

## EVALUATION OF TEMPEH AS A POTENTIAL ALTERNATIVE PROTEIN SOURCE IN THE DIETS FOR JUVENILE TIGER GROUPER, *EPINEPHELUS FUSCOGUTTATUS*

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**ABSTRACT** A 56-day feeding trial was conducted to evaluate the potential of replacing fishmeal (FM) with tempeh (TMP) in the diets for juvenile tiger grouper (TG), *Epinephelus fuscoguttatus*. Five isoproteic (50%) and isolipidic (16%) diets containing FM replaced by TMP at 0, 30, 40, 50% (namely TMP0, TMP30, TMP40, TMP50, respectively), and by 30% soybean meal supplemented with phytase (SBM30) were fed to triplicate group of fish (10.09±0.15g). Weight gain (WG) and feed intake (FI) of TMP0 group were significantly higher (201.42% and 1 g fish<sup>-1</sup> day<sup>-1</sup>, respectively) than those fed with TMP-based diets and SBM30 ( $P < 0.05$ ). On the other hand, fish fed with TMP40 attained highest WG (165.55%) among all groups fed with TMP-based diets. Despite the poorer WG and FI, TMP40 and TMP50 groups resulted in better feed conversion ratio (FCR), protein efficiency ratio (PER) and net protein utilization (NPU) ( $P < 0.05$ ) than other diets. Moderate soybean-induced distal intestine enteritis was observed in all fish fed with diets containing soy protein but signs of inflammation became increased as TMP inclusion level increased. The current findings indicated that TMP could be used as a potential alternative protein source in the diet of TG, preferably with addition of essential amino acids and feed stimulants.

**ABSTRAK** Satu percubaan pemakanan selama 56 hari telah dijalankan untuk menilai potensi penggunaan tempeh (TMP) sebagai pengganti tepung ikan (FM) dalam diet juvenil kerapu harimau (TG), *Epinephelus fuscoguttatus*. Lima diet (masing-masing mengandungi 50% protein dan 16% lemak) telah dirumuskan untuk menggantikan FM dalam diet pada aras 0, 30, 40, 50% dengan menggunakan TMP (masing-masing TMP0, TMP30, TMP40, TMP50), dan juga 30% dengan menggunakan tepung kacang soya (SBM30) berserta dengan penambahan enzim phytase. Pada akhir eksperimen, kumpulan TMP0 menunjukkan perbezaan signifikan ( $P < 0.05$ ) dalam penambahan berat (WG) dan jumlah pengambilan makanan (FI) (masing-masing 201.42% and 1g ikan<sup>-1</sup> hari<sup>-1</sup>) berbanding dengan kumpulan lain yang diberi protein soya dalam diet. Sebaliknya, TMP40 menunjukkan penambahan berat paling tinggi (165.55%) dalam kumpulan diet TMP. Walaupun mencapai WG dan FI yang kurang baik, kumpulan TMP40 dan TMP50 menunjukkan nisbah penukaran makanan (FCR), kadar efisiensi protein (PER), dan utilisasi protein bersih (NPU) yang lebih baik dari kumpulan lain ( $P < 0.05$ ). Dalam eksperimen ini, semua ikan yang diberi protein soya dalam diet menunjukkan inflamasi usus yang sederhana tetapi kesan-kesan inflamasi semakin ketara dengan penambahan TMP dalam diet. Keputusan kajian ini menunjukkan bahawa TMP berpotensi menjadi sumber protein alternatif dalam diet juvenil TG sekiranya rasa TMP dipertingkatkan.

**(Keywords:** *Epinephelus fuscoguttatus*, tempeh, soybean meal, feed utilization, palatability)

### INTRODUCTION

Tiger grouper, *Epinephelus fuscoguttatus* is a grouper species which has high commercial value in Asia [1-2]. Lau and Parry-Jones [3] reported that tiger grouper alone made up of 7% (approximately 1,730 t) of the total annual import volume (by weight) of fish imported into Hong Kong in 1997. The common trading size of the fish in Hong Kong reef fish food market is in between 25-50 cm length [4]. Tiger grouper is a popular cultured grouper species because it grows faster than either humpback grouper (*Cromileptes altivelis*) or coral trout, *Plec-*

*tropomus* spp. [2]. Besides that, matured tiger grouper is an important species together with giant grouper (*Epinephelus lanceolatus*) in the production of tiger grouper x giant grouper (TGGG) hybrid grouper, *E. fuscoguttatus* x *E. lanceolatus* [5]. Being carnivorous fish, the fish required high protein diets. The optimal dietary protein and lipid requirement of juvenile tiger grouper have been determined to be 50% and 16%, respectively [6]. Feed is one of the major production cost in grouper culture. In grouper farming, the fish are commonly fed with trash fish or high-fishmeal compound feed [2]. Increasing price of trash fish, poor feed efficiencies and other

disadvantages of relying on trash fish as sole protein source in feeding urged the need to develop cost-effective compound feed for tiger grouper from other alternative protein sources [7].

Soybean protein has been recognized as one of the top protein source in animal diets due to its consistent availability, high yield, relatively high crude protein (CP) and reasonable price [8-9]. Soybean protein has been successfully replaced fishmeal (FM) at approximately 20 to 40% for several carnivorous fish species without reducing growth performance and nutrient utilization including Japanese flounder (*Paralichthys olivaceus*) [10], Florida pompano (*Trachinotus carolinus*) [11], gilthead seabream (*Sparus aurata* L.) [12], Korean rockfish (*Sebastes schlegeli*) [13], Atlantic salmon (*Salmo salar* L.) [14], cobia (*Rachycentron canadum*) [15], parrot fish (*Oplegnathus fasciatus*) [16], tiger grouper (*Epinephelus fuscoguttatus*) [17], and humpback grouper (*Cromileptes altivelis*) [18]. Higher FM replacement level by soybean meal (SBM) in fish feed were proved to be difficult due to the shortage of essential amino acids such as methionine and lysine [19], the present of heat-labile and heat-stable antinutritional factors (ANFs) such as lectins, phytate, saponins [8, 20] as well as lower palatability [21]. Intestinal inflammation or soybean meal-induced enteritis in the distal intestine was observed in several fish species which fed on diet containing SBM [9, 22-24]. One or more of the alcohol soluble components of soy are suspected to be the causative agents of soybean meal-induced enteritis. These factor(s) that induces enteritis in salmonid fish can be removed by alcohol washing using elaborated production of soy protein concentrates [25-26].

