Malaysian Applied Biology (2022) 51(6): 73-83
https://doi.org/10.55230/mabjournal.v51i6.2466

Research Article

The Performances of Hybrid Grouper, *Epinephelus fuscoguttatus* × *E. lanceolatus* Fed with Defatted Soybean Meal-Based Feeds with Supplementation of Phytase

Norfazreena Mohd Faudzi[1], Rossita Shapawi[1], Shigeharu Senoo[1,2], Amal Biswas[3] and Annita Seok Kian Yong[1*]

[1]Borneo Marine Research Institute, Universiti Malaysia Sabah, Jalan UMS, 88400, Kota Kinabalu, Sabah, Malaysia
[2]UMS-Kindai Aquaculture Development Centre, Kindai University, Shirahama, Wakayama, 649-2211, Japan
[3]Fisheries Laboratory, Kindai University, Uragami, Wakayama, 649-5145, Japan
*Corresponding author: annitay@ums.edu.my

ABSTRACT

Fish meal is the primary protein ingredient in fish feed and this material is expensive and non-renewable. However, the use of plant protein in marine fish feed such as soybean meal products are limited due to anti-nutritional factor that hindered fish performances. Therefore, hybrid grouper, a cross between tiger grouper (*Epinephelus fuscoguttatus*) and giant grouper (*E. lanceolatus*) was evaluated using defatted soybean meal (DSM) based feed and phytase. DSM- based feeds (30% of protein replacement) with supplementation of phytase at 0 and 2000 FTU/kg dosages, as well as a control feed (CON) comprised completely of fish meal (FM) was given to the hybrid grouper with an initial body weight of 6.2±0.0g. Each experimental feed was formulated with 50% of crude protein and 12% of crude lipid. The fish were raised in 100L fiberglass tanks equipped with a flow-through water system for each triplicate treatment. The hybrid grouper was fed with the respective feeds twice a day until the perceived satiation level for 12 weeks. Hybrid grouper fed CON and DSM-based feed did not exhibit any significant difference in growth. However, hybrid grouper fed with DSM-based feed grew larger compared to that fed with CON. Hybrid grouper fed with DSM-based feed showed significantly higher feed intake, lower net protein utilization, hepatosomatic index, viscerosomatic index, and apparent digestibility coefficient (ADC) protein values ($p<0.05$). The value of ADC of phosphorus was slightly higher in DSM-based feed supplemented with phytase at a dosage of 2000 FTU/kg. No significant effect was observed on body proximate composition, morphological condition of the intestine, and ADC of lipids in all the treatments. The results show that hybrid grouper can effectively consume 30% DSM-based feed and supplementing phytase did not affect the fish performances.

Key words: Defatted soybean meal, fish performance,hybrid grouper, phytase

Article History
Accepted: 14 December 2022
First version online: 31 December 2022

Cite This Article:
Mohd Faudzi, N., Shapawi, R., Senoo, S., Biswas, A. & Yong, A.S.K. 2022. The performances of hybrid grouper, *Epinephelus fuscoguttatus* × *E. lanceolatus* fed with defatted soybean meal-based feeds with supplementation of phytase. Malaysian Applied Biology, 51(6): 73-83. https://doi.org/10.55230/mabjournal.v51i6.2466

INTRODUCTION

In the aquaculture feed industry, a variety of soybean products are used as a protein replacement for fish meal (FM) (Biswas *et al*., 2017; Mohd Faudzi *et al*., 2017; Yong *et al*., 2020). Defatted soybean meal (DSM) is one of the soybean products that are not only commonly used in aquaculture feed but also for livestock (Dei, 2011; National Research Council, 2011). It is easily available and cheaper than FM and other soybean products. In aquaculture, DSM can partially replace 20 to 60% of FM in the feeds for marine carnivorous fish species such as red sea bream (*Pagrus major*), sharpsnout sea bream (*Diplodus puntazzo*), Japanese flounder (*Paralichthys olivaceus*), pompano (*Trachinotus blochii*) and Korean rockfish (*Sebastes schlegeli*) (Choi *et al*., 2004; Lim *et al*., 2004; Deng *et al*., 2006; Biswas *et al*., 2007; Hernàndez *et al*., 2007; Pham *et al*., 2007; Nguyen *et al*., 2021). Tiger grouper (*Epinephelus fuscoguttatus*) can tolerate 20 to 30% of DSM protein in formulated feed (Shapawi *et al.,* 2013a). Meanwhile, Chor *et al*. (2015) and Garcia-Ortega *et al*. (2016) have reported that feeding juvenile tiger grouper and giant grouper (*E. lanceolatus*) with DSM frequently results in irritation of intestine or soybean meal-induced enteritis in the distal intestine, which reduces the distal intestine's capacity to absorb and digest the nutrients.

