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Short Communication

Improved growth performance of hybrid grouper (*Epinephelus fuscoguttatus* ♀× *E. lanceolatus* ♂) fed with green pea meal based diets

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Abstract

Five experimental diets were formulated to determine the effects of green pea meal (GPM) inclusion in the formulation on the growth performance of hybrid grouper (*Epinephelus fuscoguttatus* ♀ X *E. lanceolatus* ♂) juveniles. GPM was included at the expense of soybean meal (SBM) at 0 (GP0), 5 (GP5), 10 (GP10), 15 (GP15) and 20% (GP20) replacement levels, which are equivalent to 0, 3.2, 6.5, 9.7 and 12.9% of the diet. GPM used in the present study had a higher lysine content than fish meal or SBM. Overall, the GPM-based diets performed significantly better than the control. In particular, juvenile hybrid grouper fed GP10 yielded the best growth and feed utilization. Therefore, the use of GPM at 6.5% of the diet is recommended to improve growth and feed utilization of hybrid grouper juveniles.

Keywords: green pea meal, soybean meal, *Epinephelinae*, hybrid grouper, lysine

1. Introduction

Recently, hybrid grouper (*Epinephelus fuscoguttatus* x *E. lanceolatus*) has become one of the most farmed grouper species in the Asia Pacific region. Like other grouper species, it requires high protein content of about 50% to achieve the optimum growth (Yong, Mohd Faudzi, Senoo, & Shapawi, 2019). In an effort to reduce the cost of marine fish feed, the industry has turned to alternative protein sources to replace some portion of fish meal (FM) in the diet formulation. Soybean meal (SBM) is among the most widely used alternative protein sources to replace fish meal in the diets of many marine fish species due to its consistent supply and relatively low price compared to fish meal. However, high inclusion levels of SBM, especially in the raw form in carnivorous marine fish feeds, is difficult because of its poor digestibility, low palatability and lack of certain essential amino acids such as methionine and lysine (Hardy, 2010; Kader *et al*., 2012; Lim, Yong, & Shapawi, 2014). It also contains anti-nutritional substances that may inhibit the normal growth of fish (Grant, 1989). One strategy to overcome this issue is to use multiple feed ingredients with different nutritional profiles in the formulation.

Several plant resources such as legumes, beans and nuts are characterized as having good amino acid profiles. Green pea is one of the legumes with a good biological value (Ratnayake, Hoover, & Warkentin, 2002) and relatively high lysine content (Hickling, 2003). Green pea is also easily available in the local market. Studies on the use of green pea meal (GPM) in aquaculture feeds have indicated that it can be used to replace fishmeal at about 10 to 20% replacement levels (Borlongan, Eusebio, & Welsh, 2003; Ganzon-Naret,

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2013) and exhibits high digestibility in terms of protein (Thiessen, Campbell, & Adelizi, 2003). In the present study, diets were developed combining FM and SBM as the main protein sources. SBM was replaced with GPM at 5-20% replacement levels to determine the significant effects on growth performance of the hybrid grouper.

2. Materials and Methods

2.1 Experimental Designs

Five experimental diets containing different levels of green pea meal were formulated to be isonitrogenous (50% of crude protein) and isolipidic (12% of crude lipid). These diets were labeled with the codes GP0, GP5, GP10, GP15 and GP20 to represent 0%, 5%, 10%, 15% and 20% of SBM replaced with GPM; equivalent to 0%, 3.2%, 6.5%, 9.7% and 12.9% of the diet, respectively. Calculated gross energy of the experimental feeds was in the range 371–377 Kcal using the standard physiological fuel values of 9 Kcal/g for lipid, 4 Kcal/g for carbohydrate and 4 Kcal/g for protein. Dietary ingredients, formulated feeds and whole-body of the fish were subjected to proximate analysis of crude protein, crude lipid, moisture, crude ash and crude fiber using AOAC (1990) methods. The proximate and amino acid compositions of experimental diets are presented in Table 1.

