chalamaiah et al., 2010; Kristinsson & Rasco, 2000; Zhong et al., 2007). The use of commercial enzymes instead of chemicals or endogenous enzymes allows good control of hydrolysis resulting in a superior quality product (Chalamaiah et al., 2010; Kristinsson & Rasco, 2000).

Fishmeal was replaced by up to 50% TMP ingredient in juvenile red sea bream diets without negatively affecting growth (Uyan *et al.*, 2007). When comparing the post and pre hydrolyzed TMP ingredients, it was found that the amino acid profile of the two ingredients did not significantly differ. Protein content of the hydrolyzed TMP was higher due to the solubilization of protein during hydrolysis, the removal of insoluble undigested non-protein substance, and the partial removal of lipid after hydrolysis (Guerard *et al.*, 2002; Safari *et al.*, 2009; Benjakul & Morrissey, 1997). Lipid content of the TMP-H ingredient was reduced due to the process of hydrolysis.

Results of the present experiment indicated that processing TMP through enzymatic hydrolysis, and supplementing diets with it, can improve feed utilization of red sea bream. The highest feed intake was observed in fish fed the TMP-H250 diet. The improved feed intake in fish fed the TMP-H can be attributed to the free amino acid and nucleotides produced during the hydrolysis of the TMP (Aksnes *et al.*, 2006) and the low molecular acidic peptide monosodium glutamate (MSG) potentiating activity (Kristinsson & Rasco, 2000). Enzyme hydrolysis of food proteins allows the improvement or modification of the physicochemical, functional and sensory properties of the native protein without affecting its nutritional value (Chalamiah *et al.*, 2012). The increased feed intake in fish fed the TMP-H diet may also explain the improved weight gain of fish in that treatment as higher feed intake would increase the amount of protein and energy available for fish growth (Mamauag *et al.*, 2011).

The maximum inclusion level of 250 g/kg TMP-H was based on previous studies. Other reports also showed enhanced growth, survival, and feed efficiency when 19% of fish hydrolysate was added in the diet for 10 day old European sea bass, *D. labrax* (Cahu *et al.* 1999). In juvenile Atlantic salmon, *S. salar*, a 20% inclusion of fish hydrolysate (Savoie *et al.*, 2006), and 15% inclusion (Refstie *et al.*, 2004), was suggested. A higher level of inclusion can lead to an inferior growth caused by the increased absorption of free amino acid, di- and tri-peptides that saturate the intestinal transporter mechanisms and imbalance amino acid absorption (Ganapathy *et al.*, 1994; Berge *et al.*, 1999; Aragão *et al.*, 2004; Cahu *et al.*, 1999).

Blood plasma TBB, GOT, GPT, BUN and TCH which are indicative of liver health as well as the oxidative stress indicators d-ROM and BAP were similar in all treatments and their values within the range reported others (Kader *et al.*, 2010; Kader *et al.*, 2011). Blood parameters are important indicators of physiological stress responses as well as general fish health deriving from their nutritional intake. The inclusion of TMP-H did not alter the normal range of blood parameters and did not cause any deleterious effect to fish health. This was also seen in Coho salmon *Oncorhynchus kisutch* (Murray *et al.*, 2003).

The proteolytic enzyme activity of the treated fish did not vary among the dietary treatments, suggesting good adaptation to the diets and an acceptable composition of the dietary treatment (Mamauag *et al.*, 2011).

The apparent digestibility coefficient of protein was improved when the ingredient was hydrolyzed. The ADC of an ingredient is an indicator of the digestive efficiency of ingredients in feeds and their contribution to maintenance and growth (Eusebio *et al.*, 2004). Digestibility of protein and essential amino acids improved when fish hydrolysate was incorporated in the diet of Atlantic salmon (Hevroy *et al.*, 2005; Berge & Storebakken, 1996).