One of the naturally occurring anti-nutritional components in plant proteins such as soybean meal, canola meal, and others is phytate (Makkar & Becker 2009; Kumar *et al*., 2012, Ávila *et al*., 2015). Generally, the presence of phytate in feed containing plant proteins causes phosphorus to bind to the materials and chelate with other nutrients, which inhibits fish development, feed utilization, and nutrient digestion (Kumar *et al*., 2012). Because fish is not capable of breaking down phytate, important nutrients are lost and discharged into the environment (Baruah *et al*., 2004; Kumar *et al*., 2012). Due to this, utilization of DSM as FM replacement in formulated feed becomes limited, especially for marine carnivorous species (Kumar *et al*., 2012; Mohd Faudzi *et al*., 2017). Additionally, it has been found that supplementing 2000 FTU/kg phytase together with 30% of DSM may boost the growth and feed utilization of tiger grouper (Shapawi *et al*., 2013b). The growth performances, feed utilization, and nutrient digestibility of the red sea bream were also shown to be enhanced by the supplementing of 2000 FTU/kg phytase when fed with DSM-based feeds (Biswas *et al*., 2007). However, supplementing phytase to DSM-based feed at a dosage of 1000 and 2000 FTU/kg did not enhance the growth performances, protein digestibility, and energy retention of seabass (*Dicentrarchus labrax*) (Olivia-Teles *et al*., 2001). Therefore, the previous study suggested that supplementation of phytase as an exogenous enzyme source can help to boost phosphorus digestibility, thereby improving the availability of nutrients, the utilization of feed, and the growth performance of fish (Maas *et al*., 2021).

The hybrid grouper, a cross between tiger grouper and giant grouper has demonstrated better growth performances, survival, and feed utilization than other grouper species, making it one of the most successful hybrid groupers (Chor *et al*., 2015; Anthonius *et al*., 2017; Mohd Faudzi *et al*., 2017; Ebi *et al*., 2018; Yong *et al*., 2019). The previous study revealed that hybrid grouper can feed on DSM without any drawback on palatability until 60% of protein replacement (Firdaus *et al*., 2016). Similarly, it was found that hybrid grouper consumed an experimental feed containing a high level (60%) of soy protein concentrate (SPC) (Mohd Faudzi *et al*., 2017). However, when the DSM and SPC were increased from 40 to 80% (Firdaus *et al*., 2016) and 30 to 60% (Mohd Faudzi *et al*., 2017), a tendency for declining growth of hybrid grouper was observed. Additionally, no information is available on the supplementation of phytase in DSM feed on hybrid grouper. In the Southeast Asia region including Malaysia, Indonesia, and Singapore, the hybrid grouper is regarded as a significant aquaculture fish. Thus, the objective of this study is to determine the performances of hybrid grouper fed with DSM-based feeds with supplementation of phytase. The performances are measured through growth, survival, feed utilization, body proximate composition and condition indices, morphological condition of intestine and apparent digestibility coefficients.

MATERIALS AND METHODS

Experimental feed preparation

Experimental feeds (crude protein: 50%; crude lipid: 12%) (Mohd Faudzi *et al*., 2017; Yong *et al*., 2020) were formulated with 30% of DSM protein replacement at two concentrations of phytase: 0 and 2000 FTU/kg (DSM_0 and DSM_{2000}) and feed with 100% of FM protein was used as a control feed (CON). Danish FM (crude protein: 74.8%, crude lipid: 6.5%) and DSM (crude protein: 49.0%, crude lipid: 0.3%) were used as the source of protein while industrial-grade fish oil from Dexchem Sdn. Bhd. aided as the main lipid source in the experimental feeds. Corn gluten meal was supplemented with the DSM-based feed to ensure that the essential amino acids are balanced for the fish (Biswas *et al*., 2017). All the ingredients were then homogenized to form the experimental feeds (Table 1). Chromium oxide was introduced (at a concentration of 0.5%) as an inert marker for the apparent digestibility coefficient (ADC) nutrient analysis. Distilled water was used to dissolve the phytase (Natuphos® 10000G, BASF). After that, it was combined with the mixture of ingredients to form a moist dough. A 3-mm dice meat mincer (Orimas®, TBS 200, Taiwan) was used to force the moist dough through. The moist pellet was then dried in a 40 °C oven (Ming-Li Electric MFG, BS-2210, China). The condition of experimental feeds was maintained in an air-tight container and kept in the fridge (4 °C) until further use. Table 1 shows the proximate composition of the experimental feeds.

Fish rearing

Prior to the experiment, hybrid grouper with an initial body weight of 6.2±0.0 g were given CON feed to acclimate to the experimental condition for a week. The fish were stocked at 20 fish per tank into a 100 L of conical fiberglass tank at random. For each treatment, three replicate groups of fish were fed with respective feeds at 0830 and 1430 until they appeared to be full. Throughout the feeding experiment, a flow-through water system (flow rate: 5 L/min) was used. The growth and survival of the fish were assessed every two weeks.