Fermentation, a traditional food preservation method, on the other hand has been regards as cheaper alternative methods to improve nutritional values of legume protein sources and producing new and high quality product [27-28]. During fermentation, a diverse of microbial flora play roles in developing flavor, effecting the chemical composition through substrate modification and synthesis of vitamins [29]. Besides that, fermentation of soybean has been reported to be able to boost up non-specific immune system and prevent various physiological abnormalities in fish fed with diets containing fermented soybean (FSBM) [30-31]. These improvements in nutritional and functional properties of fermented soybean compare to

unfermented soybean are probably due to the degradation of soybean allergens and antinutritional factors by microbial proteolytic enzyme and various treatments during fermented soybean production [28, 32-33].

Tempeh (TMP) is a fermented food originated from Indonesia consisting of soybean partially digested and bound together by mycelium of *Rhizopus* spp. mainly *R. oligosporus* [34]. Fermentation of soybean by *R. oligosporus* increased total soluble solids, vitamins, free fatty acids, soluble nitrogen, and free amino acids while no significant changes were observed in total nitrogen and amino acid composition, which allow higher nutrient digestibility by the consumer [27, 32, 35-36]. Kovač and Raspor [27] also reported that during the production of TMP, almost all of the antinutrients such as protease inhibitors, tannins, phytates, lectins are removed, and hence reducing the restriction of nutrient absorption by body. Furthermore, certain undesirable odors and flavors of soybean are destroyed or masked after fermentation [37]. The present study was undertaken to evaluate the potential of TMP as alternative protein source for fish meal (FM) in the diet of juvenile tiger grouper.

## MATERIALS AND METHODS

### Diet preparation

Ingredients and proximate composition of experimental diets are presented in Table 1. Five isoproteic and isolipidic experimental diets (50% crude protein and 16% crude lipid, respectively according to [6]) were formulated to replaced FM by TMP at 0, 30, 40, 50% (namely TMP0, TMP30, TMP40, TMP 50, respectively) and by 30% SBM (SBM30) supplemented with 2000FTU/g phytase (Natuphos® 10000G) as described by [17], to compare the effects of SBM and FSBM in the diet of juvenile tiger grouper. TMP was obtained from local supplier in Kota Kinabalu, Sabah (manufactured through fermentation of soybean using Raprima Tempe Inoculum). Fresh TMP was oven-dried at 40°C for 6 hours before ground into fine powder form. In preparing experimental diets, all dry ingredients were mixed thoroughly followed by approximately 40% of water and lipid sources to become dough. Chromic oxide (Cr<sub>2</sub>O<sub>3</sub>), which was used as an inert marker for digestibility determination, was dissolved in water during mixing. The dough was pelleted through a meat

mincer with the die size of 3mm diameter. The pellets were then dried in oven at 40°C for 6 hours.

All diets were sealed in bags and stored at -20°C until used.

**Table 1.** Diet formulation and approximate composition of the experimental diets

Ingredient (g per 100 g diet)	Diets				
	TMP0	TMP30	TMP40	TMP50	SBM30
Fish meal	66.15	46.30	39.69	33.07	46.30
Soybean meal	0.00	28.82	38.42	48.03	29.97
Fish oil	0.00	0.00	0.76	0.70	0.00
Soybean oil	8.43	3.79	1.49	0.00	10.40
Carboxymethyl cellulose	1.50	1.50	1.50	1.50	1.50
Vitamin Premix*	2.00	2.00	2.00	2.00	2.00
Mineral Premix**	1.00	1.00	1.00	1.00	1.00
Dicalcium phosphate	1.00	1.00	1.00	1.00	1.00
Phytase	0.00	0.00	0.00	0.00	0.02
Chromic oxide	1.00	1.00	1.00	1.00	1.00
Alpha cellulose	12.77	8.99	7.73	6.47	1.28
Tapioca	6.15	5.59	5.41	5.22	5.52
<i>Proximate analysis</i>					
Crude protein	49.95	50.22	50.17	50.31	50.72
Crude lipid	15.83	16.5	15.5	17.02	15.71
Ash	12.32	10.13	9.33	8.33	11.59
Moisture	9.07	10.57	10.37	11.75	11.29

\*Contained (as/g/kg): ascorbic acid, 45; inositol, 5; choline chloride, 75; niacin, 4.5; riboflavin, 1; pyridoxine. HCL, 1; thiamin mononitrite, 0.92; calcium d-panthothenate, 3; retinyl acetate, 0.6; cholecalciferol, 0.083; menadione sodium bisulphate, 1.67; DL- $\alpha$ -tocopheryl acetate (poweder 500IU/g), 8; d-biotin, 0.02; folic acid, 0.09; vitamin B12, 0.00135; cellulose, 854.11.

\*\* Reagent grade. Contained (as g/kg): calcium phosphate. H2O (MDCP), 397.65; calcium lactate, 327; ferrous sulphate.H2O, 25; magnesium sulphate. 7H2O, 137; potassium chloride, 50; sodium chloride, 60; potassium iodide, 0.15; copper sulphate. 5H2O, 0.785; manganese oxide, 0.8; cobalt carbonate, 0.1; zinc oxide, 1.5; sodium selenite. 5H2O, 0.02.

