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Partial replacement of fish meal by soybean protein in diets for grouper *Epinephelus coioides* juveniles

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Abstract: A 56-day feeding trial was conducted to evaluate the potential of replacing white fish meal (WFM) with fermented soybean meal (FSBM) and soybean meal (SBM) in experimental diets for juvenile grouper *Epinephelus coioides* of initial weight 9.4 ±0.1g. Six isonitrogenous diets were formulated to contain 52 % protein and 12 % lipid. WFM was replaced by FSBM at four inclusion levels of 28 %, 21 %, 14 % and 7 % (diets 1-4). SBM was tested at one level of 20 % (diet 6). The control diet (diet 5) contained 68 % of WFM as a sole protein source. Grouper juveniles were cultured in the floating net-cages (1.5m ×1m ×1.5m) and fed to satiation once daily. Weight growth (WG) and special growth ratio (SGR) were maximized when fish were fed with a combination of 7 % FSBM and 63 % WFM but they were not significantly different from fish fed with the diet containing 14 % FSBM and the control diet, respectively. Feeding efficiency (FE) and protein efficiency ratio (PER) decreased with increasing inclusion of FSBM. FSBM and SBM substitutes had no significant effect on whole body composition, HSI and VSI. At the same inclusion level, fish fed the diets containing 21 % FSBM had higher growth, FE and PER than fish fed the 20 % SBM diets, indicating that FSBM was a kind of better protein source for fish growth than SBM. Based on broken-line regression analysis of WG (WG versus replacement level of WFM with FSBM), 10 % of WFM can be replaced by FSBM with no effect on growth, FE, PER and body composition of *Epinephelus coioides*.

Key words: *Epinephelus coioides* juveniles; fermented soybean meal; soybean meal; white fish meal; growth
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Grouper is economically a kind of important fish species because of their fast growth, efficient feed conversion and high market value[1, 2], and regarded as being the primary candidate for offshore cage culture in southern China. Studies on its nutritional requirement have been initiated and reported protein requirements vary between 40 % and 60 %[1, 3-5].

In the diets for carnivorous fish, fish meal is the main protein source. However, due to increasing prices and declining production of fish meal, nutritionists are seeking its substitutes for providing protein and reducing cost of diets. Among these, SBM is the most widely studied. However, due to amino acid imbalances, presence of anti-nutritional factors and low palatability, a high inclusion level of SBM is not well accepted[6, 7]. Some authors suggested that

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fermentation could improve protein digestibility, EAAI and PER of SBM[8]. However, little information is available about the effect of the addition of FSBM on fish growth performance and body composition. Therefore, the present experiment was undertaken to determine the potential of FSBM and SBM as partial substitutes for WFM in the diet for *E. coioides*.

1 Materials and methods

1.1 Experimental diets

The diets were produced at the Veterinary Medicine Institute, Guangdong Academy of Agriculture Sciences. Six practical diets were formulated to be isonitrogenous (52 % protein) contain 12 % lipid. FSBM was tested at four inclusion levels of 28 %, 21 %, 14 % and 7 % to replace 29.4 %, 22.1 %, 14.7 %, 7.4 % of WFM (diets 1-4), SBM was tested at a level of 20 % to replace 22.1 % of WFM (diet 6). The control diet contained 68 % WFM as the sole protein source (diet 5). The composition and nutrient contents of the experimental diets are given in Tab.1.

The dry ingredients (ground to pass 120μm sieve) were thoroughly mixed in a food mixer, followed by the addition of oil and phospholipid which had also been manually blended for 5 min to obtain an emulsion. Distilled water was added to (40 %, v/w) obtain a stiff dough. The moist diet was then extruded through a pelletizer with a 2.5mm die. The resultant pellets were dried in an oven at 45 ℃ until the moisture was reduced to less than 10 %. The dry pellets were placed in covered plastic bags and stored in a refrigerator at - 20 ℃ until being fed.

