Aquaculture Research

Aquaculture Research, 2017, **48**, 1904–1914 doi: 10.1111/are.13028

Supplementations of poultry by-product meal and selenium yeast increase fish meal replacement by soybean meal in golden pompano (*Trachinotus ovatus*) diet

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Abstract

The effects of poultry by-product meal (PBM) and selenium yeast (Se-yeast) supplementations on fish meal replacement by soybean meal (SBM) in the diet of golden pompano (*Trachinotus ovatus*) were examined. In trial I, a 2 × 3 layout including two PBM levels (100 and 170 g kg^{-1}) and three levels of fish meal replacement (0, 40 or 60%) was used. At 100 g kg^{-1} PBM, fish fed the basal diet exhibited the highest weight gain (WG) and nitrogen retention efficiency (NRE) and the lowest feed conversion ratio (FCR). At 170 g kg^{-1} PBM, no significant differences were found in WG and NRE between fish fed the basal diet and diet with 40% of fish meal replaced by SBM. In trial II, the basal diet containing 170 g kg^{-1} PBM (trial I) served as a reference. A 2 × 2 layout included two levels of fish meal replacement (40 or 60%) and two levels of Se-yeast addition (0 and 1 g kg^{-1}). No significant differences were found in WG, feed intake, FCR and NRE between fish fed the reference diet and diet with 40 or 60% fish meal replacement plus 1 g kg^{-1} Se-yeast addition. This study indicates that supplementations of PBM and Se-yeast can enhance the level of fish meal replacement by SBM in golden pompano diet. Dietary fish meal level can be reduced to 140 g kg^{-1} by optimizing inclusion of SBM, PBM and Se-yeast.

Keywords: antinutritional factor, fish meal, nitrogen retention efficiency, selenium, soybean meal, weight gain

Introduction

Global marine fish farming has been expanded rapidly in the past a few decades due to the increased demand on seafood and the success in domestication of marine fish species (Duarte, Marba & Holmer 2007). Most commercially cultured marine fish species are carnivorous and require 30–50 g kg^{-1} dietary fish meal (Wang, Guo, Bureau & Cui 2006; Naylor, Hardy, Bureau, Chiu, Elliott, Farrell, Forster, Gatlin, Goldburg, Hua & Nichols 2009). Replacement of fish meal with terrestrial plant or animal ingredients in fish diets has been recognized a key approach to sustainable marine fish aquaculture (Naylor *et al.* 2009). Various ingredients, such as poultry by-product meal (PBM), meat and bone meal, feather meal and soybean meal (SBM), have been evaluated as fish meal substitutes in fish feed (Wang, Kong, Li & Bureau 2006; Wang, Guo *et al.* 2006). The level of fish meal replacement by these ingredients varies greatly among studies due to differences in the source and quality of fish meal and alternative ingredients as well as basal diet formulation (Choi, Wang, Park, Lim, Kim, Bai & Shin 2004; Lim, Choi, Wang, Kim, Shin, Min & Bai

2004; Tomas, De la Gandara, Garcia-Gomez, Perez & Jover 2005; Hernandez, Martinez, Jover & Garcia 2007; Martinez-Llorens, Vidal, Garcia, Torres & Cerda 2009; Wang, Kong, Li & Bureau 2010; Lim, Kim, Ko, Song, Oh, Kim, Kim & Lee 2011; Rossi & Davis 2012). The factors limiting the inclusion of alternative ingredients in fish feed include low digestible protein, unfavuorable composition of essential amino acids, presence of antinutritional factors and deficiency of growth-promoting factors (Hertrampf & Piedad-Pascual 2000; Gatlin, Barrows, Brown, Dabrowski, Gaylord, Hardy, Herman, Hu, Krogdahl, Nelson, Overturf, Rust, Sealey, Skonberg, Souza, Stone, Wilson & Wurtele 2007; Glencross, Booth & Allan 2007; Kaushik & Seiliez 2010). However, the underlying mechanisms limiting the amount of fish meal replacement have rarely been tested in rigorously designed experiments with valid controls.

Golden pompano *Trachinotus ovatus* is widely cultured along the coast of the South China Sea (Wang, Han, Wang & Ma 2013). This fish requires more than 210 g kg^{-1} dietary fish meal when PBM, SBM or soy protein concentrate are used alone as a fish meal subsitute (Ma, Wang, Han, Wang & Lin 2014; Wu, Han, Qin & Wang 2015). Recently, the requirment of selenium (Se) in fish nutrtion has been recognized, as Se is an essential constituent of various enzymes with important physiological functions (Yoshida, Haratake, Fuchigami & Nakayama 2011). Selenium content is much higher in fish meal than in PBM and soy ingredients (Hertrampf & Piedad-Pascual 2000). The low level of fish meal replacement by PBM and soy ingredients (Ma *et al.* 2014; Wu *et al.* 2015) may be attributed to Se deficiency, and it is possible that Se supplementation can increase the amount of fish meal replacement by terrestrial proteins. On the other hand, PBM is a well-sourced protein in the diet of many marine fish species (Takagi, Hosokawa, Shimeno & Ukawa 2000; Wang, Guo *et al.* 2006; Wang, Wang, Ji, Han & Li 2015). The inclusion of more PBM may improve the growth of fish fed the diets with fish meal replaced by increasing the ratio of animal protein to plant protein in diet formulation (Reigh & Ellis 1992). The purpose of this study was to test if supplementations of PBM and Se can increase the level of dietary fish meal replacement by SBM in diets for golden pompano. The result will provide insight into the knowledge how basal diet formulation affect the replacement of fish meal with plant protein.

