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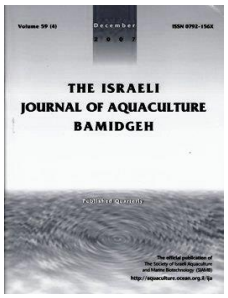

Effect of Replacing Canola Meal for Fish Meal on the Growth, Digestive Enzyme Activity, and Amino Acids, of Ovate Pompano, *Trachinotus ovatus*

Hongyan Kou, Shuying Xu, An-Li Wang*

Key Laboratory of Ecology and Environment Science in Guangdong Higher Education, Guangdong Provincial Key Laboratory for Healthy and Safe Aquaculture, College of Life Science, South China Normal University, Guangzhou 510631, People's Republic of China

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Abstract

Ovate Pompano (*Trachinotus ovatus*) is one of the most highly valued aquaculture species due to its commercial value, rapid growth, high quality flesh, and suitability for cage culture. Ovate Pompano were fed diets containing graded levels of canola meal to replace the fish meal. Six diets containing 45% crude protein and 12% crude lipid were fed to the fish during the 8-week feeding trial. Weight gain of the fish was negatively related to canola meal ratio in the diets. Protein efficiency and feed conversion ratio were inversely related to the canola meal inclusion in the diets. The crude lipid of the fish was positively related to the dietary canola meal, while an inverse trend was found in the crude protein of the fish. Protease activity and amylase activity showed a similar trend to the crude protein, while the lipase activity showed an inverse trend. No significant differences were observed in the moisture, ash, or fillet yield of the fish among dietary treatments. The "delicious amino acid " i.e. those eliciting Umami flavor (UEAA), e.g. glutamic acid and others, essential amino acid, and total amino acid content of the fish were inversely related to the canola meal ratio in the diets. The results of the experiment indicated that canola meal can replace 18.17 % fish meal without affecting growth. Higher substitution levels of canola meal produced adverse influences on growth, digestive enzyme activity, and amino acid content of Ovate pompano.

* Corresponding author. An-Li Wang Tel.: +86 2085216862; Fax: +86 2085216862

e-mail: (wangalok@163.com (An-Li Wang))

Introduction

Ovate pompano, *Trachinotus ovatus* is an economically important warm-water farmed marine fish species worldwide. In recent years, ovate pompano culture has developed rapidly and widely along the southern coast of China in tropical and subtropical climates (Zhang et al., 2014).It is widely cultured in the south of China due to its fast growth, high quality flesh, and excellent taste. *T. ovatus* is a carnivorous fish which preys mainly on zooplankton and small crustaceans, shellfish, and fish (Liu and Chen, 2009; Lin et al., 2013). Feed with high protein content is required to meet the metabolic needs of this fish, and the protein source is very important.

Fish meal is an excellent but expensive macro-ingredient for fish feed formulation (Wang et al., 2006). It is a good source of protein and this has enhanced its global demand (Rivas-Vegaa et al., 2006). Fish meal has the advantage of high protein quality and palatability compared to fish meal replacements. However, due to increasing cost and declining production of fish meal, there has been much research into substitute products (Hu et al., 2008, Kissil and Lupatsch, 2004). Research has been undertaken to find other protein substitutes such as soybean meal (Lim and Lee, 2008; Lin et al., 2013; Yu et al., 2013), pea meal (Davies and Gouveia, 2010; Øverland,et al., 2009), kernel meal (Brett et al., 2004) and other substitutes (Ai et al., 2006).

Canola meal is the by-product of vegetable oil extraction from canola seed. Canola is widely cultured in China. Canola meal in China is produced with protein content ranging between 32% and 45% of dry matter (Canola council of Canada). Protein content of canola meal is higher than soybean meal (SBM) which is considered a possible fishmeal substitute (Webster et al., 2000). Based on the essential amino acid index the protein quality of canola meal (CM) is equivalent to that of herring meal and higher than that of SBM and cottonseed meal (Higgs et al. 1990). Canola meal contains low levels of erucic acid and glucosinolates (Vermorel et al., 1986) and could potentially become a substitute for fishmeal in feeds.