Three hours after the experimental fish was fed with the respective feeds, the rearing tanks were cleansed and cleaned to remove uneaten feed. The fresh feces were then collected from the fecal collector. Distilled water was used to rinse the collected fecal sample. The collected fecal sample was then tapped to dry with dry tissue and stored at -80 °C for the following analysis. At the end of the 12-week experiment, the fish growth was measured and the surviving fish was counted. The fish from each treatment (n=9) were fasted for 24 hours and sacrificed to calculate the body indices. The value of feed conversion ratio (FCR), protein efficiency ratio (PER) and net protein utilization (NPU) were also calculated. All of the parameters were calculated according to Mohd Faudzi *et al.* (2017) as follows:

$$\text{Weight gain (\%)} = \frac{\text{Final body weight (g)} - \text{Initial body weight (g)}}{\text{Initial body weight (g)}} \times 100$$

$$\text{Specific growth rate (SGR) (\% day}^{-1}) = \frac{\text{Ln Final body weight (g)} - \text{Ln Initial body weight (g)}}{\text{Time (days)}} \times 100$$

$$\text{Survival} = \frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100$$

$$\text{Condition factor} = \frac{\text{Final body weight (g)}}{\text{Total length (cm)}^3} \times 100$$

$$\text{Hepatosomatic index (HSI)} = \frac{\text{Liver weight (g)}}{\text{Body weight (g)}} \times 100$$

$$\text{Viscerosomatic index (VSI)} = \frac{\text{Visceral weight (g)}}{\text{Body weight (g)}} \times 100$$

$$\text{Intraperitoneal fat (IPF)} = \frac{\text{Intraperitoneal fat (g)}}{\text{Body weight (g)}} \times 100$$

$$\text{Intestosomatic index (ISI)} = \frac{\text{Intestine (g)}}{\text{Body weight (g)}} \times 100$$

Feed intake (g)= Total feed intake for 12 weeks

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake (g)}}{\text{Wet weight gain (g)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Wet weight gain (g)}}{\text{Total protein intake (g)}}$$

$$\text{Net protein utilization (NPU)} = \frac{\text{Final fish body protein} - \text{Initial fish body protein}}{\text{Total protein intake}} \times 100$$

The fish sample was kept frozen at -20 °C for whole-body proximate analysis. The distal of the fish intestine (n=6) was sampled from each treatment for histological examination.

Proximate composition

The experimental feeds and fish samples were analyzed proximally. The digested sample was analyzed by Kjeltec-Protein Analyzer (Kjeltec™ 2300, Foss, Sweden) to determine the protein content (AOAC, 1999). Meanwhile, the lipid content was determined by using a solvent (diethyl ether, 40-60 °C boiling) and analyzed using Soxtec-Lipid Analyzer (Soxtec™ 2043, Foss, Sweden) (AOAC, 1999). A semi-auto fiber analysis instrument (Fibertec™ System, Foss Analytical, Sweden) was used to assess the fiber content of the experimental feeds (AOAC ,1999). The moisture content was determined by oven-drying for 24 hours at 105 °C (AOAC, 1999). The sample was burned in a muffle furnace for 6 hours at temperature of 550 °C to determine the ash content.

Hydrochloric acid (HCl, 6N) was used to hydrolyzed the samples of ingredients and feeds at temperature of 110 °C for 24 hours in order to analyze the amino acids content (Ovissipour *et al.*, 2010). The samples were then derived with o-phthaldialdehyde (OPA) and high-performance liquid chromatography (HPLC) (Shimadzu Corporation, Japan) was used to analyzed. The chromatographic peaks were integrated, identified and quantified by comparison to that amino acid standards and alpha-amino butyric acid (Sigma, USA) using Shimadzu Class-VP™ software (Version 6.1).

Apparent digestibility coefficient (ADC) analysis

The nutrient content of feeds and feces was determined following AOAC (1999). Meanwhile, the ADC of phosphorus was determined using the Amidol method by Egsgaard (1948) and measured with a spectrophotometer at 750nm. Following Furukawa and Tsukahara (1966), the chromic oxide in feeds and feces was determined using the acid digestion method and calculated as followed:

$$\text{ADCs of nutrient} = 100 \times \left[1 - \left(\frac{\text{\% faeces nutrient}}{\text{\% dietary nutrient}}\right) \times \left(\frac{\text{\% dietary chromic oxide}}{\text{faeces chromic oxide}}\right)\right]$$

Histological examination

Bouin’s solution which made by formaldehyde 37%, saturated picric acid solution, acetic acid at a ratio of 5:15:1 was used to preserved the intestine for 24 hours (Bancroft, 2008). The sample was then dehydrated and embedded according to histological standard practices. The intestinal sample was sliced into 6-μm thickness by using a microtome and stained with haematoxylin and eosin. A digital microscope (Dino-Lite AM3113T, AnMo Electronic Corporation, Taiwan) was used to measure the intestine diameter (Id) and villus height (Vh). The ratio of Id:Vh was then calculated. Light microscopy examination of the sample allowed for the identification of the histological alteration in the intestine epithelial tissues.