Material and methods

Feed ingredients and test diets

The PBM was supplied by the Hong Kong Office, National Renderer Association. The selenium yeast (Se-yeast) was purchased from the Angel Yeast Co., Ltd (Yichang, China). The other feed ingredients were purchased from the Haihuang Feed Company (Hangzhou, China). The proximate composition of the feed ingredients is shown in Table 1.

Two feeding trials were conducted. In trial I, a 2 × 3 layout included two PBM levels (P1: 100 g kg^{-1}, P2: 170 g kg^{-1}) and three levels of fish meal replacement (F1: 0%, F2: 40%, F3: 60%). At each PBM level, a basal diet was formulated to contain 350 g kg^{-1} fish meal, and the fish meal content was reduced to 210 or 140 g kg^{-1} in the other two diets by replacing the fish meal in the basal diet with SBM at an equal protein basis respectively. The six test diets in trial I were abbreviated as P1F1, P1F2, P1F3, P2F1, P2F2 and P2F3. In trial II, the basal diet P2F1 (in trial I) served as a reference. A 2 × 2 layout included two levels of Se-yeast addition and two levels of fish meal replacement. The test diets were formulated to contain 210 or 140 g kg^{-1} fish meal by replacing 40% (P2F2) or 60% (P2F3) of the fish meal in the reference diet (P2F1) with SBM respectively. At each fish meal replacement level, Se-yeast was added at 0 (S0) or 1 (S1) g kg^{-1}. Se content of the Se-yeast was 1600 mg kg^{-1}. The 1 g kg^{-1} Se-yeast addition provided 1.6 mg kg^{-1} dietary Se, which was higher than the Se requirement for Malabar grouper (Lin & Shiau 2005), but

Table 1 Proximate composition (g kg^{-1}) of feed ingredients

Ingredient	Dry matter	Crude protein	Crude lipid	Ash
Fish meal	923	689	55	215
Soybean meal	906	468	28	60
Poultry by-product meal	892	661	133	135
Selenium yeast	961	439	20	141
Brews yeast	939	462	6	61
Rapeseed meal	879	408	5	72
Wheat flour	873	127	11	7

Crude protein, crude lipid and ash are expressed on dry ingredient (in air) basis.

lower than the safe level of dietary Se for Atlantic salmon parr (Lorentzen, Maage & Julshamm 1994). Four test diets in trial II were abbreviated as S0-P2F2, S0-P2F3, S1-P2F2 and S1-P2F3. The diets S0-P2F2 and S0-P2F3 (trial II) were same to the diets P2F2 and P2F3 (trial I) respectively. The test diets were formulated to contain 480 g kg^{-1} crude protein (Wang *et al.* 2013), and crystalline DL-Met and L-Lys were added to balance the dietary amino acids (Ma *et al.* 2014). A single screw extruder was used to make the test diets, and the extruding temperature was controlled at 80–100°C as described in Wang, Kong *et al.* (2006). Formulation, proximate composition and gross energy content of the test diets are shown in Table 2.

Fish, net pens and feeding procedure

The feeding trials were conducted in Shenao Bay, Nanao, China. The golden pompano fingerlings were purchased from a local marine fish hatchery. After arrival, the fish were reared in commercial net pens (3 × 3 × 2 m) and fed minced raw fish for 2 weeks. Prior to the feeding trials, 840 fish of similar size were acclimated in 24 experimental pens (1 × 1 × 1.5 m) at 35 fish pen^{-1}. The fish were fed with diet P1F1 twice daily during the 4-week acclimation.

In trial I, fish (16.7 ± 0.2 g, mean ± SD, n = 18) were deprived of feed for 24 h prior to experiment implementation. Eighteen net pens were stocked with 30 fish each and were randomly assigned with six diet treatments (P1F1, P1F2, P1F3, P2F1, P2F2 and P2F3) in three replicates. In trial II, 12 net pens were stocked with 30 fish (16.6 ± 0.2 g, mean ± SD, n = 12) and randomly assigned with four diet treatments (S0-P2F2, S0-P2F3, S1-P2F2 and S1-P2F3) in three replicates. In addition, three pens stocked with 30 fish fed the basal diet containing 170 g kg^{-1} PBM without fishmeal replacement (P2F1) were used as

Table 2 Formulation (g kg^{-1}), proximate composition (g kg^{-1}) and energy content (MJ kg^{-1}) of the test diets