Canola meal is high in protein content, but contains many anti-nutritional factors, such as fiber, phytic acid, and phenolic compounds (Fenwick et al., 1986; Vermorel et al., 1986) and can therefore only partially replace fish meal.

Although many studies on pompano have been published, investigation into the replacement of fish meal with protein substitutes in fish feed is limited and is focused on the replacement with soy-based products (Riche and Williams, 2011; Lin et al., 2013; Quintero et al., 2012) and poultry by-product meal (Rossi and Davis, 2012). The objective of this study is to estimate the effects of increasing dietary canola meal on the growth, feed utilization, digestive enzyme activity, and amino acid profile of ovate pompano, *Trachinotus ovatus*.

Material and methods

Fish and experiment conditions. An 8 week growth trial was conducted in the summer of 2006 at Da Yawan Laboratory in Huizhou, Guangzhou, China. Six diets containing 45% crude protein, and 12% crude lipid, were formulated by replacing fish meal with 0% (the control，C0), 10% (C1), 20% (C2), 30% (C3), 40% (C4), and 50% (C5) canola meal, respectively. Feed composition is shown in Table 1.

Table1. Formulation and proximate composition of the experiment diets

Ingredients	*Diets*					
	C0(0%)	*C1(10%)*	*C2(20%)*	*C3(30%)*	*C4(40%)*	*C5(50%)*
casein	14	14	14	15	15	17
Fish meal	44	40	36	31	27	22
Canola	0	8.2	16.4	24.6	32.8	41
Wheat flour	26	23	19.85	15.1	9.35	5.85
Lecithin	1	1	1	1	1	1
Soybean oil	4.1	4.2	4.35	4.55	4.85	4.9
Fish oil	4.1	4.2	4.35	4.55	4.85	4.9
$Ca(H_2PO_4)_2$	2	2	1.4	1.55	2	0.7
Cellulose	2.15	0.75	0	0	0.5	0
Vitamin	0.1	0.1	0.1	0.1	0.1	0.1
Mineral	0.5	0.5	0.5	0.5	0.5	0.5
Choline	0.5	0.5	0.5	0.5	0.5	0.5
	Proximate analysis (%dry matter)					
Crude protein (%)	45.1	45.07	44.99	45.06	45.12	45.04
Crude lipid (%)	12.06	11.98	11.997	12.004	12.06	11.999

1. Vitamin premix (mg / kg diet): VB_1, 10.5mg; VB_2, 25mg;VB_6, 30mg;VB_{12}, 0.1mg; K, 30mg; inositol, 77.5mg; pantothenic acid, 51.5mg; niacin acid, 61mg; folic acid, 1mg; H, 2mg; VD_3, 1.2mg;VC, 500mg; antioxidant, 150mg.
2. Mineral premix: Mineral premix (mg / kg diet): NaCl, 0.4mg; KI, 0.032mg; $CoCl_2.6H_2O$, 0.02mg; $CuSO_4.5H_2O$, 0.4 mg; $FeSO_4.H_2O$, 3.2 mg; $ZnSO_4.H_2O$, 2mg; $MnSO_4.H_2O$, 2.4mg; $MgSO_4.7H_2O$, 48 mg.

The fish (initial weight 9.38± 0.53g) were obtained from a commercial fishery. Prior to the experiment, the fish were fed the control diet for 2 weeks to acclimate to the experimental conditions. At the beginning of the growth trial, healthy fish were randomly assigned to the floating cages (60cm×60cm×70cm) in a large tank.

Average water temperature was kept at 25.8-30.5°C, dissolved oxygen concentration was 4.5mg/L, pH7.8-8.1, salinity was maintained at 24-30‰ during the entire experimental period.

Each cage was stocked with 15 fish. The fish were hand fed twice a day until apparent satiation. Small amounts at a time were fed to prevent wastage. Satiety was defined as the point at which 2–3 uneaten pellets remained at the bottom of the cages. Feed intake was recorded. Each treatment had three replicates. The floating cages were siphoned daily in the morning to improve water quality. At the end of the feeding trial, fish were fasted for 24 h, then counted and weighed to determine weight gain and specific growth rate (SGR).