Table 1. Proximate compositions and amino acids in FM, SBM, GPM

	Dietary Ingredient		
	FM	SBM	GPM
Component (%)			
Crude protein	65.04	47.76	21.11
Crude lipid	8.66	1.60	2.84
Crude Fiber	NA	19.20	20.55
Moisture	11.45	9.76	9.14
Crude ash	9.23	6.98	3.07
NFE	5.62	14.7	43.29
Amino **a**cid (%)			
Arginine	3.98	0.00	1.87
Cysteine	2.26	1.40	1.55
Isoleucine	2.59	1.74	3.03
Leucine	4.40	2.99	3.70
Lysine	2.94	2.71	3.41
Methionine	0.79	1.29	1.42
Phenylalanine	3.08	1.61	2.16
Threonine	2.20	1.61	1.97

The feeding trial was conducted at the Fish Hatchery of Universiti Malaysia Sabah for 7 weeks. A 10 tonnes fiber glass tank was used with 15 of 100L cages randomly placed inside the tank that had a running seawater system. The flow rate was about 16.90 L/minute. The stocking density of the experimental fish was set at 15 fish per cage (4.20 - 4.40 g) and the treatments were run in triplicates. Experimental feeds were provided *ad libitum* twice daily (0800 and 1700 h). The water quality (dissolved oxygen, pH, salinity, and temperature) was measured twice daily using a HANNA multi-parameter device to ensure that optimum water conditions were provided to the experimental fish, and the values were kept at 6.31 ± 0.91 mgL^{-1}, 6.96 ± 0.65, 30.21 ± 0.37 ppt and 29.50 ± 0.86ºC, respectively. About 10% of the water was exchanged daily to maintain good water quality in the tank.

Fish were measured individually for the initial and final body weights and for total length. They were weighed in bulk every two weeks using a digital electronic balance to observe the growth performance of the fish. Weight gain (WG), specific growth rate (SGR), survival, feed intake (FI), feed conversion ratio (FCR), net protein utilization (NPU), protein efficiency ratio (PER), hepatosomatic index (HSI) and the viserosomatic indices (VSI) were calculated at the end of the experiment. For whole body proximate analysis, 20 fish from the stock at the beginning of the experiment and 10 fish from each cage at the end of the experiment were randomly sacrificed, and kept in a -20°C freezer until the analysis. Three fish from each replicate were sampled for determination of the body indices: hepatosomatic index (HSI) and viscerosomatic index (VSI).

The fish growth performance indicators were calculated according to the following formulas:

WG, % = (final weight – initial weight) × 100 / initial weight

SGR, %/day) = {[Ln (final weight) – Ln (initial weight)]/ duration in days} × 100

Survival (%) = 100 × (final no. of fish / initial no. of fish)

FI (g/fish) = (dry diet given – dry remaining diet recovered) / no. of fish

FCR = total dry feed fed (g) /wet weight gain (g)

PER = wet weight gain (g)/total protein intake (g)

NPU = 100×(final−initial fish body protein)/total protein intake

HSI = (Liver weigh/body weight)×100

VSI = (Visceral weight/body weight)×100

2.2 Amino acid analysis

FM, SBM and GPM samples were hydrolysed with 6N HCl. After sample digestion, they were analyzed using High-Performance Liquid Chromatography (HPLC) with a PCX 5200 Post-Column Derivatizer (Pickering Laboratories). It was equipped with fluorescence detector (SHIMADZU RF-10 AXL) with ultraviolet (UV) absorption (wavelength excitation 330nm, emission 465nm) and auto-injector (SHIMADZU SIL-10ADvp) equipped with high pH compatible Tefzel ® or PEEKTM seals, for the liquid chromatograph (SHIMADZU LC-10AD VP). The chromatography separation was carried out by using a sodium-ion exchange column (3.0 x 250nm) run with methanol-water (60:40, v/v) eluent at a flow rate of 0.4 mL/min. The temperatures of the column and the reactor were 53°C and 45°C, respectively. Chromatographic peaks obtained were analyzed with Breeze™ software by comparing them to known standards (Amino Acid Calibration Standard, protein hydrolyzate).