Ingredient	P1F1	P1F2	P1F3	P2F1	P2F2	P2F3	S1-P2F2	S1-P2F3
Fish meal	350	210	140	350	210	140	210	140
Soybean meal	150	360	455	50	260	355	260	355
Poultry by-product meal	100	100	100	170	170	170	170	170
Selenium yeast	0	0	0	0	0	0	1	1
Casein	2	11	21	2	11	21	11	21
Rapeseed meal	100	100	100	100	100	100	100	100
Brewer's yeast	30	30	30	30	30	30	30	30
Choline	2	2	2	2	2	2	2	2
Wheat flour	168	84	47	205	121	84	120	83
Seaweed powder	30	30	30	30	30	30	30	30
$CaHPO_4$	15	15	15	15	15	15	15	15
L-Lys	1	2	2	1	2	2	2	2
DL-Met	1	2	3	1	2	3	2	3
Vitamin premix	15	15	15	15	15	15	15	15
Mineral premix	15	15	15	15	15	15	15	15
Fish oil	21	24	25	14	17	18	17	18
Dry matter	902	906	902	901	892	880	904	889
Crude protein	479	477	482	488	472	477	487	493
Crude lipid	65	66	68	58	54	62	57	58
Ash	156	134	123	157	139	129	138	130
Phosphorus	24	20	18	25	21	18	21	20
Gross energy	20.9	20.9	21.7	21.0	21.5	22.1	21.3	21.9

Diets P2F1, P2F2 and P2F3 in trial I are same in formulation and ingredients as diets reference, S0-P2F2 and S0-P2F3 in trial II respectively.

Crude protein, crude lipid, ash, phosphorus, gross energy and selenium are expressed as dry feed (in air) basis.

Vitamin premix composition (kg^{-1} diet): vitamin A, 2500 IU; vitamin D_3, 2000 IU; vitamin E, 50 mg; vitamin K_3, 1 mg; vitamin B_{12}, 0.02 mg; vitamin C, 250 mg; biotin, 0.14 mg; choline, 1000 mg; folic acid, 1 mg; niacin, 10 mg; D-Calcium pant acid, 20 mg; vitamin B_6, 5 mg; riboflavin, 6 mg; vitamin B_1, 1 mg; astaxanthin, 50 mg.

Mineral premix composition (mg kg^{-1} diet): $Ca(H_2PO_4)_2{\cdot}H_2O$, 400; calcium lactate, 1000; ferric citrate, 100; $MgSO_4{\cdot}7H_2O$, 400; K_2HPO_4, 700; $NaH_2PO_4{\cdot}H_2O$, 250; $AlCl_3{\cdot}6H_2O$, 20; $ZnCl_2$, 60; $CuSO_4{\cdot}5H_2O$, 30; $MnSO_4{\cdot}4H_2O$, 20; KI, 20.

the reference. Totally, 24 net pens were used since treatment P2F2 and P2F3 (in trial I) were overlapped with treatment S0-P2F2 and S0-P2F3 (in trial II) respectively. Three groups with three fish each were sampled from the pooled population prior to each trial as the initial sample. After measurement of condition factor, hepatosomatic index (HSI) and viscerosomatic index (VSI), the fish were stored at −20°C for analysis of initial body composition.

The duration of trials I and II was 8 weeks, during which fish were hand fed at 0800 and 1600 h daily with the method described in Wang, Guo *et al.* (2006). Fish were not fed in the days with heavy rainfall. The dead fish were accounted and weighed. Water temperature was measured daily and fluctuated from 21.2 to 28.6°C (24.8 ± 2.3°C, mean ± SD, $n = 56$). Salinity was measured weekly and fluctuated from 28 to 34 ppt (28.9 ± 3.0 ppt, mean ± SD, $n = 8$).

At the end of the feeding trials, fish in each pen were deprived of feed for 24 h, and then weighed in bulk. Three fish were sampled from each pen to determine condition factor, HSI, VSI and final body composition. The sampled fish were stored at −20°C until chemical analysis.

Chemical analysis

Prior to the chemical analysis, fish were autoclaved at 120°C for 20 min, homogenized, and then dried at 75°C. The contents of moisture, crude protein, crude lipid, ash and phosphorus of the feed ingredients, test diets and fish were analyzed using the method described in AOAC (1995). The contents of gross energy of the test diets and fish were measured using a Parr-6200 calorimeter (Parr Instrument Company, Moline, IL, USA). The Se content of Se-yeast was analyzed with an Agilent 7700× inductively coupled plasma-mass spectrometry (Agilent Technologies, Santa Clara, CA, USA).

Calculation and statistical analysis

Feed intake, weight gain (WG), feed conversion ratio (FCR), nitrogen retention efficiency (NRE), phosphorus retention efficiency (PRE), energy retention efficiency (ERE), condition factor, HSI, VSI, nitrogen waste (NW) and phosphorus waste (PW) were calculated as below:

$$\text{Feed intake (\% day}^{-1}) = 100 \times I/[(W_0 + W_t)/2 \times t]$$

$$\text{Weight gain (g)} = (W_t/N_t - W_0/N_0)$$

$$\text{Feed conversion ratio} = I/(W_t - W_0)$$

$$\text{Nitrogen retention efficiency (\%)} = 100 \times (W_t \times C_{Nt} - W_0 \times C_{N0})/(I \times C_{Nf})$$

$$\text{Phosphorus retention efficiency (\%)} = 100 \times (W_t \times C_{Pt} - W_0 \times C_{P0})/(I \times C_{Pf})$$

$$\text{Energy retention efficiency (\%)} = 100 \times (W_t \times C_{Et} - W_0 \times C_{E0})/(I \times C_{Ef})$$