Tab.1 Ingredients and proximate composition of experimental diets

ingredients	diet no.					
	1	2	3	4	5	6
WFM (imported from New Zealand)	48	53	58	63	68	53
FSBM	28	21	14	7	0	0
SBM	0	0	0	0	0	20
fish oil (imported from New Zealand)	5.5	5.2	4.9	4.6	4.3	4.9
cellulose	0	2.3	4.6	6.9	9.2	3.6
others	18.5	18.5	18.5	18.5	18.5	18.5
proximate analysis						
moisture (%)	8.64	8.93	8.82	8.00	7.16	7.80
protein (%DM)	51.78	51.99	51.47	51.03	51.46	50.48
lipid (%DM)	11.25	12.96	11.70	12.51	12.31	13.71
ash (%DM)	13.64	14.26	14.46	14.87	15.44	14.16

Notes: vitamin mix according to Chou et al.[9]; mineral mix according to Wanakowat, et al.[10] others (%): dextrin, 10; sodium alginate, 2; vitamin mix, 1; mineral mix, 2; phospholipid, 2; choline chloride (50 %), 1; ascorbic acid phosphate ester, 0.5

1.2 Experimental procedures

The experiment was carried out in an inlet of South China Sea, the experimental base of Guangdong Evergreen Group. Juvenile grouper were obtained from a local fish fry dealer and stocked in the floating cages (1.5m × 1m × 1.5m) for 2 weeks prior to the trial, in order to condition the fish to cage conditions. During the acclimatization period, the fish were fed minced trash fish mixed with the control diet. The amount of trash fish was more than that of the diet at the start of acclimatization but the amount of the diet was enhanced step by step until grouper juveniles were utterly acclimatized to the diet. At the start of the experiment, twenty uniform-sized fish (mean weight: 9.4 ± 0.1g) with good health condition were stocked in every cage (six treatments with three replicate each). Each diet was supplied *ad libitum* to three cages. In view of the situation that waste feed could not be collected, would sink and escape from the cage, fish were fed slowly to minimize the loss of the diet. Based on visual observation, fish were fed to apparent satiation once daily (10:00 a.m.) during a 45-minute period and adjusted the daily ration for the subsequent day according to uneaten feed observed daily. The amount of feed consumed in each cage was recorded daily. In order to minimize the stress fish were counted and weighed every month. The cages were cleaned as needed. The feeding trial lasted for

56 days. On the termination of the experiment, fish from each cage were counted and weighed collectively.

The experiment was conducted under conditions of natural photoperiod. Temperature, dissolved oxygen and total ammonia, and salinity were monitored at weekly intervals during the experiment and had values of water temperature from 23 ℃ to 28 ℃, salinity 18 - 26, dissolved oxygen ≥5.0 mg · L^{-1}, total ammonia-nitrogen 0.25 - 0.52 mg · L^{-1}.

1.3 Sample collection and chemical analysis

At the end of the experiment, fish were starved for one day prior to sampling. Total number and weight of fish in each cage were determined. Eight fish from each cage were randomly collected for proximate analysis, five for analysis of whole body composition, and three for analysis of dorsal muscle, liver composition and for calculation of CF, HSI and VSI. The samples were analyzed in duplicate for moisture (105 ℃, overnight), crude protein (N × 6.25, using an Auto Kjeldahl System, 1030-Auto-analyzer, Tecator, Sweden), crude lipid (the ether-extraction method by Soxtec System HT6, Tecator, Sweden) and ash (550 ℃, overnight).

1.4 Statistical analysis

The results were presented as means ± standard error of the mean (SEM). The data were analyzed by one-way ANOVA using the software of the Statistica (Release 5.0, Tulsa, USA). Duncan's multiple range test was used to compare differences among means and the level of significance was chosen at $P < 0.05$. The optimal dietary replacement level of WFM with FSBM was determined by broken-line regression model[11] using WG results.

2 Results

2.1 Growth and survival

The fish growth performance, FE, PER, and survival are shown in Tab. 2. All fish quickly accepted the experimental diets and fed actively for the duration of the experiment. WG and SGR of fish fed with diets 3, 4 and 5 were significantly higher than those of the other dietary groups. The lowest WG and SGR were found in fish fed with diets containing 20 % SBM but they were comparable to that of fish fed with diets containing 28 % FSBM. The relationship of WG to the replacement level of WFM with FSBM was expressed by broken-line model and a breakpoint at 10.7 % was thought to be optimal for FSBM to replace the percentage of WFM (Fig. 1).