### Experimental animals and feeding trial

The feeding trial was conducted in Fisheries Department of Sabah, Tanjung Badak, Tuaran, Sabah. Tiger grouper (*Epinephelus fuscoguttatus*) juveniles were purchased from private fish farm in Medan, Indonesia. The fish were quarantined and acclimatized in experimental condition and fed with control diet (TMP0) for 2 weeks before feeding trial. At the beginning of feeding trial, the fish were starved for 24 hour and weighed after being anesthetized with Transmore (Nika). 300 of fish with an initial weight of 10.09±0.15 g were randomly distributed into 15 cylindrical floating cages (50 cm depth, 50 cm diameter) placed in 2 tanks of 3 tonnes fiberglass tank, with 20 fish per

cage. Continuous supply of aeration and sand-filtered water (30 L min<sup>-1</sup>) were provided into the tanks. Triplicate groups of fish were hand-fed with experimental diets until apparent satiation twice daily at 0800 hours and 1500 hours for 8 weeks. Numbers of uneaten feed particles were recorded 30 minutes after each feeding for each cage. The average weight of each feed particle was calculated by weighing 3 X 50 particles of each diet. Amount of consumed feed by fish and mortality were checked daily. Uneaten feed and fecal matter were siphoned out after amount of uneaten feeds were recorded. Interval measurements of each replicate were carried out by bulk-weighing once every 2-week throughout feeding trial.

### **Samples collection**

At the end of feeding trial, the fish were starved for 24-hour before harvest. Total number of fish and individual measurements in each cage were recorded. A sample of 5 fish at the beginning of feeding trial and 4 fish per cage at the end were sacrificed and stored frozen (-20°C) for determination of viscerosomatic index (VSI), hepatosomatic index (HSI) and proximate whole body composition. Another fish from each cage were sacrificed and dissected to obtain fish intestine for histological examination. Intestine samples were then fixed in Bouin's solution for 24 hours before transferred to 70% ethanol for further processing.

The remaining fish from same treatment were pooled and distributed into 5 fecal collection tanks respectively. The fish were acclimatized to this new environment for 5 days. The fecal collection tank design was based on [38] with slight modification. Briefly, the 150-L conical-shaped tank has a sloping bottom leading to a centrally located drainage slot, and the effluent feces were directed to and collected in a fecal collection column. Tanks were supplied with aeration and sand-filtered sea water at the rate of 6-L/ min. After acclimatization, the fish were hand-fed to apparent satiation at 0800 hours and 1500 hours. One hour after last daily feeding, the drainpipe and fecal collection column were brush thoroughly to remove feed residues and feces from the tank. Two-third of the water in the tank was drained to ensure complete cleaning. Fecal samples were collected 0730 hours in the following day before daily first feeding and treated as described by [39]. Daily fecal samples from each tank were pooled until sufficient sample for chemical analysis was obtained.

### **Chemical analysis**

Proximate composition analysis on feed ingredients, experimental diets, experimental fish and faecal were performed by the standard methods of AOAC (Association of Official Analytical Chemists, 1995). Dry matter of samples was determined by oven-drying (Memmert Modell 500) at 105°C for 24 hours. Protein was determined by using the Kjeldahl method after acid digestion using and auto Kjeldahl system (Foss Kjeltex 2300 Auto Distillation and Foss Tecator Digester), lipid by ether extraction using Soxhlet method (Soxhlet TM 2043 Extraction unit, Foss Analytical, Sweden), ash by incineration

at 550°C for 5-h in furnace (Carbolite CWF 1300). Cr<sub>2</sub>O<sub>3</sub> content of diets and feces were determined for apparent digestibility coefficients (ADC) of experimental diets by wet-acid method of [40].

For histological examinations of intestine, segment of distal intestine were subsequently dehydrated in ethanol and embedded in paraffin according to standard histological procedures. The distal intestines (DI) were then cross-sectioned into 6µm and mounted on glass-slides, dried briefly and then incubated overnight at 60°C to enhance adherence to the slides. Each slide contained 2 rows of approximately 8 contiguous serial sections. Slides were stained with hematoxylin and eosin (H&E) after dried. Each slide was examined under a light microscope equipped with camera (Nikon Eclipse 80i) for structural changes of mucosal and lamina propria as well as the extends of cellular infiltration into the mucosa of the intestinal folds.

### **Statistical Analysis**

All data (except the ADC of experimental diets) were presented as mean±SD and subjected to one-way ANOVA using SPSS ver21. Duncan's new multiple range test was used to test the differences among individual means. The difference was regarded as significant when  $P < 0.05$ .

## **RESULTS**

### **Growth performances and feed utilization**

Growth performances and feed utilization over the 56-day feeding trial are summarized in Table 2. Among the treatments, TMP0 group showed the highest WG and SGR (201.42% and 1.97, respectively) ( $P < 0.05$ ), followed by SBM30 (175.26% and 1.81, respectively) and TMP 40 (165.55% and 1.74, respectively). An increased in TMP in the diet was associated with subsequent FI reduction ( $P < 0.05$ ). At the highest TMP replacement level (TMP50), fish ate only 44% as much feed as those fish fed on TMP0 ( $P < 0.05$ ). Despite showing lower growth and feed intake, higher TMP level in the diets (TMP40 and TMP50) resulted in better feed utilization compared to other diets ( $P < 0.05$ ). High FCR, PER and NPU values were observed in fish fed with both TMP40 (1.84, 1.12 and 20.67%, respectively) and TMP50 (1.85, 1.09, and 20.49%, respectively). Mortality was observed in each group of fish fed with soy protein-based diet ( $P < 0.05$ ) but not in fish fed with fishmeal-based diet



(100% survival rate). Condition factor (CF) were significantly lower ( $P < 0.05$ ) in fish fed with TMP-based diets (Table 3). Lowest viserosomatic index (VSI) value ( $P < 0.05$ ) was observed in fish fed

with TMP50 (7.45%) but showed no significant difference ( $P > 0.05$ ) in hepatosomatic index (HSI) among treatments.