$$\text{Condition factor} = 100 \times W_s/L_s^3$$

$$\text{Hepatosomatic index (\%)} = 100 \times W_l/W_s$$

$$\text{Viscerosomatic index (\%)} = 100 \times W_v/W_s$$

$$\text{Nitrogen waste [g N (kg fish gain)}^{-1}] = 1000 \times (I \times C_{Nf}) \times (1 - NRE)/[(W_t - W_0) \times 6.25]$$

$$\text{Phosphorus waste [g P (kg fish gain)}^{-1}] = 1000 \times (I \times C_{Pf}) \times (1 - PRE)/(W_t - W_0)$$

where I (g) is total amount of the diets fed on a dry matter basis; W_0 (g) is the total initial body weight and W_t (g) is the total final body weight; t (d) is the duration of the feeding trials; N_t is number of fish at the end of the feeding trials and N_0 at the start; C_{Nt} (%) is crude protein content of whole body at the end of the feeding trials and C_{N0} (%) at the start; C_{Nf} (%) is crude protein content of the test diets; C_{Pt} (%) is phosphorus content of the whole body at the end of the feeding trials and C_{P0} (%) at the start; C_{Pf} (%) is phosphorus content of the test diets; C_{Et} (%) is energy content of the whole body at the end of the feeding trials and C_{E0} (%) at the start; C_{Ef} (%) is energy content of the test diets; W_s (g), L_s (cm), W_v (g) and W_l (g) are body weight, body length, viscera weight and liver weight of sampled fish at the start or end of the feeding trials.

In both trial I and trial II, two-way ANOVA was used to examine the differences in survival, final body weight, WG, feed intake, FCR, retention efficiencies of nitrogen, phosphorus and energy, condition factor, HSI, VSI, body components (contents of moisture, crude protein, crude lipid, ash and energy), NW and PW among treatments. The differences between the treatments were compared either with Tukey's honestly significant difference test (HSD test) if significant differences main treatment factor were detected in ANOVA. The feed intake, NRE, PRE, ERE, HSI, VSI and body components were arcsine-transformed. In trial II, student's *t*-test was used to examine the differences in WG, feed intake and nutrient retention efficiencies between fish fed the reference diet and other four diets. The significant level was set at $P < 0.05$.

Results

Trial I

Table 3 shows survival, feed intake, growth, feed utilization of fish in trial I. The survival was 98–100% across all the treatments. The final body weight, WG, feed intake, FCR, NRE, ERE and PRE were dependent on fish meal replacement level; and feed intake, FCR and PRE were dependent on PBM level (two-way ANOVA, $P < 0.05$). The final body weight, WG, feed intake, FCR, NRE and PRE were dependent on the interaction between fish meal replacement and PBM levels (two-way ANOVA, $P < 0.05$). At the same level of fish meal replacement, the WG, NRE and ERE increased, whereas the FCR decreased with the increase in PBM level (HSD test, $P < 0.05$), except fish fed the basal diets (P1F1 and P2F1). At the same PBM level, the WG, NRE and ERE tended to decrease, whereas the FCR tended to increase, with the decrease in fish meal level (HSD test, $P < 0.05$). The WG, NRE and ERE were highest, and the FCR was lowest in fish fed diet P1F1 (HSD test, $P < 0.05$). No significant differences were found in the WG, NRE and ERE between fish fed diets P2F1 and P2F2 or between fish fed diets P1F2, P1F3 and P2F3 (HSD test, $P > 0.05$). The WG, NRE and ERE were higher, but the FCR was lower in fish fed diet P2F2 than in fish fed diets P1F2, P1F3 and P2F3 (HSD test, $P < 0.05$).

Table 4 shows morphological parameters and body composition of fish in trial I. At the end of the trial, the condition factor, VSI and body composition (moisture, crude protein, crude lipid and ash) were independent on both fish meal replacement and PBM levels (two-way ANOVA, $P > 0.05$).

Figure 1 shows NW and PW of fish in trial I. Nitrogen waste and PW were significantly affected by both fish meal replacement and PBM levels (two-way ANOVA, $P < 0.05$,). At the same PBM level, the NW tended to increase, whereas the PW tended to decrease, with the decrease in fish meal level (HSD test, $P < 0.05$).

Trial II

Table 5 shows survival, feed intake, growth, feed utilization of fish in trial II. The survival was 98 to 100% across the treatments. The final body weight, WG, feed intake, FCR and PRE were dependent on fish meal replacement level, and the final body weight, WG, feed intake and FCR were dependent on Se-yeast addition (two-way ANOVA, $P < 0.05$). The feed intake was significantly affected by the interaction between fish meal replacement level and Se-yeast addition (two-way ANOVA, $P < 0.05$). The final body weight and WG were higher in fish fed diet S1-P2F2 than in fish fed diet S0-P2F3 (HSD test, $P < 0.05$), whereas no significant differences were found in the final body weight and WG between fish fed diets S0-P2F2, S1-P2F2 and S1-P2F3 (HSD test, $P > 0.05$). The feed intake and FCR were higher in fish fed diet S0-P2F3 than in fish fed diets S0-P2F2, S1-P2F2 and S1-P2F3 (HSD test, $P < 0.05$). No significant

Table 3 Final body weight (FBW g fish^{-1}), weight gain (g fish^{-1}), feed intake (% day^{-1}), feed conversion ratio (FCR), nitrogen retention efficiency (NRE %), energy retention efficiency (ERE %), phosphorus retention efficiency (PRE %) and survival (%) of golden pompano in trial I (Mean ± SD, $n = 3$)