Statistical analysis

One-way analysis of variance (ANOVA) using Tukey’s multiple range tests in Statistical Packages of Social Sciences Version 21.0 was used to determine the significant differences of data that obtained in this study.

RESULTS

Table 1 shows the proximate composition of experimental feeds. The protein and lipid contents were corresponded to the dietary requirement. The ash content and nitrogen-free extract were almost similar in all treatments, while the fiber content was slightly higher in the DSM-based feed than in other feed. The results of amino acid composition in FM, DSM and experimental feeds are shown in Table 2. With exception of tyrosine, the content of other amino acid was higher in FM than in DSM.

Table 1. Ingredients (g kg^{-1} dry weight basis) and proximate composition (%) of experimental feeds fed to hybrid grouper juvenile

Ingredients	Experimental Feeds		
	CON	DSM_0	DSM_{2000}
Fish meal	669.0	397.0	397.0
Defatted soybean meal	-	306.0	306.0
Corn gluten meal	-	80.0	80.0
Fish oil	77.0	78.0	78.0
Lecithin	-	20.0	20.0
Taurine	-	12.0	12.0
Mineral[a]	15.0	20.0	20.0
Vitamin[b]	30.0	30.0	30.0
Dicalcium phosphate	10.0	10.0	10.0
Carboxyl methyl cellulose (CMC)	20.0	20.0	20.0
Alpha starch	180.0	27.0	27.0
Phytase (Natuphos, BASF)	0.0	0.0	0.2
Chromic oxide	5.0	5.0	5.0
Proximate Composition			
Crude protein (%)	50.4	50.7	50.7
Crude lipid (%)	12.0	12.2	12.2
Moisture (%)	3.8	2.9	3.7
Ash (%)	12.7	11.7	12.0
Fiber (%)	0.86	1.76	1.75
Nitrogen-free extract (NFE)	20.2	20.7	19.7
Energy (MJ kg^{-1})	19.5	19.8	19.6
Protein:Energy (g MJ^{-1})	25.8	25.6	25.9

[a]Mineral premix (Dexchem Industries Sdn. Bhd.), contains (g kg^{-1} dry weight): calcium phosphate monobasic 270.98 g; calcium lactate 327 g; ferrous sulphate 25 g; magnesium sulphate 132 g; potassium chloride 50 g; potassium iodide 0.15 g; copper sulphate 0.785 g; manganese oxide 0.8 g; cobalt carbonate 1 g; zinc oxide 3 g; sodium selenite 0.011 g; calcium carbonate 129.27 g
[b]Vitamin premix (Dexchem Industries Sdn. Bhd.), contains (g kg^{-1} dry weight): ascorbic acid 45 g; inositol 5 g; choline chloride 75 g; niacin 4.5 g; riboflavin 1 g; pyridoxine HCl 1 g; thiamine HCl 0.92 g; dicalcium pantothenate 3 g; retinyl acetate 0.6 g; vitamin D3 0.08 g; menadione 1.67 g; dialpha tocopherol acetate 8 g; d-Biotin 0.02 g; folic acid 0.09 g; vitamin B12 0.001 g; cellulose

Table 2. Amino acids composition (g kg^{-1} dry weight basis) of fish meal (FM), defatted soybean meal (DSM) and experimental feeds

Amino Acids	FM	DSM	Experimental Feeds		
			CON	DSM_0	DSM_{2000}
Aspartic acid	78.84	50.37	49.41	50.01	50.48
Threonine	34.11	17.51	22.01	22.26	22.83
Serine	37.20	23.17	23.68	23.71	23.10
Glutamic acid	114.21	82.71	73.08	85.08	80.55
Glycine	54.94	20.59	38.23	33.40	31.67
Alanine	51.80	19.91	35.22	32.84	31.74
Cystine	83.50	18.85	53.16	24.99	24.04
Methionine	21.46	4.69	13.08	8.53	7.87
Isoleucine	36.03	12.22	23.37	24.41	23.39
Leucine	62.62	28.11	41.28	42.94	39.92
Tyrosine	25.90	31.45	13.91	14.79	14.78
Phenylalanine	33.00	19.37	21.19	20.73	20.39
Lysine	72.11	26.46	48.41	42.47	41.39
Arginine	54.88	5.69	35.82	35.63	34.27

The levels of amino acids such as glycine, alanine, cysteine, methionine, phenylalanine and lysine decreased when FM was substituted with DSM at 30% level in the DSM-based feed. Meanwhile, the levels of amino acids such as glutamic acid, aspartic acid and tyrosine increased when 30% DSM was introduced. The content of other amino acids such as threonine, serine, isoleucine, leucine and arginine were almost similar among the experimental feeds.