Performance parameters included:

Weight gain (WG) = (Final body weight - Initial body weight) (g)/ The number of fish

Feed conversion ratio (FCR) = Feed intake (g)/Weight gain (g)

Protein efficiency rate (PER) = Weight gain (g)/Crude protein intake (g)

Specific growth rate (SGR) = 100× (ln Final weight – ln Initial weight)/ day

Measurement of digestive enzyme activity. The intestinal tracts were removed from the fish, and chyme was extracted with distilled water, and then stored frozen at -80°C until analysis. Samples were weighed, then homogenized in ice-cold 0.75% saltwater in the ratio of 1:5 (w/v). Following Centrifugation (1800 × g, 10 min, 4°C), the supernatants were removed and kept at 4°C for analysis.

Intestinal protease and lipase activity were analyzed following analytical procedures and using commercially available kits (Jiancheng Bioengineering Institute, Nanjing, China). Alpha-amylase (E.C. 3.2.1.1) activity of the tissue supernatants was measured using specific analytical procedures using commercially available kits (Jiancheng Bioengineering Institute, Nanjing, China).

Statistical analysis: All data were analyzed using Sigma Stat statistical software from SPSS 13.0. One-way analysis of variance was used to determine the mean difference among treatments at 5% significance level.

Chemical analysis of the fish and diets. Proximate analysis of the diets and whole body composition of the fish were performed using standard methods (AOAC, 1995). Proximate composition analysis was carried out in triplicate for each diet. The fish samples were analyzed in triplicate. Moisture of the fish and diets was analyzed by drying at 105°C for 24 h; crude lipid of the fish and diets was measured by ether-extraction method; crude protein was determined by measuring nitrogen (N×6.25) using the Kjeldahl method; crude ash was determined after heating in a muffle furnace, at 550°C for 4h.

The content of amino acid. Fish samples were hydrolyzed for 24h at 110°C in an oven and their amino acid composition determined by high performance liquid chromatography (HPLC, HP1100, USA).

Results

Growth performance. Weight gain (WG), specific growth rate (SGR), feed intake, protein efficiency ratio (PER) and feed conversion ratio (FCR) are shown in Table 2.

Table 2. The growth performance and feed utilization of Ovate Pompano fed

Diets	*Weight gain (WG, g)*	*Protein efficiency ratio (PER)*	*Feed conversion ratio(FCR)*	*Specific growth ratio (SGR)*
C0	45.33±4.57^{a}	1.58±0.016^{a}	1.49±0.19^{d}	0.91±0.053^{a}
C1	45.79±1.60^{a}	1.53±0.016^{a}	1.47±0.025^{d}	0.90±0.117^{a}
C2	44.19±4.05^{a}	1.55±0.013^{a}	1.50±0.029^{d}	0.84±0.017^{ab}
C3	38.67±1.98^{b}	1.41±0.010^{ab}	1.59±0.008^{c}	0.75±0.054^{b}
C4	36.34±0.31^{bc}	1.31±0.014^{b}	1.75±0.110^{b}	0.73±0.006^{bc}
C5	32.97±3.66^{c}	1.08±0.031^{c}	2.69±0.102^{a}	0.61±0.054^{c}

Values in a column with different letters are significantly different (P<0.05).
Where C0= Control, C1= 10% canola meal, C2 = 20% canola meal, C3=30% canola meal etc.

Specific growth rate (SGR) of the fish was negatively related to the canola meal inclusion in the diets (P<0.05). No significant differences were observed in the fish fed the diets which containing 20%, 30%and 40% canola meal. Specific growth rate of the fish significantly decreased with the increasing dietary canola meal. There was no significant difference in SGR between the control group and the fish fed the C1 and C2 diets. SGR was significantly lower than the control when substitution was 30% or higher.

The PER was inversely related to the increasing dietary canola meal levels. Fish fed the control diet showed best PER when the substitution levels of canola meal were 40% or more, the PER was significantly lower than the control group (P<0.05).