Diet	FBW	Weight gain	Feed intake	FCR	NRE	ERE	PRE	Survival
P1F1	59.7 ± 1.0^{a}	43.0 ± 0.9^{a}	3.02 ± 0.03^{d}	1.50 ± 0.05^{d}	23.8 ± 1.1^{a}	25.6 ± 1.5^{a}	15.0 ± 0.6^{bc}	100
P1F2	51.3 ± 0.5^{c}	34.6 ± 0.6^{c}	3.37 ± 0.03^{ab}	1.86 ± 0.03^{a}	19.3 ± 0.3^{c}	21.0 ± 1.1^{ab}	14.0 ± 0.6^{c}	100
P1F3	50.8 ± 0.8^{c}	34.3 ± 0.7^{c}	3.41 ± 0.04^{a}	1.87 ± 0.03^{a}	19.3 ± 0.4^{c}	19.4 ± 1.7^{b}	15.8 ± 0.6^{ab}	100
P2F1	56.4 ± 0.3^{b}	39.6 ± 0.3^{b}	3.12 ± 0.01^{c}	1.62 ± 0.01^{c}	21.9 ± 0.3^{b}	23.5 ± 2.4^{ab}	14.1 ± 0.2^{c}	100
P2F2	55.0 ± 0.4^{b}	38.3 ± 0.4^{b}	3.16 ± 0.01^{c}	1.66 ± 0.01^{c}	21.9 ± 0.6^{b}	22.6 ± 0.4^{ab}	17.0 ± 0.3^{a}	100
P2F3	52.5 ± 1.0^{c}	35.9 ± 1.1^{c}	3.29 ± 0.01^{b}	1.76 ± 0.04^{b}	20.3 ± 0.5^{bc}	20.2 ± 2.0^{b}	16.2 ± 0.5^{ab}	98 ± 2
Fish meal replacement	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	NS
PBM level	NS	NS	$P < 0.05$	$P < 0.05$	NS	NS	$P < 0.05$	NS
Interaction	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	NS	$P < 0.05$	NS

Feed intake and FCR are expressed on a dry feed (in air) basis.
Values with different superscripts within the same column are significantly different (HSD test, $P < 0.05$).

Table 4 Condition factor (CF g cm^{-3}), hepatosomatic index (HSI %), viscerasomatic index (VSI %), proximate composition (g kg^{-1}) and energy content (MJ kg^{-1}) in whole body of golden pompano in trial I (Mean ± SD, n = 3)

Diet	CF	HSI	VSI	Moisture	Crude protein	Crude lipid	Ash	Phosphorus	Gross energy
Initial	2.81 ± 0.09	1.13 ± 0.04	4.89 ± 0.18	760 ± 6	170 ± 2	36 ± 7	46 ± 2	7.4 ± 0.1	5.2 ± 0.3
P1F1	3.78 ± 0.08	1.88 ± 0.21	6.39 ± 0.29	710 ± 6	171 ± 4	85 ± 4	38 ± 2	6.0 ± 0.1^{bc}	7.3 ± 0.3
P1F2	3.49 ± 0.11	1.82 ± 0.23	6.99 ± 0.73	715 ± 7	171 ± 0	81 ± 4	37 ± 1	5.9 ± 0.2^{c}	7.2 ± 0.3
P1F3	3.49 ± 0.02	2.01 ± 0.20	7.53 ± 0.62	716 ± 11	173 ± 2	79 ± 7	37 ± 3	6.0 ± 0.1^{bc}	7.0 ± 0.5
P2F1	3.61 ± 0.33	2.17 ± 0.29	7.66 ± 0.22	715 ± 14	172 ± 1	83 ± 8	30 ± 1	6.2 ± 0.1^{ab}	7.2 ± 0.5
P2F2	3.45 ± 0.08	1.82 ± 0.26	6.87 ± 0.38	710 ± 3	171 ± 3	84 ± 2	37 ± 0	6.4 ± 0.0^{a}	7.2 ± 0.1
P2F3	3.51 ± 0.19	1.74 ± 0.28	6.95 ± 0.81	712 ± 12	170 ± 3	82 ± 8	38 ± 1	5.9 ± 0.2^{bc}	7.1 ± 0.5
Fish meal replacement	NS	NS	NS	NS	NS	NS	NS	NS	NS
PBM level	NS	NS	NS	NS	NS	NS	NS	NS	NS
Interaction	NS	NS	$P < 0.05$	NS	NS	NS	NS	NS	NS

Crude protein, crude lipid, ash, phosphorus and gross energy are expressed on a wet weight basis.
SD < 0.5 are expressed as SD = 0, and SD < 0.05 are expressed as SD = 0.0.
Values with different superscripts within the same column are significantly different (HSD test, $P < 0.05$).

