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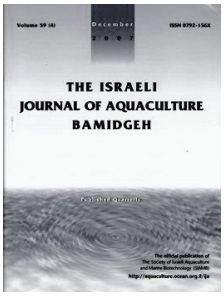

Effects of Dietary Arachidonic Acid Levels on Growth Performance, Whole-Body Proximate Composition, Digestive Enzyme Activities and Gut Morphology of Juvenile Golden Pompano *Trachinotus ovatus.*

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Keywords: juvenile golden pompano; arachidonic acid; growth performance; digestive enzymes; gut morphology.

Abstract

The effects of dietary arachidonic acid (ARA) levels on growth performance, whole-body proximate composition, digestive enzyme activities, and gut morphology were studied in juvenile golden pompano *Trachinotus ovatus*. Six diets were formulated with six levels of ARA. Fish were fed twice daily to apparent satiation for 56 days (8 weeks). Weight gain (WG), specific growth rate (SGR), protein efficiency ratio (PER), feed conversion ratio (FCR), and viscerosomatic index (VSI) were significantly affected by dietary ARA levels ($P < 0.05$). WG, SGR and PER increased with the increasing levels of ARA ($P < 0.05$), and thereafter slightly declined ($P > 0.05$); they were highest in fish fed the diet with 0.51% ARA and the lowest in fish fed the diet containing 0.15% ARA. Linear regression analysis on SGR indicated that the recommended optimum dietary ARA level for optimal growth of juvenile golden pompano was 0.53%. Whole body protein significantly declined when dietary ARA levels increased from 0.15% to 0.88% ($P < 0.05$), and were lowest in fish fed the diet containing 0.88% ARA. The whole body lipid content showed an opposite trend compared with whole body protein. Pepsin activities showed no significant differences among treatments ($P > 0.05$), while lipase activities of fish were significantly influenced by dietary ARA levels ($P < 0.05$). The number of goblet cells and intestinal villus length increased with increasing levels of ARA from 0.15% to 0.51% ($P < 0.05$), and decreased thereafter. Goblet cells of fish fed diets with 0.36%, 0.51%, 0.71% ARA were higher than in the other groups ($P < 0.05$).

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Introduction

Essential fatty acids (EFAs) are one of the most important nutrients for finfish. Dietary deficiency of EFAs results in manifestation of diseases, inhibition of growth and reproduction, and eventually death (Das, 2006). Reports in juveniles and sub-adults of freshwater fish species indicate that both n-3 and n-6 series of polyunsaturated fatty acids are important to freshwater fish (Bell et al., 1986). These can desaturate and elongate oleic acid (C18:1 n-9), linoleic acid (LA) (C18:2 n-6), and linolenic acid (LNA) (C18:3 n-3) into highly unsaturated fatty acids, such as arachidonic acid (ARA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), and can satisfy their requirements for n-6 and n-3 series of fatty acids, respectively (Bell et al., 1986). However, in some marine fish these conversions do not occur and for these marine species polyunsaturated fatty acids (EPA and DHA) are essential. The effect of dietary EPA and DHA on growth performance and development of marine fish has been documented in a range of marine species (Hossain et al., 2012). Optimum dietary levels of DHA and DHA/EPA have been studied in some fish (Glencross & Rutherford, 2011; Ma et al., 2014). Reports regarding the physiological and biochemical functions of ARA are limited. Since the required level of ARA may be lower than that of DHA and EPA, the importance of ARA has been neglected (Bell & Sargent, 2003). Reports indicate that ARA plays a critical role in development, reproduction, stress resistance, and regulation of immune function of finfish (Mandal et al., 2013; Hurtado et al., 2009; Furuita et al., 2003). Some of these studies have suggested that dietary ARA levels could influence growth, survival, and tissue fatty acid profile of fish (Fountoulaki et al., 2003; Xu et al., 2010; Carrier et al., 2011). Although there have been reports that suitable dietary ARA levels have improved growth performance of fish and influenced fish immune response, no data is available on the effect of dietary ARA levels on digestive enzyme activities and gut morphology of finfish.

Golden pompano *Trachinotus ovatus* is a carnivorous fish, found in China, Japan, Australia, and other countries (Liu & Chen, 2009). Culture of this fish is widespread in China's coastal areas and annual production has surpassed 100,000 tons. The study of the requirements of various nutrients for golden pompano *T. ovatus* is necessary to save feed costs and improve farming efficiency. Few studies have been reported regarding the nutrient requirements of this species.

The optimum protein requirement of golden pompano ranges from 43% to 49% (Liu et al., 2011), lipid from 6% to 6.5% (Wang et al., 2014), and carbohydrate from 11.2% to 16.8% of diet (Zhou et al., 2015). The optimal requirements of methionine and lysine for golden pompano have been determined (Lin et al., 2015; Niu et al., 2013). However, no studies have been conducted to determine the optimal requirement of n-6 long chain polyunsaturated fatty acid, arachidonic acid (ARA),for this species.

Materials and methods

Experimental diets. Levels of dietary protein and lipid were adjusted to satisfy fish nutrient requirements according to previous studies (Lin et al., 2015). The composition of the experimental diets is shown in Table 1. Six isonitrogenous and isolipidic diets (Diet 1, Diet 2, Diet 3, Diet 4, Diet 5 and Diet 6) were formulated to contain 0.15%, 0.36%, 0.51%, 0.71%, 0.88%, 0.96% ARA (Table 2) on a dry matter basis by progressively adding ARA oil (ARA content, 46.2% of total fatty acid, Inner Kingdomway Pharmaceutical Ltd., China) as a substitute for lard oil. Fatty acid composition of the experimental diets was determined by Gas Chromatography Mass Spectrometry (GCMS-QP2010 plus, Shimadzu, Japan).