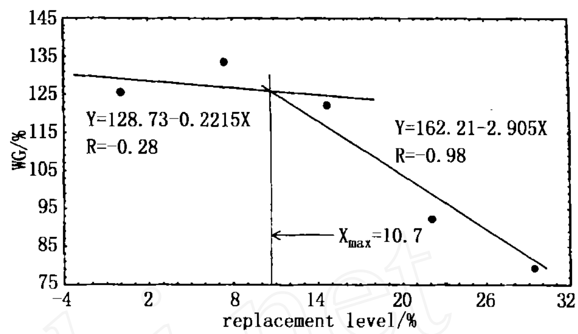

Fig. 1 The effect of replacement level of WFM with FSBM on WG of *Epinephelus coioides* juveniles, based on broken-line regression analysis

In the present experiment, FE and PER were probably underestimated because feed waste could not be collected. But with good control of feeding, loss of waste feed could be minimized. FE and PER followed a similar pattern as described above for WG. These parameters were the highest when fish fed with diets containing 7 % FSBM but not markedly different from that of fish fed with the control diet and 14 % FSBM diet, respectively.

2.2 Body composition

The body composition of fish after 8 weeks of growth trial is given in Tab. 3 and Tab. 4. No significant differences were found among the treatments for any of the whole body constituents ($P > 0.05$). However, a slight but insignificant decrease in whole body lipid content was observed in grouper fed with diets containing SBM.

Inclusion levels of FSBM and SBM had no significant effect on moisture contents of dorsal muscle and liver ($P > 0.05$). However, fish fed the control diet had significantly higher lipid content of liver ($P < 0.05$). SBM substitution led to the lowest lipid content of dorsal muscle ($P < 0.05$).

2.3 CF, HSI and VSI

HSI ranged between 2.58 and 2.76, VSI varied from 8.37 to 8.66 (Tab. 5). There were no significant differences in HSI and VSI among all the treatments ($P > 0.05$). Slight yet significant fluctuations were obtained in CF ($P < 0.05$). The highest CF was found in fish fed with the control diet but it was not statistically significant compared to fish fed with diets containing 7% and 14% FSBM, respectively.

Tab. 2 Effect of partial replacement of WFM with FSBM and SBM on growth performance, FE, PER and survival in juvenile grouper

	diet no.					
	1	2	3	4	5	6
IBW	9.43 ±0.12	9.65 ±0.35	9.28 ±0.28	9.18 ±0.60	9.40 ±0.74	9.25 ±0.00
FBW	16.94 ±0.24	18.59 ±1.22	20.63 ±0.59	21.40 ±0.86	21.23 ±2.13	16.24 ±0.39
WG	79.57 ±4.48a	92.56 ±6.84b	122.23 ±4.77c	133.52 ±13.3c	125.53 ±5.42c	75.84 ±4.24a
SGR	1.05 ±0.04a	1.17 ±0.06b	1.43 ±0.04c	1.51 ±0.10c	1.45 ±0.04c	1.01 ±0.04a
FE	60.21 ±4.78ab	74.32 ±5.24b	95.68 ±5.37bc	108.52 ±10.36c	104.61 ±7.49c	56.83 ±3.36a
PER	1.18 ±0.22a	1.45 ±0.23ab	1.90 ±0.20bc	2.15 ±0.36b	2.05 ±0.28b	1.15 ±0.18a
survival	69.37	76.08	76.90	68.69	90.28	77.01

Notes: values within the same row with different letters are significantly different ($P < 0.05$); IBW, initial fish mean body weight; FBW, final fish mean body weight; weight gain (WG) = 100 × (final mean weight - initial mean weight) / initial mean weight; specific growth rate (SGR) = 100 × ln (final mean weight) - ln (initial mean weight)) / days; FE = weight gain (g) ×100/ food intake; PER = weight gain/ protein intake; survival = 100 × (final fish number) / (initial fish number)