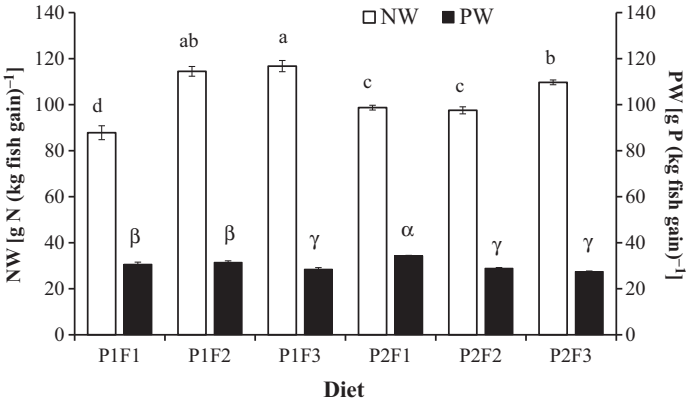

Figure 1 Nitrogen waste (NW) and phosphorus waste (PW) of golden pompano in trial I. The column represents mean value and error bar represents standard deviation (n = 3). The values with different letters are significantly different (HSD test, $P < 0.05$).

differences were found in the NRE, PRE and ERE between fish fed diets S0-P2F2, S0-P2F3, S1-P2F2 and S1-P2F3 (HSD test, $P > 0.05$, Table 5). In addition, no significant differences were found in the WG, feed intake, FCR, NRE, phosphorus retention efficiency and ERE between fish fed the reference diet (P2F1) and diets S1-P2F2 or S1-P2F3 (Student's t-test, $P > 0.05$, Table 5).

Table 6 shows morphological parameters and body composition of fish in trial II. At the end of the trial, the condition factor, VSI and body composition (moisture, crude protein, crude lipid and ash) were independent on both fish meal replacement level and Se-yeast addition (two-way ANOVA, $P > 0.05$).

Figure 2 shows NW and PW of fish in trial II. Nitrogen waste and PW were dependent on by both fish meal replacement and Se-yeast addition (two-way ANOVA, $P < 0.05$). At the same Se-yeast level, the NW increased with the decrease in fish meal level (HSD test, $P < 0.05$).

Discussion

Nutrient requirement of fish is generally determined as the lowest dietary nutrient content for fastest growth of fish. Besides dietary composition, some environmental factors such as water temperature, dissolved oxygen and salinity also affect fish growth (Bowyer, Qin & Stone 2013; Bowyer, Booth, Qin, D'Antignana, Thomason & Stone 2014). Therefore, nutrient requirement of fish should be evaluated under an environmental condition that favours fish growth. In this study, the

Table 5 Final body weight (FBW g fish^{-1}), weight gain (g fish^{-1}), feed intake (% day^{-1}), feed conversion ratio (FCR), nitrogen retention efficiency (NRE %), nitrogen retention efficiency (PRE %), energy retention efficiency (ERE %), phosphorus retention efficiency (PRE %) and survival (%) of golden pompano in trial II (Mean ± SD, n = 3)

Diet	FBW	Weight gain	Feed intake	FCR	NRE	ERE	PRE	Survival
Reference	56.4 ± 0.3	39.6 ± 0.3	3.12 ± 0.01	1.62 ± 0.01	21.9 ± 0.3	23.5 ± 2.4	14.1 ± 0.2	100
S0-P2F2	55.0 ± 0.4^{a}	38.3 ± 0.4^{ab}	3.16 ± 0.01^{b}	1.66 ± 0.01^{b}	21.9 ± 0.6	22.6 ± 0.4	17.0 ± 0.3	100
S0-P2F3	52.5 ± 1.0^{b}	35.9 ± 1.1^{b}	3.29 ± 0.01^{a}	1.76 ± 0.04^{a}	20.3 ± 0.5	20.2 ± 2.0	16.2 ± 0.5	98 ± 2
S1-P2F2	56.9 ± 1.1^{a}	40.4 ± 1.3^{a}	3.12 ± 0.02^{b}	1.59 ± 0.04^{b}	21.8 ± 0.5	21.4 ± 3.0	17.4 ± 0.5	100
S1-P2F3	55.0 ± 1.1^{a}	38.2 ± 1.3^{ab}	3.16 ± 0.04^{b}	1.65 ± 0.05^{b}	20.5 ± 0.9	20.5 ± 3.4	19.6 ± 2.5	99 ± 2
Fish meal replacement	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	NS	NS	$P < 0.05$	$P < 0.05$
Se-yeast addition	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	NS	NS	NS	NS
Interaction	NS	NS	$P < 0.05$	NS	NS	NS	NS	NS

Diets reference, S0-P2F2 and S0-P2F3 are same in formulation and ingredients as diets P2F1, P2F2 and P2F3 used in trial I respectively.

Feed intake and FCR are expressed on a dry feed basis.

Values with different superscripts within the same column are significantly different (HSD test, $P < 0.05$). Diet reference was not involved in ANOVA and HSD test.

Se-yeast, Selenium yeast.