In conclusion, 250 g/kg hydrolysate generated from tuna meat by-product can be utilized as a partial substitution for fishmeal since it improves feed intake, growth and digestibility of feed in juvenile red sea bream. Fractionation and utilization of the bioactive peptides from fish hydrolysate should be conducted to elucidate the specific effect of peptide on fish growth, immune response and nutrient utilization. Furthermore, an economic analysis should be conducted on the utilization of fish hydrolysates on a commercial scale.

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References

- Kader A, M., Koshio S., Ishikawa M., Yokoyama S., Bulbul M., Honda Y., Mamauag R. and A. Laining,** 2011. Growth, nutrient utilization, oxidative condition, and element composition of juvenile red sea bream, *Pagrus major*; fed with fermented soybean meal and scallop by-product blend as fishmeal replacement. *Fisheries Sci.* 77, 119-128.
- Aksnes A., Hope B., Høstmark Ø. and S. Albrektsen,** 2006. Inclusion of size fractionated fish hydrolysate in high plant protein diets for Atlantic cod, *Gadus morhua*. *Aquaculture* 261, 1102-1110.
- Association of Official Analytical Chemists (AOAC),** 1995. Official Methods of Analysis of the the Association of Official Analytical Chemists 15th ed. Association of Official Analytical Chemists, Arlington, VA., USA.
- Aragão C., Conceição L.E.C., Martins D., Rønnestad I., Gomes E. and M.T. Dinis,** 2004. A balanced dietary amino acid profile improves amino acid retention in post-larval Senegalese sole, *Solea senegalensis*. *Aquaculture* 233, 293-304.
- Benjakul S. and M.T. Morrissey,** 1997. Protein Hydrolysates from Pacific Whiting Solid Wastes. *J. Agricult. Food Chem.* 45, 3423-3430.
- Berge G.E., Bakke-McKellep A.M. and E. Lied,** 1999. *In vitro* uptake and interaction between arginine and lysine in the intestine of Atlantic salmon, *Salmo salar*. *Aquaculture* 179, 181-193.
- Berge G.M. and T. Storebakken,** 1996. Fish protein hydrolyzate in starter diets for Atlantic salmon, *Salmo salar*, fry. *Aquaculture* 145, 205-212.
- Cahu C.L., Zambonino Infante J.L., Quazuguel P. and M.M. Le Gall,** 1999. Protein hydrolysate vs. fish meal in compound diets for 10-day old sea bass, *Dicentrarchus labrax*, larvae. *Aquaculture* 171, 109-119.
- Chalamaiah M., Rao G.N., Rao D.G. and T. Jyothirmayi,** 2010. Protein hydrolysates from meriga, *Cirrhinus mrigala*, egg and evaluation of their functional properties. *Food Chem.* 120, 652-657.
- Eusebio P.S., Coloso R.M. and R.E.P. Mamauag,** 2004. Apparent digestibility of selected ingredients in diets for juvenile grouper, *Epinephelus coioides* (Hamilton). *Aquacult. Res.* 35, 1261-1269.
- Ganapathy V., Brandsch M. and F.H. Leibach,** 1994. Intestinal transport of amino acids and peptides. In: Johnson, L.R. (Ed.), *Physiology of the Gastrointestinal Tract*. Raven Press, New York, 1773-1794.
- Guerard F., Guimas L. & A. Binet,** 2002. Production of tuna waste hydrolysates by a commercial neutral protease preparation. *J. Mol. Catal. B: Enzymatic* 19-20, 489-498.
- Hevroy E.M., Espe M., Waagbø R., Sandnes K., Ruud M. and G.I. Hemre,** 2005. Nutrient utilization in Atlantic salmon, *Salmo salar*, fed increased levels of fish protein hydrolysate during a period of fast growth. *Aquacult. Nutr.* 11, 301-313.
- Kader M.A., Koshio S., Ishikawa M., Yokoyama S. and M. Bulbul** 2010. Supplemental effects of some crude ingredients in improving nutritive values of low fishmeal diets for red sea bream, *Pagrus major*. *Aquaculture* 308, 136-144.
- Kotzamanis Y.P., Gisbert E., Gatesoupe F.J., Zambonino Infante J. and C. Cahu,** 2007. Effects of different dietary levels of fish protein hydrolysates on growth, digestive enzymes, gut microbiota, and resistance to *Vibrio anguillarum* in European sea bass, *Dicentrarchus labrax*, larvae. *Comp. Biochem. Phys. A.: Molecular & Integrative Physiology* 147, 205-214.
- Kristinsson H.G. and B.A. Rasco,** 2000. Biochemical and Functional Properties of Atlantic Salmon, *Salmo salar*, Muscle Proteins Hydrolyzed with Various Alkaline Proteases. *J. Agricult. Food Chem.* 48, 657-666.