Tab. 3 Effect of partial replacement of WFM with FSBM and SBM on whole body composition in juvenile grouper

diet no.	moisture (%)	protein (%DM)	lipid (%DM)	ash (%DM)
1	75.86 ±1.33	63.67 ±0.85	14.29 ±0.64	20.58 ±1.27
2	74.27 ±0.59	62.44 ±1.92	14.64 ±1.02	19.39 ±0.73
3	75.60 ±1.52	63.69 ±0.33	14.76 ±0.60	19.34 ±1.16
4	75.13 ±0.77	64.60 ±1.21	14.29 ±1.45	19.52 ±1.21
5	74.83 ±0.97	64.92 ±1.97	14.98 ±0.42	18.69 ±1.09
6	75.49 ±0.64	63.65 ±1.33	13.39 ±0.89	20.70 ±1.26b

Notes: values within the same column with different letters are significantly different ($P < 0.05$)

Tab. 4 Effect of partial replacement of WFM with FSBM and SBM on dorsal muscle and liver composition in juvenile grouper

diet	dorsal muscle			liver	
no.	moisture (%)	protein (% DM)	lipid (% DM)	moisture (%)	lipid (%DM)
1	79.77 ±0.28	87.73 ±1.10ab	4.39 ±0.06a	74.16 ±0.87	15.22 ±0.59a
2	79.82 ±0.37	87.63 ±0.86a	4.58 ±0.10a	73.49 ±0.60	15.36 ±0.87a
3	79.89 ±0.47	88.10 ±0.41ab	4.62 ±0.25a	73.65 ±0.82	16.00 ±0.38a
4	80.04 ±0.66	89.12 ±0.36ab	4.45 ±0.25a	73.42 ±0.53	15.20 ±0.53a
5	79.43 ±0.46	89.18 ±0.44b	4.67 ±0.29a	72.95 ±0.83	20.01 ±0.38b
6	79.97 ±0.59	87.95 ±1.09ab	3.60 ±0.23b	73.91 ±1.02	14.84 ±1.05a

Notes: values within the same column with different letters are significantly different ($P < 0.05$)

Tab. 5 Effect of partial replacement of WFM with FSBM and SBM on CF, HSI and VSI in juvenile grouper

	diet no.					
	1	2	3	4	5	6
CF	2.92 ±0.06a	2.88 ±0.17a	2.99 ±0.11ab	3.02 ±0.06ab	3.15 ±0.10b	2.83 ±0.14a
HSI	2.70 ±0.17	2.58 ±0.14	2.76 ±0.15	2.60 ±0.15	2.64 ±0.20	2.53 ±0.18
VSI	8.66 ±0.46	8.52 ±0.38	8.84 ±0.28	8.38 ±0.52	8.37 ±0.16	8.42 ±0.61

Notes: values within the same row with different letters are significantly different ($P < 0.05$); condition factor (CF) = 100 × (live weight, g) / (body length, cm)^3; hepatosomatic index (HSI) = 100 × (liver weight) / (body weight); viscerasomatic index (VSI) = 100 × (viscera weight) / (body weight)

3 Dicussion

Survival at the present study ranged between 68 % and 90 %. This seemed reasonable because grouper was a rather sensitive species and got frightened easily as mentioned in a previous report[12]. They became extremely excited whilst being handled and during cleaning operations. At the same time *E. coioides* sometimes attack each other and injure themselves, which might have enhanced fish loss and reduced survival. In fact, the value was similar to or higher than *Anguilla anguilla* (survival, 66 % - 86 %)[13], and *E. malabaricus* (survival, 41 % - 75 %)[4].

Data on growth performance of *E. coioides* with similar fish sizes have not been published to date. Still, Millamena[2] reported that WG and SGR of *E. coioides* cultured in the laboratory with initial mean weight of 6.1 ±0.55g ranged between 440 % and 570 %, 2.8 and 3.1, respectively. In the present experiment WG and SGR of *E. coioides* weighing 9.4 ±0.1g ranged between 75 % and 135 %, 1.01 and 1.51, respectively, which were lower than those reported by Millamena[2]. The discrepancy of growth performance between the two experiments was attributable to the differences between fish sizes, cultural conditions and formulation of diets.