FCR was positively related to canola meal inclusion in the diets. When the substitution was 0%, 10%, and 20% no significant difference in FCR was observed (P>0.05). FCR was significantly higher than the control group when the substitution level of canola meal was 30% or more.

Weight gain was significantly (P<0.05) reduced with the addition of 30% or more CM

in the diet. This result is identical to that of SGR (Fig.1)

Table 3. Whole-body composition of Ovate Pompano fed diets with different levels of canola meal

Diets	*Moisture (%)*	*Crude protein (%)*	*Crude lipid (%)*	*Ash (%)*	*fillet yield (%)*
C0	69.56±3.48	69.48±0.203^{a}	24.288±0.55^{f}	1.294±0.303	30.85±3.05
C1	65.40±5.69	64.61±0.240^{b}	27.99±0.66^{e}	2.328±0.229	38.55±5.68
C2	70.23±5.37	64.08±0.98^{b}	28.76±0.77^{d}	1.969±0.238	36.34±5.09
C3	71.63±4.58	58.35±0.14^{c}	32.96±0.41^{b}	3.745±0.139	30.66±2.68
C4	66.71±5.83	56.79±0.13^{d}	30.04±0.45^{c}	3.093±1.238	35.19±5.26
C5	68.91±4.95	52.13±0.077^{e}	36.71±0.83^{a}	2.13±0.123	36.63±7.67

Values in a column with different superscript letter are significantly different (P<0.05).
Where C0= Control, C1= 10% canola meal, C2 = 20% canola meal, C3=30% canola meal, etc.

Growth of fish fed the C1and C2 diets was comparable to the control group. Substitution of fishmeal with increasing canola meal resulted in suppression of weight gain (Fig. 1).

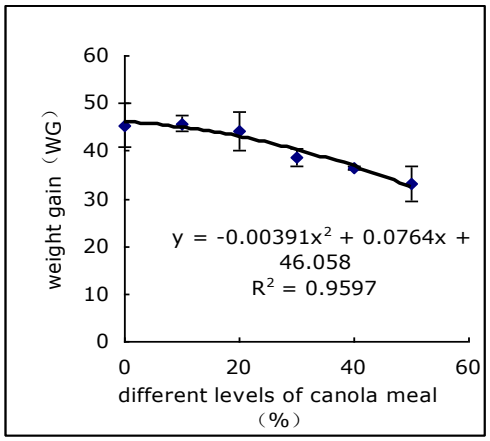

Fig 1: The relation between weight gain and different levels of canola meal.

No significant difference (P>0.05) was observed in WG of the fish fed the diets with 30% and 40% canola meal.

Whole body composition. Moisture, ash, fillet yield, crude protein and crude lipid of the fish fed the diets are presented in Table 3.

The differences between the control group and treatment groups in moisture, ash or fillet yield were not significant (P>0.05). In general, the crude protein of the fish was negatively related to increasing canola meal in the diets (P<0.05). The crude protein of the fish fed the control diet showed the highest value. The crude lipid of fish fed the control diet was the lowest and in general decreased with increasing dietary canola meal (P<0.05). Significant differences were observed in the crude lipid of the fish between the diets (P<0.05).

Digestive enzyme activities. Protease activity, lipase activity, and amylase activity are given in Figs. 2, 3, & 4.

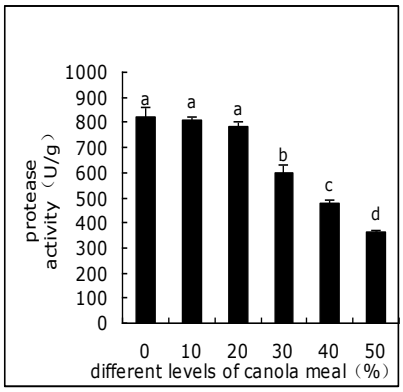

Fig 2: The relation between protease activity and dietary canola meal.