**Table 2.** Growth performance and feed utilization of fish fed with experimental diets

	Diets				
	TMP0	TMP30	TMP40	TMP50	SBM30
WG	201.42±21.33 <sup>a</sup>	146.3±2.47 <sup>cd</sup>	165.55±12.38 <sup>bc</sup>	135.57±3.30 <sup>d</sup>	175.26±11.56 <sup>b</sup>
SGR	1.97±0.13 <sup>a</sup>	1.61±0.02 <sup>cd</sup>	1.74±0.08 <sup>bc</sup>	1.53±0.03 <sup>d</sup>	1.81±0.08 <sup>b</sup>
FI	1.00±0.07 <sup>a</sup>	0.64±0.03 <sup>c</sup>	0.54±0.03 <sup>d</sup>	0.44±0.00 <sup>e</sup>	0.84±0.06 <sup>b</sup>
FCR	2.98±0.42 <sup>a</sup>	2.45±0.14 <sup>b</sup>	1.84±0.10 <sup>c</sup>	1.85±0.01 <sup>c</sup>	2.84±0.05 <sup>a</sup>
PER	0.69±0.07 <sup>a</sup>	0.81±0.05 <sup>b</sup>	1.12±0.06 <sup>c</sup>	1.09±0.03 <sup>c</sup>	0.71±0.05 <sup>ab</sup>
NPU	13.69±1.15 <sup>b</sup>	15.10±0.33 <sup>b</sup>	20.67±2.04 <sup>a</sup>	20.49±0.53 <sup>a</sup>	14.20±0.78 <sup>b</sup>
Survival	100±00 <sup>a</sup>	93.33±7.64 <sup>ab</sup>	91.67±5.77 <sup>b</sup>	88.33±2.89 <sup>ab</sup>	85.00±5.00 <sup>b</sup>

Values are mean±S.D. of three replicates and values within the same row with different letters are significantly different ( $P < 0.05$ ). Weight gain, WG (%) = 100 x (final mean weight-initial mean weight)/initial mean weight; specific growth rate, SGR (% day-1) = 100x [ln(final mean weight)- ln(initial mean weight)]/days; feed intake, FI (g fish-1 day-1); feed conversion ratio, FCR= feed intake/ (final mean body weight- initial mean body weight); protein efficiency ratio; PER= g gain/ g protein fed; net protein utilization, NPU (%)= 100 x (final fish body protein-initial fish body protein)/ g protein fed; survival (%) = 100 x (final fish number)/ (initial fish number).

**Table 3.** Effect of different experimental diets on condition factor, viscerosomatic index and hepatosomatic index in juvenile tiger grouper

	Diets				
	TMP0	TMP30	TMP40	TMP50	SBM30 CF
CF	1.65±0.08 <sup>a</sup>	1.54±0.02 <sup>b</sup>	1.56±0.01 <sup>b</sup>	1.53±0.02 <sup>b</sup>	1.64±0.05 <sup>a</sup>
VSI	8.93±0.63 <sup>ab</sup>	9.37±1.36 <sup>a</sup>	9.29±0.95 <sup>a</sup>	7.45±1.57 <sup>b</sup>	8.04±1.51 <sup>ab</sup>
HSI	1.05±0.18	1.25±0.21	1.12±0.28	1.05±0.32	1.25±0.29

Values are mean±S.D. of three replicates and values within the same row with different letters are significantly different ( $P < 0.05$ ). Condition factor, CF (%) = 100 x (body weight, g)/ (body length, cm)<sup>3</sup>; viscerosomatic index, VSI (%) = 100x (viscera weight)/ (body weight) ; hepatosomatic index, HSI (%) = 100 x (liver weight)/ (body weight).

### Whole body composition

Whole body composition showed varied responses to dietary treatments (Table 4). Fish fed with TMP0 and SBM30 have the highest protein content (18.25%) compared to TMP-based diets ( $P < 0.05$ ). All fish fed with soy protein-based diets showed significantly lower whole body lipid ( $P < 0.05$ )

compared to TMP0 and the lipid content of fish fed with TMP-based diet decreased as TMP inclusion level increased in the diets. Fish fed TMP0 has the lowest moisture content (69.80%) ( $P < 0.05$ ). Ash content were not significantly different among treatments ( $P > 0.05$ ), ranging from 4.96 to 5.34%.

**Table 4: Effect of different experimental diets on whole body composition in tiger grouper**

	Diets				
	TMP0	TMP30	TMP40	TMP50	SBM30
<i>Whole body</i>					
Protein	18.25±0.22 <sup>a</sup>	17.63±0.38 <sup>b</sup>	17.68±0.41 <sup>b</sup>	17.87±0.49 <sup>ab</sup>	18.25±0.28 <sup>a</sup>
Lipid	6.31±0.22 <sup>a</sup>	5.05±0.67 <sup>b</sup>	4.73±0.69 <sup>b</sup>	4.50±1.52 <sup>b</sup>	5.18±0.36 <sup>b</sup>
Moisture	69.80±0.56 <sup>c</sup>	71.79±1.80 <sup>a</sup>	71.00±0.82 <sup>ab</sup>	71.68±1.17 <sup>a</sup>	70.46±0.74 <sup>b</sup>
Ash	5.24±0.32	4.96±0.26	5.34±0.35	5.20±0.60	5.13±0.25

Values are mean±S.D. of three replicates and values within the same row with different letters are significantly different ( $P<0.05$ ).

**Digestibility of dry matter and nutrients**

Apparent digestibility of dry matter, crude protein and lipid of dietary treatments for tiger grouper are shown in Table 5. Apparent digestibility of dry

matter, crude protein and crude lipid among diet are relatively high (ranging 81.78-89.87%, 93.18-94.83% and 91.36-99.23%, respectively) except for fish fed with TMP50 resulted in poor apparent lipid digestibility (78.21%).