Table 3 shows the growth performances, survival and feed utilization of hybrid grouper fed with experimental feeds over a period of 12 weeks. The growth of hybrid grouper was not significantly affected (p>0.05) by the experimental feeds. Hybrid grouper fed with the DSM-based feeds grew slightly higher than fed with CON feed. However, the growth performance of hybrid grouper fed with DSM-based feed with or without the supplementation of phytase remained unchanged. The feed intake was significantly higher (p=0.04) in hybrid grouper fed with DSM-based feeds than fed with CON feed. The feed conversion ratio (FCR) and protein efficiency ratio (PER) of hybrid grouper fed with DSM-based feeds were not significantly different (p>0.05) from that of CON feed. However, hybrid grouper fed with DSM-based feeds exhibited significantly lower net protein utilization (NPU) than those fed with CON feed (p=0.04). The survival of hybrid grouper ranged from 96% to 100% and was unaffected by the experimental feeds.

Condition factor, intraperitoneal fat (IPF) and intestosomatic index (ISI) of hybrid grouper were not significantly (p>0.05) affected by the dietary treatments (Table 4). However, feeding the hybrid grouper with DSM-based feeds significantly (p<0.05) reduced the value of hepatosomatic index (HSI) and viscerosomatic index (VSI) compared to CON feed. The measured body protein, lipid, moisture and ash were not significantly different (p>0.05) for all the treatment groups (Table 5), with values ranging from 16.7 to 17.8%, 4.2 to 6.1%, 68.6 to 70.7% and 14.6 to 16.5%, respectively.

Table 3. Growth performances, survival and feed utilization of hybrid grouper juvenile fed with DSM based feeds with different inclusion level of phytase for 12 weeks

Parameter	Experimental feed		
	CON	DSM_0	DSM_{2000}
Final BW (g)	119.08±6.65	127.12±7.45	128.39±10.46
Weight Gain (%)	1821.92±105.06	2037.70±86.87	2036.12±68.64
SGR (%/day)	3.52±0.06	3.60±0.07	3.60±0.11
Survival (%)	98.33±2.89	98.33±2.89	96.67±2.89
Total FI (g fish^{-1})	116.87±2.04 ^{a}	139.80±6.15 ^{b}	142.88±3.71 ^{b}
FCR	1.04±0.06	1.11±0.01	1.13±0.02
PER	1.92±0.11	1.78±0.01	1.75±0.03
NPU	34.03±0.59 ^{b}	29.48±1.33 ^{a}	29.80±0.78 ^{a}

Mean (±SE) values with different superscripts within the row are significantly difference (p<0.05)
BW, body weight; SGR, specific growth rate; FI, feed intake; FCR, feed conversion ratio; PER, protein efficiency ratio; NPU, net protein utilization

Table 4. Body condition indices of hybrid grouper juvenile fed with DSM based feeds with different inclusion level of phytase for 12 weeks

Parameter	Experimental feed		
	CON	DSM_0	DSM_{2000}
Condition Factor	3.02±0.36	2.62±0.23	3.03±0.35
Hepatosomatic Index	2.43±0.55 ^{b}	1.51±0.20 ^{a}	1.41±0.24 ^{a}
Viscerosomatic Index	13.20±0.96 ^{b}	10.90±1.09 ^{a}	11.00±0.55 ^{a}
Intraperitoneal Fat	3.22±0.72	2.91±0.92	2.16±0.84
Intestosomatic Index	4.26±0.84	3.67±0.64	4.15±0.59

Mean (±SE) values with different superscripts within the row are significantly difference (p<0.05)

Table 5. Whole body proximate composition (%) of hybrid grouper juvenile fed with DSM based feeds with different inclusion level of phytase for 12 weeks

Parameter	Experimental Feed		
	CON	DSM_0	DSM_{2000}
Protein	17.53±0.26	17.09±1.00	17.76± 0.71
Lipid	6.01±2.76	6.12±1.42	5.27±1.51
Moisture	69.55±1.80	70.08±1.25	68.60±1.11
Ash	14.86±3.67	14.55±2.35	16.45±2.72

Mean (±SE) values with different superscripts within the row are significantly difference (p<0.05)

The ADC of protein, lipid and phosphorus in this study are presented in Table 6. Hybrid grouper fed with CON feed exhibited significantly higher (p=0.03) ADC of protein than those subjected to other treatments. No significant effect (p>0.05) was observed on the ADC of lipid and phosphorus. However, the ADC of phosphorus was slightly higher in hybrid grouper fed with DSM_{2000} compared to that fed with DSM_0 and CON (p>0.05). The tissue section of intestine is shown in Figure 1. No significant difference (p>0.05) was observed in the intestinal diameter (Id), villus height (Vh) and Id/Vh ratio (Table 7) among hybrid grouper fed with the dietary treatments, indicating that feeding the fish with DSM-based feed does not alter the intestinal condition.