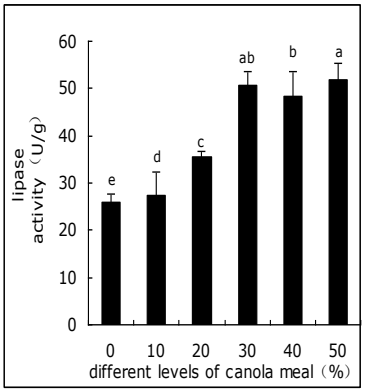

Fig 3: The relation between lipase activity and dietary canola meal.

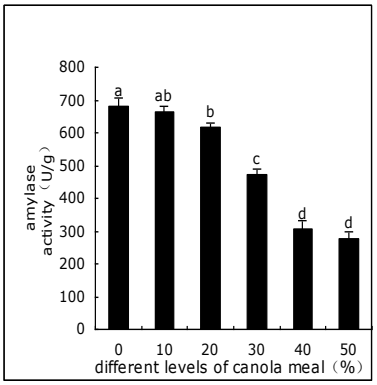

Fig 4: The relation between amylase activity and dietary canola meal.

Protease activity was significantly ($P<0.05$) reduced when dietary canola meal was greater than 20%. No significant differences were found when the levels of canola meal were 20% or less.

Lipase and amylase activity were also influenced by dietary canola meal ($P<0.05$). Lipase activity significantly increased in relation to the added dietary canola meal. No significant differences were observed in the C3 and C4 diets ($P>0.05$). Fish fed the control diet showed the highest lipase activity. Amylase and protease activity showed a similar trend. No significant differences were found between the control diet and the C1 diet.

Amino acid content. The contents of essential amino acid, *delicious* (eliciting umami flavor e.g. glutamic acid) amino acid, and total amino acid, are given in Table 4.

Table 4. The amino acid levels of the Ovate Pompano fed diets containing canola meal substituted at 0, 10%, 20%, 30%, 40% and 50% of fish meal in the control diet

Amino acid	*C0 (mg/g)*	*C1(mg/g)*	*C2(mg/g)*	*C3(mg/g)*	*C4(mg/g)*	*C5(mg/g)*
Asparagine(Asp)	30.25	26.92	25.03	23.45	23.52	21.69
Glutamic acid(Glu)	42.32	38.64	32.21	26.49	25.69	24.08
Histidine(His)	30.04	21.37	19.65	8.70	8.34	7.47
*Threonine(Thr)	31.16	15.62	8.34	7.09	6.96	6.84
*Arginine(Arg)	32.39	28.07	24.22	22.12	21.60	16.29
Alanine(Ala)	9.25	8.95	6.19	5.71	5.58	5.18
Tyrosine(Tyr)	29.15	25.62	22.11	19.07	18.38	17.26
*Valine(Val)	28.77	7.71	6.28	6.57	6.48	6.90
*Methionie(Met)	10.40	8.21	7.50	6.85	5.69	4.77
*Phenylalanine(Phe)	18.03	19.82	11.81	10.00	10.47	9.84
*Isoleucine(Ieu)	23.41	26.78	15.90	13.84	12.39	11.98
*Leucine(Leu)	8.40	7.90	5.20	4.19	4.20	4.70
*Lysine(Lys)	36.05	26.16	25.50	22.71	20.23	18.39
Delicious amino acid	11.3	9.01	7.12	6.27	5.98	5.78
Essential amino acid	18.86	13/03	10.48	9.33	8.80	7.67
Total amino acid	32.96	24.34	20.99	17.68	16.75	15.54

All these decreased in relation to the added canola meal ratio in the diets. Total amino acid, *delicious* amino acid, and essential amino acid of the fish fed the control diet was higher than that of the fish fed other diets.

Discussion

When fish meal substitution levels were 30% or less, PER was similar. These results are consistent with reports on other fish species (Davies et al., 1990; Webster et al., 1997). This indicates that the protein of the diets with up to 30% canola meal was similar to that of the control diet. The reasons for the decrease in feed utilization and PER could be the indigestibility of canola meal.