2.3 Statistical analysis

The data were analyzed using SPSS version 22.0 with one-way analysis of variance (ANOVA) and Duncan's post-hoc test with significant difference requirement of $p < 0.05$.

3. Results and Discussion

The GPM used in the present study contained 21.11%, 2.84%, 20.55%, 9.14%, 3.07% and 43.29% crude protein, crude lipid, crude fiber, moisture, crude ash, and nitrogen free extract (NFE), respectively. The amino acid data show that green pea had high lysine and methionine contents (3.41% and 1.42%, respectively). Cysteine, leucine and threonine contents in the green pea were 1.55%, 3.70% and 1.97%, respectively in contrast to 2.26%, 4.40% and 2.20% in the fish meal. Overall, GPM presented a higher percentage of essential amino acids than SBM (Table 1). The proximate compositions of the experimental diets in Table 2 show that the analyzed protein and lipid contents correspond to the calculated values (50% and 12%, respectively). The moisture content in the diets ranged from 9.21 ± 0.34% to 9.76 ± 0.22%, while ash content was in the range from 11.23 ± 0.07% to 11.73 ± 0.08%.

The weight of hybrid grouper increased from 4g to 35g during the 7 weeks of feeding trial. Fish fed GP10 attained the highest WG and SGR at 740.49 % and 4.34%/day, respectively, followed by GP5 (593.28% and 3.955%/day), GP15 (527.24% and 3.74%/day), GP20 (491% and 3.63%/day) and GP0 (395 % and 3.27 %/day). Fish survival was high throughout the feeding period with no significant differences detected, ranging from 88.89% (GP0 and GP5) to 91.11% (GP10, GP15 and GP20). Dietary treatments significantly influenced the feed intake and FCR, and the best FCR was also for the GP10 treatment. Similarly, PER and NPU were also affected by the dietary treatments with values ranging from 13.4 to 22.14 and from 1.73 to 1.93, respectively. In contrast, HSI and VSI were independent of the diets (Table 3). There was no significant difference in the whole-body proximate composition of hybrid grouper juveniles caused by the inclusion of GPM in the diets (Table 4). Moisture, protein, lipid and ash of the final fish ranged

Table 2. Feed formulations and proximate compositions of the experimental diets (g/100 g diet)

Dietary Ingredient (g/100 g diet)	GP0	GP5	GP10	GP15	GP20
Danish FM	47.65	47.65	47.65	47.65	47.65
Soybean meal	28.34	26.92	25.51	24.09	22.67
Green pea meal	0	3.23	6.46	9.69	12.91
Fish oil	7.42	7.35	7.28	7.21	7.14
CMC[a]	1.50	1.50	1.50	1.50	1.50
Vitamin premix[b]	3.00	3.00	3.00	3.00	3.00
Mineral premix[c]	2.00	2.00	2.00	2.00	2.00
Tapioca Starch	10.09	8.35	6.60	4.86	3.12
GE (Kcal)[d]	376.81	375.50	374.18	372.87	371.55
Component (%)					
Crude protein	50.33 ± 0.23	50.22 ± 0.17	50.17 ± 0.19	50.13 ± 0.27	50.14 ± 0.22
Crude lipid	12.16 ± 0.31	12.11 ± 0.22	12.09 ± 0.25	12.07 ± 0.19	12.06 ± 0.26
Crude Fiber	19.93 ± 0.29	20.76 ± 0.54	21.21 ± 0.34	23.35 ± 0.46	25.67 ± 0.87
Moisture	9.29 ± 0.15	9.24 ± 0.23	9.72 ± 0.21	9.76 ± 0.22	9.21 ± 0.34
Crude ash	11.23 ± 0.07	11.36 ± 0.37	11.49 ± 0.11	11.29 ± 0.24	11.73 ± 0.08

a, carboxymethyl cellulose.
b & c, vitamin and mineral premix, respectively as-used basis (Luo *et al*.,, 2004; Shapawi *et al*. 2014).
d, calculated gross energy (Kcal).
GP0-GP20, experimental diets with different GPM inclusion levels