**Table 5. Apparent digestibility coefficients of the experimental diets in digestibility test**

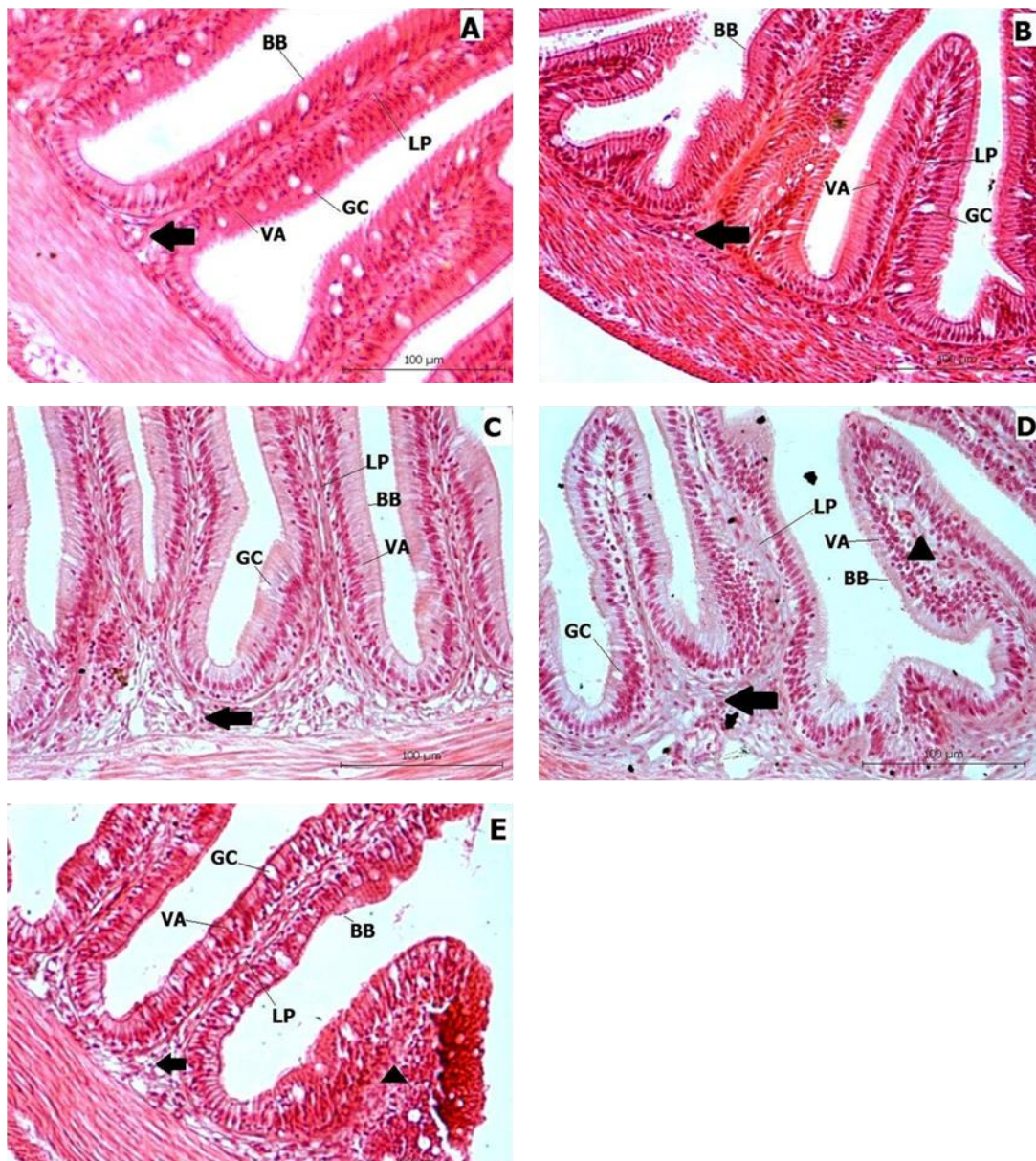
	Diets				
	TMP0	TMP30	TMP40	TMP50	SBM30
DM%	86.13	88.01	87.52	81.78	89.87
CP%	93.18	94.83	94.38	93.81	94.05
CL%	99.23	91.36	96.16	78.21	93.26

ADC of dry matter (%) = 100- [chromium oxide in feed (%) / chromium oxide in fecal (%)] ; ADC of nutrient = 100- [100 x (chromium oxide in feed (%) / chromium oxide in fecal (%)) x (nutrient in fecal (%) / (nutrient in feed (%))]

**Microscopy**

Feeding of soy protein-based diets during the feeding trial have slightly altered distal intestine histology (Figure 1). Widening and shortening of villi in DI were observed in all fish fed with soy protein-based diets but increased signs of inflammation (increasing cellular infiltration intensity and width of lamina propria and submucosa layer) were observed in fish fed with TMP50 and SBM30. Fish fed with TMP30 showed almost similar DI tract histology to TMP0 except the shortening of villi. Fish fed with SBM30 showed shorter villi, mild thickening

of lamina propria but normal cellular infiltration. Reduction of number of goblet cell was observed in TMP40 and TMP50 as TMP level increased in the diets. Compared to unfermented soybean meal diet (SBM30), FM replaced by TMP at the same inclusion level (TMP30) showed partial improved soybean-induced enteritis in distal intestine of tiger grouper.



**Figure 1.** Distal intestine histology of fish fed TMP0 (A), TMP30 (B), TMP40 (C), TMP50 (D) and SBM30 (E). Fish fed soy protein-based diets exhibit overall shortening and fusion of mucosal folds. Cellular infiltration (arrow) and width of lamina propria (triangle) increased as TMP inclusion level increased. VA, absorptive vacuole; GC, goblet cell; LP, lamina propria; BB, brush border.