In the present study WG and SGR of fish fed with 14 % of FSBM were not significantly different from those of fish fed with the control diet. Based on broke-line regression analysis, 10 % of fish meal could be replaced by FSBM in the diet for grouper. Unlike SBM, FSBM is not commonly used as protein source for fish and its effect is rarely known. However, according to some documents[8] FSBM should be better than SBM in providing protein for fish, and thus the replacement level obtained in the current experiment was lower than several fish species, such as, tin foil barb[14], hybrid striped bass[15], red drum[12] and yellowtail[16], but similar to chinook salmon[17] and higher than tiger shrimp[18]. One plausible explanation for this is that the dietary protein levels in the grouper diets were higher than those of other studies, and, at the same time, it also indicated the wide variation possible in the nutritional value of FSBM and SBM for various fish species. It will be worth evaluating the possible further beneficial effects of supplementary amino acid on grouper, since in most of the studies mentioned previously, amino acid (methionine) supplementation has also been found to improve growth performance of fish fed with high levels of soybean meal[15, 19]. Besides, Millamena[2] reported that 80 % of fish meal protein could be replaced by meat meal and blood meal (4 :1 ratio) for *E. coioides*, so further improvements in the use of these plant meals are likely to involve their combination with other protein sources rather than as single meals.

In the present investigation growth performance, FE and PER of grouper were reduced in direct relation to the inclusion level of plant protein. The reduced growth and feed efficiency due to replacement of fish meal with SBM have also been reported in other fishes[6,7,14,16, 20, 21]. Several hypotheses have been suggested to explain this kind of phenomenon, which were also possibly the reasons in study 1. SBM contains many anti-nutritional factors, such as, trypsin inhibitors, non-digestible carbohydrates, lectins, saponins and phytates, which can adversely affect the growth of fish[22 - 24]; 2. SBM contains suboptimal amino acid balance, as it did not meet the methionine requirement in several fish species[21,25,26]; 3. Higher SBM contents may have caused the diets to be less attractive or palatable. Hajen et al.[17] reported that juvenile chinook salmon fed at 15 % and 30 % replacement levels of SBM and soy protein isolate had extremely low feed intake due to the poor palatability of these products. The poor palatability of SBM has also been reported in other fish species[7,12].

The inclusion level of FSBM and SBM had no significant effect on body composition. This is similar to other reports. For example, Refstie et al.[27] reported that no effects of dietary soy were detected on body composition but muscle protein content. Kikuchi[10] also suggested that there were no significant differences in the moisture, crude protein, and crude lipid content among the treatments fed different inclusion levels of SBM but slight fluctuations in ash content. In contrast, several authors reported that whole body moisture content increased and body fat decreased with increasing dietary SBM[14,19,23].

4 Conclusion

Results of this trial indicated that FSBM could replace 10 % of WFM without negative effects on grouper performance, FE, PER and body composition. There was marked difference between FSBM and SBM in nutritional quality as a protein source in fish diets.

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石斑鱼配合饲料中发酵豆粕和豆粕部分替代白鱼粉的研究

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摘要:在浮式海水网箱(1.5m ×1m ×1.5m)中养殖石斑鱼幼鱼(9.4 ±0.1g),在等氮(52 % CP)基础上进行以发酵豆粕和普通豆粕替代鱼粉的实验,为期 56 天。结果显示:在石斑鱼饲料中添加 14 %发酵豆粕,其增重率、特定生长率(SGR)、饲料效率和蛋白质效率与对照组没有显著性差异($P > 0.05$),以后随着发酵豆粕添加量的上升,这些指标都显著下降($P < 0.05$)。在同样替代水平下,添加 21 %发酵豆粕组,增重率,SGR,饲料效率和蛋白质效率都比添加 20 %豆粕组高($P < 0.05$),表明对海水肉食性鱼类来说,发酵豆粕是一种比豆粕更优良的蛋白源。用折线模型分析增重率随白鱼粉替代水平的变化关系,结果表明在石斑鱼配合饲料中,发酵豆粕替代白鱼粉的最适量为 10 %。从实际生产的经济效益出发,建议在饲料中添加 14 %发酵豆粕,对石斑鱼的生长和鱼体组成不会造成显著影响。

关键词:石斑鱼幼鱼;发酵豆粕;豆粕;白鱼粉;生长

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