Table 3. WG, SGR, survival and feed utilization of juvenile hybrid groupers fed with control and test diets over 7 weeks (mean ± sd)

Parameter	**Diet**				
	GP0	GP5	GP10	GP15	GP20
Initial weight (g)	4.40 ± 0.04	4.39 ± 0.16	4.23 ± 0.08	4.30 ± 0.08	4.20 ± 0.25
Final weight (g)	21.80 ± 0.32[a]	30.43 ± 0.37[c]	35.54 ± 2.30[d]	26.97 ± 2.33[bc]	24.86 ± 2.39[ab]
WG (%)	395.97 ± 7.92[a]	593.28 ± 33.81[c]	740.49 ± 63.23[d]	527.24 ± 63.78[bc]	491.74 ± 31.86[b]
SGR (%/day)	3.27 ± 0.03[a]	3.95 ± 0.10[b]	4.34 ± 0.15[c]	3.74 ± 0.20[b]	3.63 ± 0.11[b]
Survival (%)	88.89 ± 3.85	88.89 ± 3.85	91.11 ± 3.85	91.11 ± 3.85	91.11 ± 7.70
FI (g/fish)	50.02 ± 0.61[b]	50.86 ± 1.04[bc]	53.47 ± 0.71[c]	48.14 ± 2.29[ab]	47.13 ± 1.99[a]
FCR	1.39 ± 0.06[a]	0.93 ± 0.07[bc]	0.80 ± 0.7[c]	0.99 ± 0.11[bc]	1.11 ± 0.16[b]
PER (%)	0.87[a]	1.19 ± 0.04[bc]	1.33 ± 0.10[c]	1.12 ± 0.10[b]	1.06 ± 0.15[b]
NPU (%)	13.40 ± 0.07[a]	18.79 ± 0.65[b]	22.14 ± 1.89[c]	17.58 ± 1.84[b]	15.87 ± 2.33[ab]
HSI	1.73 ± 0.31	1.91±0.16	1.93 ± 0.07	1.90 ± 0.35	1.86 ± 0.31
VSI	10.62 ± 0.85	12.14±0.28	12.38±0.65	10.69±1.02	10.83 ± 1.58

Different superscripts indicate significant differences ($p<0.05$)
GP0-GP20 are the experimental diets with different GPM inclusion levels.
n=3 fish per tank for PER, NPU, HSI & VSI

Table 4. Whole-body proximate composition of juvenile hybrid grouper after 7 weeks feeding trial (mean ± sd)

Component	Initial	Whole-body				
		GP0	GP5	GP10	GP15	GP20
Moisture (%)	72.01±1.03	68.26±0.74	72.04±0.22	69.85±0.30	69.98±1.09	71.21±0.59
Protein (%)	16.25±0.54	18.75±0.89	18.11±0.85	18.63±0.94	18.31±1.01	17.77±0.76
Lipid (%)	5.53±1.27	5.21±0.71	5.03±1.06	5.11±0.99	5.09±1.12	4.98±1.57
Ash (%)	5.65±0.24	5.12±0.43	4.98±0.81	5.04±0.51	4.78±0.60	4.92±0.91

GP0-GP20 are the experimental diets with different GPM inclusion levels.
n = 20 initial fish and 10 final fish from each treatment

from 68.26 to 71.21%, from 17.77 to 18.75%, from 4.98 to 5.21%, and from 4.78 to 5.12%, respectively.