## DISCUSSION

In the present study, fish fed with TMP-based diets attained lower growth rate compared to FM-based (TMP0) or SBM-based diet (SBM30). Among all TMP-based diets, FM substitution by TMP at 40% showed the optimum growth rate, which is higher than *E. coioides* (10% FM substitution by FSBM)

as reported by [41]. Although performances of fish fed SBM30 with the supplementation of phytase showed higher growth rate than TMP-based diets, the growth rate was not comparable to fish fed with FM-based diet as previously described [17]. This might be due to larger fish ( $44.57 \pm 0.28$ g) used in previous study has higher tolerance toward SBM-based diet than smaller fish ( $10.09 \pm 0.15$ g) used in



the present study. Despite exhibiting poor growth performances, positive results in FCR, PER and NPU indicate that fish were able to utilize nutrients in TMP better than fish fed with unfermented SBM. Several studies have reported that soluble protein, glucose, free amino acid content, vitamin and other nutrients increased sharply after fermentation and thus improving digestion and feed utilization [28, 32-33, 36]. Yamamoto *et al.* [31] reported that few studies showed nutritional value of SBM was able to improve through fermentation, though the effect on improvement of the growth performance was very limited as seen in present study. Poor palatability was suspected to be the main constraint restricting the higher inclusion of TMP in the diet based on poor feed intake in TMP-based diets. FI decreased significantly as TMP inclusion level increased in the diet, resulted in poor growth performances as described in other plant protein-based diets [12]. Results in FI and growth reduction were also reported in weanling rats fed with diets containing TMP [42]. The authors suspected that depressed acceptance of TMP-based diet was due either to the mold or something elaborated by the mold of the *Rhizopus* species. In order to achieve higher TMP inclusion level in the diet, the use of feeding stimulant should be considered in recovering depleted FI.

Fish fed TMP-based diets showed lower whole body protein and lipid and higher moisture content when compared to fish fed with TMP0, but the contents remained constant among TMP-based diets. Similar results were shown in other studies in which whole body lipid decreased inversely with the increased of whole body moisture when SBM inclusion increased in the diets of Japanese flounder and saddled bream [10, 43]. Antović *et al.* [43] suggested that the reason of depleting of whole body lipid when SBM was fed at higher level might be due to the effect of alcohol-soluble components of SBM comprise antinutrients. Soy polysaccharide, in the form of indigestible nonstarch polysaccharides, was reported to have negative effects on lipid absorption in Atlantic salmon. Although fermentation was reported to be able to reduce or eliminate the antinutrients presented in soybean, extend of the reduction and effects of remaining antinutrients in TMP to the fish are uncertain. Zhou *et al.* [44] in their study suggested that although fermentation may reduce the amount of soybean-based antinutrients, it is possible that the process may also have paved

the way for the occurrence for other antinutrients. Another possible explanation of whole body lipid reduction might be due to the catabolism of the lipid as energy source during starvation caused by low feed intake when TMP inclusion level increased in the diet [45-47].

The apparent digestibility coefficients (ADCs) of both protein and dry matter in all dietary treatments were considered high, ranging from 93.18-94.83% and 81.78-89.97%, respectively when compared to black sea bream and Chinese sucker fed on FSBM-based diets [44,48]. These indicate that the TMP and SBM are well digested by the fish as previously described [17]. However, tiger grouper showed poor apparent digestibility of lipid when FM was replaced by TMP at 50%. Elevated amount of antinutrients as TMP inclusion increased were believed to be the causative agent of poor apparent digestibility of lipid. In addition, poor nutrient absorption and digestion may be related to subsequent dysfunction of distal intestine due to soybean-induced enteritis as TMP inclusion level increased. Absorptive captivity for nutrients were reported to reduce corresponds to soybean-induced enteritis severity in Atlantic salmon [24-25]. After 56-day of introduction to soy protein in present study, tiger grouper showed only moderate inflammation compared to full distal intestine inflammation in Atlantic salmon when fed with extracted SBM-containing diet in just 21 days [49]. The absence of consistent intestinal inflammatory changes in the tiger grouper fed with soy protein diets indicates that this species might have better tolerance to diets with high levels of soy protein.

## CONCLUSION

Although growth advantage was not seen as a result of using TMP in the diets, feed utilization efficiency of TMP-based diets are comparable to or better than fishmeal-based and SBM-based diet. Fish fed with TMP resulted in partial improvement of soybean-induced enteritis compared to unfermented SBM. Results showed that growth performance could be enhanced if the palatability of the TMP-based diets can be improved to increase feed intake. The effect of supplementation of essential amino acid and feeding stimulant to improve the diets palatability should be investigated to determine the full potential of TMP as a source of protein in the diets of grouper.