DISCUSSION

This study demonstrates that FM can be partially replaced with DSM without significantly affecting the growth performances of hybrid grouper. This finding is in agreement with that of the parental fish tiger grouper fed with DSM-based feeds containing 30% DSM (Shapawi *et al*., 2013a; 2013b). Feeding the hybrid grouper with DSM-based feeds resulted in slightly higher growth and feed intake than those subjected to the CON feed. This indicates that the replacement of fish meal with 30% DSM does not affect the palatability of the feed. According to a previous study, hybrid grouper can tolerate up to 60% of the protein replacement in DSM-based feed over a 14-day experiment (Firdaus *et al*., 2016). Meanwhile, a reduction on feed intake due to poor feed palatability and the consequent effects on fish growth has been frequently reported with the use of plant-based protein replacement, such as orange-spotted grouper (*E. coioides*) and tiger grouper fed with 14% and 40% protein replacement, respectively (Luo *et al*., 2004; Shapawi *et al*., 2013a). Additionally, the DSM-based feeds were well-accepted by the hybrid grouper and have better FCR (1.10-1.13) than tiger grouper fed with DSM (1.45) and fermented soybean meal (FSM) (1.85) (Shapawi *et al*., 2013a; 2013b; Chor *et al*., 2015).

It is commonly accepted that FM-based feed promotes better growth than feed with plant-sourced protein at higher levels of replacement (Shapawi *et al*., 2013a; Yong *et al*., 2013). However, in this study, hybrid grouper fed with DSM feeds demonstrated slightly higher growth than those fed with the CON feed (full FM), despite no significant difference recorded. Similar results were also obtained for hybrid grouper fed with feeds that were partially added with SPC and duckweed meal, which resulted in higher growth performance than those subjected to full FM feed (Anthonius *et al*., 2017; Mohd Faudzi *et al*., 2017). In this study, the greater uptake of the DSM-based feeds than the CON feed supports the higher growth performance of the hybrid grouper.

This study revealed that supplementation of phytase did not significantly improve the ADC of phosphorus, although a slightly increasing trend was observed when the phytase was added in DSM feed at 2000 FTU/kg. Supplementation of phytase allows for the hydrolysis of phytate and enhances nutrient uptake from plant-based feed (Kumar *et al*., 2012; Hussain *et al*., 2017). However, the beneficial effect of phytase supplementation on hybrid grouper was not observed in this study. The dosage of phytase varies depending on several factors such as the phytase source, the fish species and feed processing technology (Cao *et al*., 2007; Hussain *et al*., 2017) and the current investigation was carried out using only one dosage of phytase. In previous studies, supplementation of phytase (2000 FTU/kg) in 30% of DSM-based feed has been reported to positively influence the growth, feed utilization and digestibility of tiger grouper (Shapawi *et al*., 2013b) and red sea bream (Biswas *et al*., 2007). Additionally, supplementation of phytase at 750 FTU/kg in canola meal-based feed was reported to enhance the digestibility of phosphorus in carp (*Labeo rohita*) (Hussain *et al*., 2017).

Based on the histological observation, the partial replacement of FM with DSM did not cause morphological changes in the intestine of the hybrid grouper. The intestine diameter and villus height in hybrid grouper fed with DSM-based feeds were comparable with those subjected to CON feed. In addition, feeding the hybrid grouper with DSM-based feed did not result in typical signs of inflammation in the intestine. It is well known that the intestinal villi play a big role in digestive function

Table 6. Apparent digestibility coefficients (%) of hybrid grouper juvenile fed with DSM based feeds with different inclusion level of phytase for 12 weeks

Parameter	Experimental feed		
	CON	DSM_0	DSM_{2000}
Protein	97.6±0.0 ^{b}	96.6±0.2 ^{a}	96.5±0.1 ^{a}
Lipid	96.5±0.6	96.4±0.5	96.4±0.3
Phosphorus	67.5±2.6	65.8±2.7	68.2±1.9

Mean (±SE) values with different superscripts within the row are significantly difference (p<0.05)