Methionine and lysine are two common limiting amino acids in alternative feed ingredients. Both are critical nutrients for promoting growth of fish, including grouper species (Li, Mai, Trushenski, & Wu, 2009; Luo *et al*., 2005). GPM has been reported by several studies as a secondary or tertiary protein source in fish diets. In the present study, GPM contained about half of the protein in SBM with similar fiber but lower ash contents. Interestingly, the amino acid content of GPM is comparable to FM and better than that of SBM. The analysis of amino acids in GPM proved that it can be a good source of the limiting essential amino acids, especially lysine. This is also supported by other studies which have reported the amounts of methionine and lysine in GPM (from 0.93% to 1.1% ; and from 5.26% to 8.1%, respectively) (Iqbal, Khalil, Ateeq, & Khan, 2006; Lisiewska, Słupski, Kmiecik, & Gębczyński, 2008) as much higher than the methionine and lysine contents in FM (1.74% and 4.78%, respectively) (Ween, Stangeland, Fylling, & Aas, 2017) or in SBM (0.3% and 3.4%, respectively) (Gorissen *et al*., 2018). The higher percentage of amino acids in a prior study than in the present study was mostly due to different quality or origin of the green pea.

There were significant differences in growth and feed utilization as a result of green pea inclusion in the diets. In general, diets with the inclusion of GPM performed better than the control without GPM, indicating a positive influence of GPM in supporting good growth, feed utilization and survival of the hybrid grouper juveniles. In fact, the weight gain of hybrid grouper fed GPM10 was almost double from the growth of the control fish. However, reduced growth trend was observed when GPM was included at the higher levels of 15% and 20%. This could be due to the reduced feed intake (indicating reduced palatability) by the fish fed GP15 and GP20 compared to the other treatments (GP0, GP5 and G10). Another causal factor might be the high fiber content of GPM which can reduce diet digestibility (Borlongan, Eusebio, & Welsh, 2003). There is limited information available on the use of GPM in grouper feeds. In general, diets with animal-based protein are more successful than those with plant-based proteins when offered to grouper species (Lim, Yong, & Shapawi, 2014).

In our previous study, SBM was able to replace fishmeal in the diet formulated for *E. fuscoguttatus* juveniles at no more than 30% replacement level (Shapawi, Ebi, & Yong, 2013), unlike when using poultry by-product meal that was successfully used to replace fish meal at much higher replacement levels of up to 70% (Shapawi, Ng, & Mustafa, 2007). Nevertheless, in the present study, GPM was included at the expense of SBM with a constant amount of fish meal in all diets. Apparently, the combination of these 3 protein sources has provided balanced nutrients in the diets which supported good growth, feed utilization and survival of the hybrid grouper juveniles.

In other fish species, the inclusion of 25 to 40% of extruded peas in the diet significantly increased the weight gain, specific growth rate, and gave similar or improved feed conversion ratio and feed intake relative to a fish meal reference diet or a commercial diet, for rainbow trout and European seabass (Burel, Boujard, Tulli, & Kaushik, 2000; Carter & Hauler, 2000; Gouveia, Teles, Gomes, & Rema, 1993; Gouveia & Davies, 1998). Borlongan, Eusebio, & Welsh (2003) reported that GPM can replace up to 20% of the total dietary protein in feed for milkfish (*Chanos chanos*). Meanwhile, replacement of fish meal with GPM at 10% was recommended in Asian seabass diets (*Lates calcarifer*) (Ganzon-Naret, 2003).

4. Conclusions

GPM can be successfully used as a feed supplement in the diets based on a mixture of fish meal and soybean meal as protein sources. Inclusion of GPM at about 6.5% of the diet is highly recommended for the juvenile hybrid groupers to yield improved growth, survival, and feed utilization. Nevertheless, more in-depth studies (e.g. a long-term feeding trial) are recommended to fully understand the full potential of GPM as a dietary ingredient for the hybrid grouper.

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