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## REFERENCES

1. Giri, N.A., Suwirya, K., and Marzuqi, M. (2004). Optimum level of dietary protein and lipid for rearing juvenile tiger grouper (*Epinephelus fuscoguttatus*). In: MA Rimmer, S McBride, K.C. Williams. Advances in grouper aquaculture. Canberra: Australian Centre for International Agricultural Research; p. 92-94.
2. Rachmansyah, Usman, Palinggi, N.N., and Wiliams, K. (2009). Formulated feed for tiger grouper grow-out. In: Wilkinson, S., editor. Aquaculture Asia Magazine Vol. XIV No.2 April-June 2009. Bangkok, Thailand: Asia-Pacific Marine Finfish Aquaculture Network-Network of Aquaculture Centres in Asia-Pacific (NACA); p 30-35.
3. Lau, P., and Parry-Jones, R. (1999). The Hong Kong trade in live fish for food. Hong Kong: TRAFFIC East Asia and World Wide Fund for Nature Hong Kong, pp 1-6.
4. Lee, C., and Sadovy Y. (1998). A taste for live fish: Hong Kong's live reef fish market. Naga, The ICLARM Quarterly. 21: 38-42.
5. Luin, M., Ching, F.F., and Senoo, S. (2013). Sexual maturation and gonad development in tiger grouper (*Epinephelus fuscoguttatus*) x giant grouper (*E. lanceolatus*) hybrid. J Aquac Res Development. 5:213. doi: 10.4172/2155-9546.1000213.
6. Shapawi, R., Ebi, I., Yong, A.S.K., and Ng, W.K. (2014). Optimizing the growth performance of brown-marbled grouper, *Epinephelus fuscoguttatus* (Forsk.), by varying the proportion of dietary protein and lipid levels. Anim Feed Sci Tech. 191: 98-105.
7. Sim, S., Rimmer, M.A., Williams, K., Toledo, J.D., Sugama, K., Rumengan, I., and Philips, M.J. (2005). A practical guide to feeds and feed management for cultured groupers. Network of Aquaculture Centres in Asia-Pacific.
8. Drew, M.D., Borgeson, T.L., and Thiessen, D.L. (2007). A review of processing of feed ingredients to enhance diets digestibility in finfish. Anim Feed Sci Tech. 138: 118-136.
9. Peng, M., Xu, W., Ai, Q.H., Mai, K.S., Liufu, Z.G., and Zhang, K.K. (2013). Effects of nucleotide supplementation on growth, immune responses and intestinal morphology in juvenile turbot fed diets with graded levels of soybean meal (*Scophthalmus maximus* L.). Aquaculture. 392-395: 51-58.
10. Deng, J.M, Mai, K.S., Ai, Q.H., Zhang, W.B., Wang, X.J., Xu, W., and Liufu, Z.G. (2006). Effects of replacing fish meal with soy protein concentrate on feed intake and growth of juvenile Japanese flounder, *Paralichthys olivaceus*. Aquaculture. 258: 503-513.
11. Quintero, H.E., Davus, D.A., and Rhodes, M.A. (2012). Soy protein concentrate as an alternative ingredient in florida pompano (*Trachinotus carolinus*) diets. J Appl Aquac. 24: 247-261.
12. Kissil, G.W., Lupatsch, I., Higgs, D.A., and Hardy, R.W. (2000). Dietary substitution of soy and rapeseed protein concentrates for fish meal, and their effects on growth and nutrient utilization in gilthead seabream *Sparus aurata* L. Aquac Res. 31: 595-601.
13. Lim, S.R., Choi, S.M., Wang, X.J., Kim, K.W., Shin, I.S., Min, T.S., and Bai, S.C. (2004). Effects of dehulled soybean meal as a fish meal replacer in diets for fingerling and growing Korean rockfish *Sebastes schlegelii*. Aquaculture. 231: 457-468.
14. Carter, C.G., and Hauler, R.C. (2000). Fish meal replacement by plant meals in extruded feeds for Atlantic salmon, *Salmo salar* L. Aquaculture. 185: 299-311.
15. Chou, R.L., Her, B.Y., Su, M.S., Hwang, G., Wu, Y.H., and Chen, H.Y. (2004). Substituting fish meal with soybean meal in diets in juvenile cobia *Rachycentron canadum*. Aquaculture. 229: 325-333.

16. Lim, S.J., and Lee, K.J. (2009). Partial replacement of fish meal by cottonseed meal and soybean meal with iron and phytase supplementation for parrot fish *Oplegnathus fasciatus*. *Aquaculture*. 290: 283-289.
17. Shapawi, R., Ebi, I., Yong, A., Chong, M., Lu, K.C., and Sade, A. (2013). Soybean meal as a source of protein in formulated diets for tiger grouper, *Epinephelus fuscoguttatus* juvenile. Part II. Improving diet performances with phytase supplementation. *Agr Sci*. 4: 19-24.
18. Rahmansyah, Usman, Makmur, and Ahmad, T. (2005). Substitution of fishmeal with soybean meal in humpback grouper, *Cromileptes altivelis* juvenile diets supplemented with phytase. *Indones Fish Res J*. 11: 73-80.
19. Alam, M.S., Tesima, S., Koshio, S., Ishikawa, M., Uyan, O., Hernandez, L.H.H., and Michael, F.R. (2005). Supplemental effects of coated methionine and/ or lysine to soy protein isolate diet for juvenile kuruma shrimp, *Marsupenaeus japonicas*. *Aquaculture*. 248: 13-19.
20. Francis, G., Makkar, H.P.S., and Becker, K. (2001). Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture*. 199: 197-227.
21. Kader, M.A., Bulbul, M., Koshio, S., Ishikawa, M., Yokoyama, S., Nguyen, B.T., and Komilus, C.F. (2012). Effect of complete replacement of fishmeal by dehulled soybean meal with crude attractants supplementation in diets for red sea bream, *Pagrus major*. *Aquaculture*. 350-353: 109-116.
22. Murray, H.M., Lall, S.P., Rajaselvam, R., Boutilier, L.A., Blanchard, B., Flight, R.M., Colombo, S., Mohindra, V., and Douglas, S.E. (2010). A nutrigenomic analysis of partial soybean meal replacement in diets for juvenile Atlantic halibut, *Hippoglossus hippoglossus*, L. *Aquaculture*. 298. 282-293.
23. Venold, F.F., Penn, M.H., Krogdahl, Å., and Overturf, K. (2012). Severity of soybean meal induced distal intestinal inflammation, enterocyte proliferation rate, and fatty acid binding protein (Fabp2) level differ between strains of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. 364-365: 281-292.
24. Sahlmann, C., Sutherland, B.J.G., Kortner, T.M., Koop, B.F., Krogdahl, A., and Bakke, A.M. (2013). Early response of gene expression in the distal intestine of Atlantic salmon (*Salmo salar* L.) during the development of soybean meal induced enteritis. *Aquaculture*. 34: 599-609.
25. Refstie, S., Sahlström, S., Bråthen, E., Baeverfjord, G., and Krogedal, P. (2005). Lactic acid fermentation eliminates indigestible carbohydrates and antinutritional factors in soybean meal for Atlantic salmon (*Salmo salar*). *Aquaculture*. 246: 331-345.
26. Refstie, S., Landsverk, T., Bakke-Mckellep, A.M., Ringø, E., Sundby, A., Shearer, K.D., and Krogdahl, Å. (2006). Digestive capacity, intestinal morphology, and microflora of 1-year and 2-year old Atlantic cod (*Gadus morhua*) fed standard or bioprocessed soybean meal. *Aquaculture*. 261: 269-284.
27. Kovač, B., and Raspor, P. (1997). The use of mould *Rhizopus oligosporus* in food production. *Food Technol Biotech*. 35: 69-73.
28. Amadou, I., Kamara, M.I., Tidjani, A., Foh, M.B.K., and L, G.W. (2010). Physicochemical and nutritional analysis of fermented soybean protein meal by *Lactobacillus plantarum* Lp6. *World J Dairy Food Sci*. 5: 114-118.
29. Nout, M.J.R., and Rombouts, F.M. (1990). Recent developments in tempe research. *J Appl Bacteriol*. 69: 609-633.
30. Kim, S.S., Galaz, G.B., Pham, M.A., Jang, J.W., Oh, D.H., Yeo, I.K., and Lee, K.J. (2009). Effects of dietary supplementation of a meju, fermented soybean meal, and *Aspergillus oryzae* for juvenile parrot fish (*Oplegnathus fasciatus*). *Asian Austral J Anim*. 22: 849-856.
31. Yamamoto, T., Iwashita, Y., Matsunari, H., Sugita, T., Furuita, H., Akimoto, A., Okamatsu, K., & Suzuki, N. (2010). Influence of fermentation conditions for soybean meal in a non-fish meal diet on the growth performance and physiological condition of rainbow trout *Oncorhynchus mykiss*. *Aquaculture*. 309: 173-180