- Mamauag R.E.P., Koshio S., Ishikawa M., Yokoyama S., Gao J., Nguyen B.T. and J.A. Ragaza,** 2011. Soy peptide inclusion levels influence the growth performance, proteolytic enzyme activities, blood biochemical parameters and body composition of Japanese flounder, *Paralichthys olivaceus*. *Aquaculture* 321, 252-258.
- Morganti P., Bruno C., Guarneri F., Cardillo A., Del Ciotto P. and F. Valenzano,** 2002. Role of topical and nutritional supplement to modify the oxidative stress. *Int. J. Cosmetic Sci.* 24, 331-339.
- Murray A.L., Pascho R.J., Alcorn S.W., Fairgrieve W.T., Shearer K.D. and D. Roley,** 2003. Effects of various feed supplements containing fish protein hydrolysate or fish processing by-products on the innate immune functions of juvenile coho salmon, *Oncorhynchus kisutch*. *Aquaculture* 220, 643-653.
- Nordgreen A., Tonheim S. and K. Hamre,** 2009. Protein quality of larval feed with increased concentration of hydrolysed protein: effects of heat treatment and leaching. *Aquacult. Nutr.* 15, 525-536.
- Onal U., and C. Langdon,** 2009. Potential delivery of water soluble protein hydrolysates to marine suspension feeders by three different microbound particle types. *Aquaculture* 296, 174-178.
- Refstie S., Olli J.J. and H. Standal,** 2004. Feed intake, growth, and protein utilisation by post-smolt Atlantic salmon, *Salmo salar*, in response to graded levels of fish protein hydrolysate in the diet. *Aquaculture* 239, 331-349.
- Safari R., Motamedzadegan A., Ovissipour M., Regenstein J., Gildberg A. & B. Rasco,** 2009. Use of hydrolysates from Yellowfin Tuna, *Thunnus albacares*, heads as a complex nitrogen source for lactic acid bacteria. *Food and Bioprocess Technol.* 1-7.
- Savoie A., Le François N.R., Cahu C., Blier P.U. & I. Andreassen,** 2006. Do protein hydrolysates improve survival and growth of newly-hatched spotted wolffish, *Anarhichas minor*, a non-metamorphic aquaculture fish species? *Aquaculture* 261, 782-788.
- Shih I., Chen L., Yu T., Chang W. & S. Wang,** 2003. Microbial reclamation of fish processing wastes for the production of fish sauce. *Enzyme Microbiol. Technol.* 33, 154-162.
- Tonheim S.K., Espe, M., Hamre, K. and I. Rønnestad,** 2005. Pre-hydrolysis improves utilisation of dietary protein in the larval teleost Atlantic halibut (*Hippoglossus hippoglossus* L.). *J. Exp. Mar. Biol. Ecol.* 321, 19-34.
- Uyan O., Koshio, S., Teshima S.I., Ishikawa M., Michael F.R., Ren T. and A. Laining,** 2007. Effects of tuna muscle powder in diet on the growth and phosphorus loading of juvenile red seabream, *Pagrus major*. *Aquacult. Sci.* 55, 29-40.
- Zhong, F., Zhang, X., Ma, J. & C.F. Shoemaker,** 2007. Fractionation and identification of a novel hypocholesterolemic peptide derived from soy protein Alcalase hydrolysates. *Food Res. Internat.* 40, 756-762.