No significant differences were observed in the WG of the fish when the canola meal level was less than 20%. Growth of the fish decreased when canola meal was up to 20%, as shown in other fish (Cheng et al., 2010). This result indicates that increasing dietary canola meal results in a reduction of growth possibly due to an imbalance of amino acids and indigestible protein found in the canola meal (Webster et al., 1997). Our findings suggest that up to 20% canola meal could replace fish meal in the Pompano diet without affecting the growth. As a function of canola meal replacement for fish meal in a practical-type formulation for Ovate Pompano, *Trachinotus ovatus*, regression analysis ($y = -0.00391x2 + 0.0764x + 46.058$; $R2 = 0.9597$) for WG suggests the optimum level of substitution for maximal WG is 18.17%. This may be important for ovate pompano culture.

FCR is an indicator of feed quality, which reflects the practical use of the feed. Poor growth and feed utilization of fish fed the diets with high levels of canola meal may be due to the presence of anti-nutritional factor, low protein digestibility, and essential amino acid deficiency in the feeds.

The moisture, ash, and fillet yield of the fish were not significantly influenced by the inclusion levels of canola meal. Crude protein of the fish decreased with increasing dietary canola meal, which may be caused by the indigestible protein in the canola meal. It was found that whole body crude protein of Florida pompano linearly decreased with level of soy protein isolate (SPI) incorporation (Riche and Williams, 2011). No differences in crude protein of Florida pompano were observed among the diets containing various percentage of poultry by-product meal (Rossi and Davis, 2012). The differences may be caused by the fish size and the replacement levels. The replacement of 5–15% fish meal by poultry by-product meal was too low to change the crude protein of Florida pompano.

The crude lipid of the fish decreased with increasing dietary canola meal. The whole body crude lipid of Florida pompano fed 0–400 g/kg fish meal protein replacement with SPI was significantly higher than that of the fish fed 600–1000 g/kg fish meal protein replacement with SPI (Riche and Williams, 2011). Fish fed the diets with high levels of canola meal must ingest more food to meet their protein requirements, and this increases lipid absorption. The excess lipid may be deposited in the liver and body of the fish.

Limited studies on the replacement of fish meal by other protein sources suggest that fish meal could be reduced to 12.5 and 25% when soybean meal and soy protein isolate were used respectively as alternative ingredients in Florida pompano diets (Riche and Williams, 2011). Fermented soy bean meal (FSM) has also been used to replace 100 g/kg of white fish meal (WFM) without negative effects on the growth of pompano, *Trachinotus ovatus* (Lin et al., 2013). The growth performance of fish given diets with 5-15% fish meal did not differ significantly. Results from these studies indicate that it is not yet

possible to totally remove fish meal from Florida pompano diets by utilizing plant based ingredients d (Rossi and Davis, 2012).

Digestive capacity depends on digestive enzyme levels. Activity of digestive enzymes on feed consumed plays an important role in determining final digestibility of nutrients (Fountoulaki et al., 2005).Protease activity, lipase activity, and amylase activity were significantly related to dietary canola meal. In our study, protease and amylase activity of pompano significantly decreased with increasing dietary canola meal, corresponding to results on Japanese sea bass (Cheng et al., 2010). Lipase activity showed an inverse trend to amylase activity. Crude protein of the fish increased with increasing dietary canola meal, paralleling the result of protease activity. Lipase activity improved when crude fat of the fish increased. As the inclusion of wheat flour in the diets decreased with increasing dietary canola meal, amylase activity of the fish decreased since the function of amylase was to hydrolyze the flour. The results of amylase activity show that canola meal can replace the fish meal up to 10%.

Free amino acids have been used as quality indicators in various fish and crustacean species (Ruiz and Moral, 2001). In our study, the essential, and total amino acids, were negatively related to increasing dietary canola meal. Essential amino acids cannot be synthesized by animals and must be supplied in the diet. Glutamic acid was the most abundant amino acid in the fish muscle. Leucine content was lowest in the fish muscle. The *delicious* amino acid was inversely related to increasing dietary canola meal inclusion. The delicious amino acid level increased until the replacement was 20% (Iwasaki et al., 1985).

In conclusion, the results of our study revealed that canola meal could replace 18.17% fish meal without affecting the growth, digestive enzyme activity, and flavor of ovate pompano. Partial replacement of fish meal by canola meal is important in reducing the cost of the feed.

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