32. Astuti, M., Meliala, A., Dalais, F., and Wahlqvist, M.L. (2000). Tempe, a nutritious and healthy food from Indonesia. *Asia Pac J Clin Nutr.* 9: 322-325.
33. Bavia, A.C.F., Silva, C.E.D., Ferreira, M.P., Santos Leite, R., Mandarino, J.M.G., and Carrão-Panizzi, M.C. (2012). Chemical composition of tempeh from soybean cultivars specially developed for human consumption. *Ciênc. Technol. Aliment.* 32: 613-620.
34. Jurus, A.M., and Sunberg, W.J. (1976). Penetration of *Rhizopus oligosporus* into soybeans in tempeh. *Appl Environ Microb.* 32: 284-287.
35. Wang, H.L., Ruttle, D.I., and Hesseltine, C.W. (1967). Protein quality of wheat and soybeans after *Rhizopus oligosporus* fermentation. *J Nutr.* 96: 109-114.
36. Babu, P.D., Bhagyaraj, R., and Vidhyalakshmi, R. (2009). A low cost nutritious food “tempeh”- A review. *World J Dairy Food Sci.* 4: 22-27.
37. Zamora, R.G., and Veum, T.L. (1988). Nutritive value of whole soybeans fermented with *Aspergillus oryzae* or *Rhizopus oligosporus* as evaluated by neonatal pigs. *J Nutr.* 118: 438-444.
38. Biswas, A.K., Seoka, M., Takii, K., and Kumai, H. (2007). Comparison of apparent digestibility coefficient among replicates and different stocking density in red sea bream. *Fisheries Sci.* 73: 19-26.
39. Lin, H.Z., Liu, Y.J., Tian, L.X., Wang, J.T., Zheng, W.H., Huang, J.N., and Chen, P.J. (2004). Apparent digestibility coefficients of various feed ingredients for grouper *Epinephelus coioides*. *J World Aquacul Soc.* 35: 134-142.
40. Furukawa, A., and Tsukahara, H. (1966). On the acid digestion method for the determination of chromic oxide as an index substance in the study of digestibility in fish feed. *B Jpn Soc of Sci Fish.* 32: 502-508.
41. Luo, Z., Liu, Y.J., Mai, K.S., Tian, L.X., Liu, D.H., and Tan, X.Y. (2004). Partial replacement of fish meal by soybean protein in diets for grouper *Epinephelus coioides* juveniles. *J Fish China.* 28: 175-181.
42. Hackler, L.R., Steinkraus, K.H., VanBuren, J.P., and Hand, D.B. (1964). Studies on the utilization of Tempeh Protein by Weanling Rats. *J Nutr.* 82: 452-456.
43. Antović, N., Kožul, Antović, and Bolotin, J. (2012). Effects of partial replacement of fish meal by soybean meal on growth of juvenile saddled bream (Sparidae). *Turk J Fish Aquat Sc.* 12: 247-252.
44. Zhou, F., Song, W.X., Shao, Q.J., Peng, X., Xiao, J.X., Hua, Y., and Owari, B.N. (2011). Partial replacement of fish meal by fermented soybean meal in diets for Black sea bream, *Acanthopagrus schegeliai*, Juveniles. *J World Aquacult Soc.* 42: 184-196.
45. Savitz, J. (1971). Effects of starvation on body protein utilization of bluegill sunfish (*Lepomis macrochirus* Rafinesque) with a calculation of caloric requirements. *T Am Fish Soc.* 100: 18-21.
46. Niimi, A.J. (1972). Changes in the proximate body composition of largemouth bass (*Micropterus salmoides*) with starvation. *Can J Zool.* 50: 815-819.
47. Jobling, M. (1980). Effects of starvation on proximate chemical composition and energy utilization of plaice, *Pleuronectes platessa* L. *J Fish Biol.* 17: 325-334.
48. Yuan, Y.C., Lin, Y.C., Yang, H.J., Gong, Y., Gong, S.Y., and Yu, D.H. (2012). Evaluation of fermented soybean meal in the practical diets for juvenile Chinese sucker, *Myxocyprinus asiaticus*. *Aquac Nutr.* 19: 75-83.
49. Marjara, I.S., Chikwati, E.M., Valen, E.C., Krogdahl, A., and Bakke, A.M. (2012). Transcriptional regulation of IL-17A and other inflammatory markers during the development of soybean meal-induced enteropathy in the distal intestine of Atlantic salmon (*Salmo salar* L.). *Cytokine.* 60: 186-196.