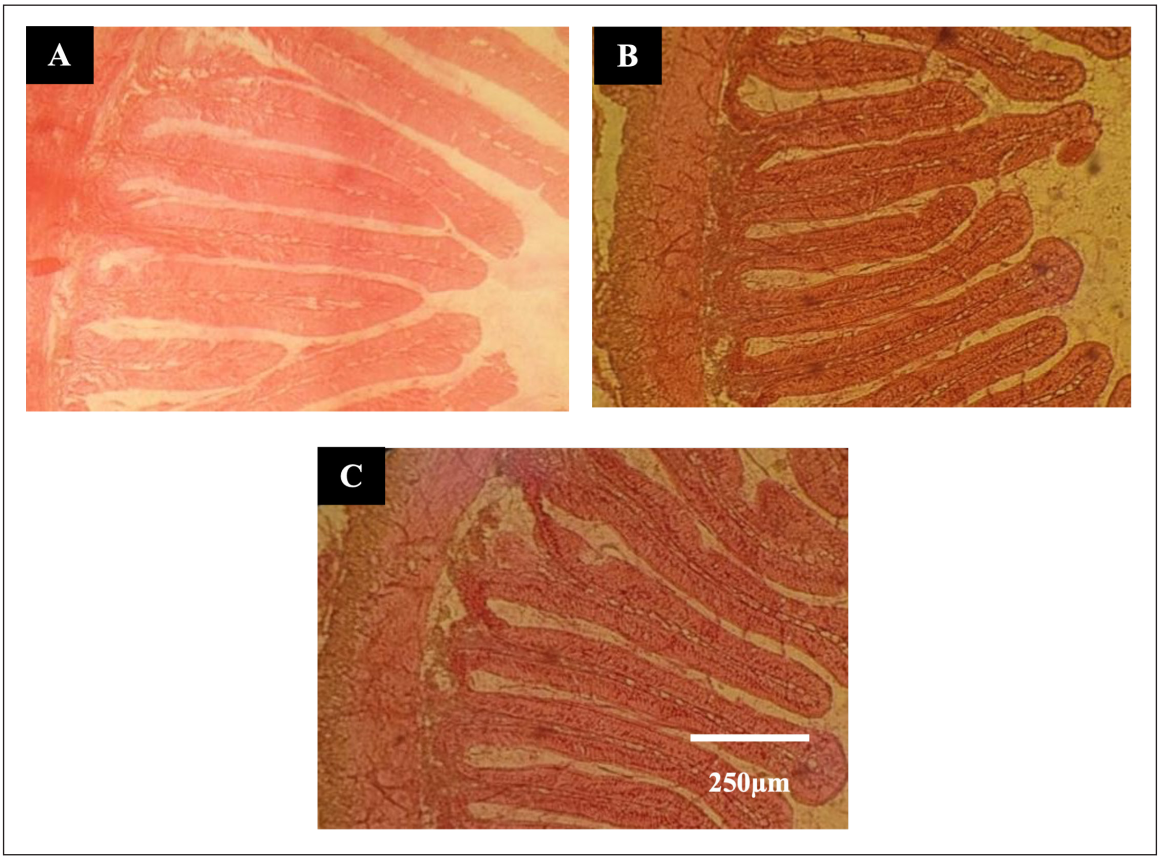

Fig. 1. Hematoxylin and eosin-stained tissue section of hybrid grouper juvenile; (A) Hybrid grouper fed CON; (B) Hybrid grouper fed DSM_0; (C) Hybrid grouper fed DSM_{2000}

Table 7. Intestine morphometric measurement of hybrid grouper juvenile fed with DSM based feeds with different inclusion level of phytase for 12 weeks

Parameter	Experimental Feed		
	CON	DSM_0	DSM_{2000}
Intestine diameter (Id) (mm)	1.92±0.18	1.95±0.14	1.93± 0.16
Villus height (Vh) (mm)	0.68±0.07	0.70±0.07	0.68±0.05
Id/Vh	2.90±0.25	2.81±0.21	2.85±0.19

Mean (±SE) values with different superscripts within the row are significantly difference (p<0.05)

(Wang *et al*., 2017), whereby the increased length of intestinal villi is associated with the increased surface area for absorption of available nutrients (Wang *et al*., 2017). Therefore, the reduction of intestinal villus height is commonly related to poor growth of fish (Wang *et al*., 2017). It was found that feeding the parental fish, the tiger grouper with DSM and FSM at 30% and 40% of replacement levels, respectively, resulted in the shortening of the villi and moderate inflammation in the intestine (Chor *et al*., 2015). The orange-spotted grouper fed with DSM at 50 and 100% replacement levels also exhibited shortened villus height and inflamed intestine (Wang *et al*., 2017). Additionally, the intestinal epithelium's goblet cells were found to be less numerous and smaller in size for the parental fish, the giant grouper fed with DSM or SPC at 20 to 80% replacement levels (Garcia-Ortega *et al*., 2016). Therefore, the findings in this study suggest that hybrid grouper are better at utilizing DSM in the feeds than the parental fish and other groupers.