Table 6 Condition factor (CF g cm^{-3}), hepatosomatic index (HSI %), viscerasomatic index (VSI %), proximate composition (g kg^{-1}) and energy content (MJ kg^{-1}) in whole body of golden pompano in trial II (Mean ± SD, n = 3)

Diet	CF	HSI	VSI	Moisture	Crude protein	Crude lipid	Ash	Phosphorus	Gross energy
Reference	3.61 ± 0.33	2.17 ± 0.29	7.66 ± 0.22	715 ± 14	172 ± 1	83 ± 8	30 ± 1	6.2 ± 0.1	7.2 ± 0.5
S0-P2F2	3.45 ± 0.08	1.82 ± 0.26	6.87 ± 0.38	710 ± 3	171 ± 3	84 ± 2	37 ± 0	6.4 ± 0.0^{ab}	7.2 ± 0.1
S0-P2F3	3.51 ± 0.19	1.74 ± 0.28	6.95 ± 0.81	712 ± 12	170 ± 3	82 ± 8	38 ± 1	5.9 ± 0.2^{b}	7.1 ± 0.5
S1-P2F2	3.52 ± 0.18	1.73 ± 0.30	5.11 ± 3.17	727 ± 19	169 ± 3	72 ± 9	38 ± 1	6.3 ± 0.1^{ab}	6.7 ± 0.7
S1-P2F3	3.48 ± 0.52	1.59 ± 0.39	6.98 ± 0.28	722 ± 20	168 ± 2	73 ± 15	40 ± 2	6.7 ± 0. 4^{a}	6.8 ± 1.0
Fish meal replacement	NS	NS	NS	NS	NS	NS	NS	NS	NS
Se-yeast addition	NS	NS	NS	NS	NS	NS	NS	NS	NS
Interaction	NS	NS	NS	NS	NS	NS	NS	NS	NS

Diets reference, S0-P2F2 and S0-P2F3 are same in formulation and ingredients as diets P2F1, P2F2 and P2F3 used in trial I respectively.

Crude protein, crude lipid, ash, phosphorus and gross energy are expressed on a wet weight basis.

SD < 0.5 are expressed as SD = 0, and SD < 0.05 are expressed as SD = 0.0.

Values with different superscripts within the same column are significantly different (HSD test, $P < 0.05$). Diet reference was not involved in ANOVA and HSD test.

Se-yeast, Selenium yeast.

survival and WG of fish fed the basal diets (P1F1 and P2F1) in trial I were excellent, suggesting that the data are reliable for evaluating the fish meal replacement by SBM on fish growth and feed utilization.

In this study, the two basal diets (P1F1 and P2F1) used in trial I contained the same level of fish meal but with different levels of PBM and SBM. The WG and NRE were higher in fish fed diet P1F1 than in fish fed diet P2F1, suggesting that nutritive quality of these diets depended on the balance of diet formulation, rather than the contents of a single ingredient, such as fish meal, PBM or SBM. This result highlights that the purpose of fish meal replacement in fish diets is to establish a nutritionally balanced diet formulation with low fish meal inclusion, rather than replacing fish meal with the alternative ingredients that are

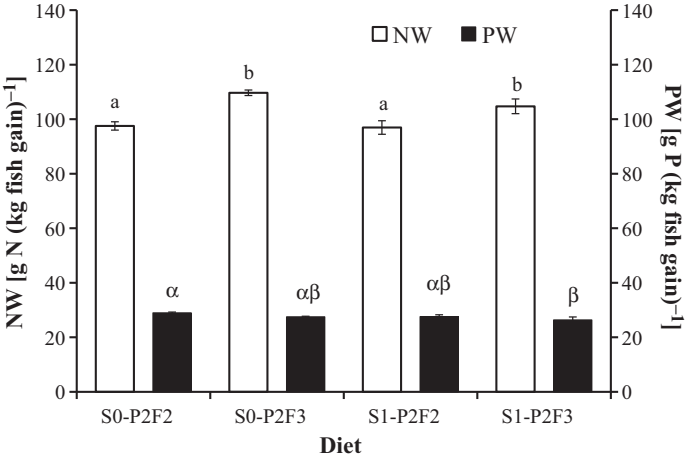

Figure 2 Nitrogen waste (NW) and phosphorus waste (PW) of golden pompano in trial II. Diets S0-P2F2 and S0-P2F3 are same in formulation and ingredients as diets P2F2 and P2F3 used in trial I respectively. The column represents mean value and error bar represents standard deviation (n = 3). The values with different letters are significantly different (HSD test, $P < 0.05$).

nutritionally equal to fish meal. The level of fish meal replacement was higher when diet P2F1 was used as a basal diet than when diet P1F1 was used as a basal diet, suggesting that the potential to replace fish meal with alternative ingredients depends on basal diet formulation (Wang, Guo *et al.* 2006, 2015).

Riche and Williams (2011) reported that the level of dietary fish meal for juvenile Florida pompano can be reduced to 190 g kg^{-1} when solvent-extracted SBM was used as a fish meal substitute. Ma *et al.* (2014) reported that more than 240 g kg^{-1} fish meal should be remained in golden pompano diet when SBM is used alone as a fish meal substitute. Wu *et al.* (2015) reported that the least dietary fish meal level was 320 g kg^{-1} for golden pompano when soy protein concentrate is used as a fish meal substitute. In this study, no significant differences were found in the WG, FCR and NRE between fish fed diets P2F1, S1-P2F2 and S1-P2F3 (trial II). This result suggests that dietary fish meal level can be reduced to 140 g kg^{-1} by supplementation of 355 g kg^{-1} SBM, 170 g kg^{-1} PBM and 1.6 mg kg^{-1} Se.