Besides, feeding the hybrid grouper with DSM-based feeds did not result in any negative effects on the whole-body proximate composition in comparison to the CON feed. It has been reported that the whole-body composition of tiger grouper was unaffected by the optimal level of FM replaced with DSM (30%) (Shapawi *et al*., 2013a; 2013b). Meanwhile, the replacement of DSM higher than 30% level had a significant impact on the whole-body composition of tiger grouper (Shapawi *et al*., 2013a). Similar results were observed in other species including rainbow trout (Kaushik *et al*., 1995; Yang *et al*., 2011) and Atlantic salmon (*Salmo salar*) (Refstie *et al*., 2001), demonstrating that DSM ingestion has an impact on the whole-body proximate composition. Feeding the fish with more than the optimum level of DSM replacement will cause low feed intake by fish and consequently leads to starvation in fish and reduction in body proximate composition (Shapawi *et al*., 2013a).

A slight decreasing trend in body lipid and intraperitoneal fat compositions was detected when the hybrid grouper was fed with DSM-based feed supplemented with phytase at 2000 FTU/kg. The VSI of hybrid grouper also decreased when fed with DSM-based feed, indicating less fat deposition in viscera compared with those subjected to CON feed. In addition, this study demonstrated that hybrid grouper fed with DSM-based feed showed a decrease in HSI, which also contributed to poor VSI. A reduction of HSI was reported in a giant grouper fed with an increasing level of DSM and SPC (Garcia-Ortega *et al*., 2016). A decreasing trend of HSI was also observed in the tiger puffer fed with DSM from 0 to 60% level of replacement (Lim *et al*., 2011). In contrast, a previous study revealed that feeding the hybrid grouper with SPC at various levels of replacement had no significant impact on the HSI (Mohd Faudzi *et al*., 2017). The HSI of tiger grouper was also unaffected by DSM and FSM feeds at 30% level of replacement (Chor *et al*., 2015). A previous study suggested that the feeding of several carnivorous fishes with increasing of plant protein may result in physiological stress and immune system disruption in the fish (Daniel, 2018). Stress in fish may result in hypermetabolism, which alters glucose uptake and decrease the ability of the liver to store, thus resulting on reducing in liver size (Li *et al*., 2020).

A slight decreasing trend in the level of several amino acids in the feeds was observed when DSM was used to replace FM at a 30% level. For instance, methionine content decreased from 13.08 g/kg (FM-based feed) to 7.87 g/kg (DSM-based feeds). The methionine in DSM-based feeds in this study is lower than the stated amount in SPC feed (30% replacement level) (9.41 g/kg) for hybrid grouper (Mohd Faudzi *et al*., 2017). However, the methionine content in DSM-based feed in this study is greater than the minimum requirement of methionine for hybrid grouper as reported by Wu *et al*. (2017) (6.68 g/kg). The methionine content in other feeds for pure species such as that of tiger grouper (11.6 g/kg) and orange-spotted grouper (13.1 g/kg) is higher than the reported content for hybrid grouper (Luo *et al*., 2005; Shapawi *et al*., 2013a). A slight decreasing trend of lysine was also observed when FM protein was replaced with DSM at a 30% level. However, the lysine content in DSM-based feed in the present study is higher than the reported amount in SPC feed at a 30% replacement level (39.46 g/kg) for hybrid grouper (Mohd Faudzi *et al*., 2017). The lysine content in this study was also higher than the stated in other feeds for pure species (tiger grouper: 34.1 g/kg; orange-spotted grouper: 28.3 g/kg) (Luo *et al*., 2005; Shapawi *et al*., 2013a). The finding indicates that the amount of methionine and lysine content in DSM-based feed is sufficient in promoting good growth for hybrid grouper.

CONCLUSION

The present study shows that DSM has the potential to replace FM in the formulation of feed for hybrid grouper at 30% protein replacement level, with or without phytase supplementation. The findings also suggest that the hybrid grouper demonstrates various degrees of utilization of DSM feeds and has lower sensitivity to anti-nutritional factors present in DSM compared to the parental fish, particularly the tiger grouper. The utilization of DSM in the feeds is not only reducing the dependency on the fish meal as the main protein source but also helps in reducing the cost of feed during the grow-out period for hybrid grouper.

ACKNOWLEDGEMENTS

The study was supported by the Ministry of Higher Education, Malaysia under the Fundamental Research Grant Scheme (FRG339-STWN 1/2013). The study also collaborated with Fisheries Laboratory (Uragami), Kindai University, Japan. The authors gratefully acknowledged the technical support provided by staff at Fish Hatchery, Borneo Marine Research Institute.

ETHICAL STATEMENT

The Malaysian Code of Practice for the care and use of animals for scientific purpose was followed when handling and caring for experimental fish.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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