Generally, excessive replacement of fish meal with SBM in fish diet results in growth decline (Choi *et al.* 2004; Lim *et al.* 2004, 2011; Tomas *et al.* 2005; Wang, Kong *et al.* 2006; Hernandez *et al.* 2007; Kasper, Watkins & Brown 2007; Martinez-Llorens *et al.* 2009). Poor digestibility of protein, unfavourable amino acid composition, presence of antinutritional factors and deficiency of growth prompting factors are recognized as the factors limiting fish meal replacement by terrestrial plant ingredients in fish diets (Hertrampf & Piedad-Pascual 2000; Gatlin *et al.* 2007; Glencross *et al.* 2007; Kaushik & Seiliez 2010). The poor digestibility of protein or deficiency in essential amino acids can be improved by increasing the level of dietary protein (Wang *et al.* 2010) and adding crystalline amino acids (Ma *et al.* 2014). Previous studies reported that no significant differences were found in the digestibility of protein and bioavailability of amino acids between SBM and fish meal for Florida pompano (Gonzalez-Felix, Davis, Rossi & Perez-Velazquez 2010; Gothreaux, Reigh, Williams & Chesney 2010; Riche & Williams 2010). In this study, protein and DL-Met contents of the test diets were formulated to satisfy the requirements of golden pompano (Wang *et al.* 2013). The SBM content (360 g kg^{-1}) of diets P1F2 and P2F3 was higher than that (260 g kg^{-1}) of diet P2F2, while fish meal content (210 g kg^{-1}) of diets P1F2 and P2F2 was higher than that (140 g kg^{-1}) of diet P2F3. However, the WG and NRE of fish fed diet P1F2 were higher than those of fish fed diet P2F2, but did not significantly differ from those of fish fed diet P2F3. These results suggest that the antinutritional factors of SBM might be a primary factor limiting the level of fish meal replacement, and SBM inclusion could not exceed 360 g kg^{-1} in golden pompano diet.

In this study, SBM inclusion decreased with the increase in PBM inclusion (trial I). The taurine content of fish meal (7.92 g kg^{-1}) and PBM (3.62 g kg^{-1}) is much higher than that (undetectable) of SBM (Y.B. Wu, Y. Wang, D.L. Jiang, Y.Y. Lin, unpublished data). Therefore, more PBM inclusion not only resulted in the reduction in the

content of antinutritional factors, but also resulted in the increase in taurine content. Rossi and Davis (2012) reported that taurine supplementation might be required for maximum growth of Florida pompano. Wu *et al.* (2015) indicated that taurine supplementation can improve fish meal replacement by soy ingredients in diets for golden pompano.

Partial replacement of fish meal with PBM can improve the growth of red sea bream (Takagi *et al.* 2000) and Japanese seabass (Wang *et al.* 2015), but result in growth decline in cuneate drum (Wang, Guo *et al.* 2006), rainbow trout (El-Haroun, Azevedo & Bureau 2009) and golden pompano (Ma *et al.* 2014). Replacement of fish meal with PBM at high levels may result in Se deficiency since Se content is higher in fish meal than in PBM (Hertrampf & Piedad-Pascual 2000). Selenium is an essential constituent of some enzymes, including glutathione peroxidase, deiodinase and thioredoxin reductase (Yoshida *et al.* 2011). Selenium supplementation can improve the growth of fish exposed to stressors (Bell, Pirie, Adron & Cowey 1986; Kücükbay, Yazlak, Karaca, Sahin, Tuzcu, Cakmak & Sahin 2009). However, excessive Se is toxic to fish (Wiseman, Thomas, Higley, Hursky, Pietrock, Raine, Giesy, Janz & Hecker 2011). Selenium requirement varies among fish species, e.g. 0.38 mg kg^{-1} for rainbow trout (Hilton, Hodson & Slinger 1980), 0.25 mg kg^{-1} for channel catfish (Gatlin & Wilson 1984) and 0.7 mg kg^{-1} for Malabar grouper (Lin & Shiau 2005). Lorentzen *et al.* (1994) reported that 2 mg kg^{-1} Se supplementation did not negatively affect the growth of Atlantic salmon parr. In this study, 1.6 mg kg^{-1} Se supplementation improved WG, NRE and ERE of fish fed the diets with fish meal replaced by SBM. This result reveals, for the first time, that Se deficiency may be a factor limiting fish meal replacement by SBM in fish diets.

Ma *et al.* (2014) reported that the incrase in HSI with the decrease in body lipid content when golden pompano was fed the diets with fish meal replaced by PBM or SBM. In this study, no significant differences were found in the condition factor, HSI, VSI and body composition between fish fed diets P1F1, P1F2 and P1F3 or between fish fed diets P2F1, P2F2 and P2F3. These results suggest that replacement of fish meal by SBM did not significantly affect morphology and body composition of golden pompano.

In conclusion, supplementation of PBM and Se-yeast can increase the amount of fish meal replaced by SBM in diets for golden pompano, and the mechanisms may include the reduction in antinutrition factors of SBM and the increase in dietary Se and taurine contents. The dietary fish meal level for golden pompano can be reduced to 140 g kg^{-1} through optimizing SBM, PBM and Se inclusions in diet formulation.

Acknowledgments

The Hong Kong Office of the National Renders Association, Inc. (NRA) and the Major State Basic Research of China (Grant No. 2009CB118706) funded this research. We thank Miss Ji Wenxiu for her help in preparing the test diets.

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