All ingredients were ground into powder and sieved through 60 mesh strainer, weighed, mixed and homogenized with an electric mixer, after which oil and distilled water were added to produce dough then pelleted with a screw-press pelletizer (F-26, South China University of Technology, Guangzhou, China) using 2.0 mm die, and air dried in an air-conditioned room for approximately 48 h to reduce the moisture to less than 10%. After drying, all diets were packed in bags and stored at -20°C until used.

Table 1 Formulation and chemical proximate composition of the experimental diets (% dry matter basis)

Ingredients	*Diet 1*	*Diet 2*	*Diet 3*	*Diet 4*	*Diet 5*	*Diet 6*
	0.15%	*0.36%*	*0.51%*	*0.71%*	*0.88%*	*0.96%*
Fish meal[1]	23	23	23	23	23	23
Soy protein concentrate[2]	20	20	20	20	20	20
Soybean meal	14	14	14	14	14	14
Peanut meal	11	11	11	11	11	11
Brewer's yeast	3	3	3	3	3	3
Wheat flour[3]	16	16	16	16	16	16
Fish oil[4]	1.4	1.4	1.4	1.4	1.4	1.4
Ethoxyquin	0.1	0.1	0.1	0.1	0.1	0.1
Vitamin premix[6]	1	1	1	1	1	1
Mineral premix[7]	1	1	1	1	1	1
Choline chloride(50%)	0.5	0.5	0.5	0.5	0.5	0.5
Lard oil	9	7.9	6.8	5.7	4.7	3.6
ARA oil[5]	0	1.1	2.2	3.3	4.3	5.4
Proximate analysis						
Dry matter	90.84	90.80	90.63	90.95	91.52	91.65
Crude protein	49.20	49.11	49.08	49.10	49.19	48.68
Crude lipid	12.62	12.70	12.77	12.76	12.76	12.68
Ash	9.83	9.80	9.78	9.97	9.59	9.69

[1] Fish meal: Provided by CFGINVESTMENTS.A.C., Plant Tambo de Mora.
[2] Soy protein concentrate: Shandong long-run biological Ltd., China.
[3] Wheat flour: Dongguan Suifeng food Ltd., China.
[4] Fish oil: Guangzhou Yongxing concentrated feed Ltd., China.
[5] ARA oil: Inner Kingdomway Pharmaceutical Ltd., China.
[6] Vitamin premix provides the following per kg of diet: Vitamin B_1 25 mg, Vitamin B_2 45 mg, Vitamin B_6 20 mg, Vitamin B_{12} 0.1 mg, Vitamin K_3 10 mg, inositol 800 mg, pantothenic acid 60 mg, nicotinic acid 200 mg, folic acid 1.2 mg, biotin 32 mg, Vitamin D_3 5 mg, Vitamin E 120 mg, Vitamin C 2.0 g, choline chloride 2.0 g, ethoxyquin 150 mg, and manna-croup 14.52 g.
[7] Mineral premix provides the following per kg of diet: NaF 4 mg, KI 1.6 mg, $CoCl_2 \cdot 6H_2O$ (1%) 100 mg, $CuSO_4 \cdot 5H_2O$ 20 mg, $FeSO_4 \cdot H_2O$ 160 mg, $ZnSO_4 \cdot H_2O$ 100 mg, $MnSO_4 \cdot H_2O$ 120 mg, $MgSO_4 \cdot 7H_2O$ 2.4 g, $Ca(H_2PO_4)_2 \cdot H_2O$ 6.0 g, NaCl 200 mg, and zeolite powder 30.90 g.

Table 2 Fatty acid composition of the experimental diets for juvenile golden pompano *Trachinotus ovatus* (% dry matter basis)

Fatty acid	*Diet 1*	*Diet 2*	*Diet 3*	*Diet 4*	*Diet 5*	*Diet 6*
	0.15%	*0.36%*	*0.51%*	*0.71%*	*0.88%*	*0.96%*
C14:0	0.40	0.39	0.37	0.36	0.36	0.36
C15:0	0.05	0.05	0.05	0.05	0.05	0.05
C16:0	2.24	2.20	2.06	2.00	2.07	2.15
C16:1n-7	0.59	0.54	0.55	0.51	0.50	0.50
C17:0	0.09	0.06	0.07	0.08	0.09	0.08
C17:1	0.02	0.02	0.03	0.03	0.03	0.03
C18:0	1.23	1.19	1.13	1.06	1.10	1.08
C18:1n-9	3.36	3.32	3.01	2.87	2.89	2.77
C18:2n-6	1.78	1.80	1.72	1.68	1.66	1.78
C18:3n-3	0.03	0.04	0.05	0.06	0.05	0.12
C20:0	0.06	0.05	0.06	0.06	0.07	0.07
C20:1n-9	0.23	0.18	0.18	0.17	0.17	0.16
C20:2n-7	0.08	0.07	0.08	0.10	0.08	0.07
C20:3n-6	0.09	0.10	0.12	0.13	0.13	0.15
C20:4n-6	0.15	0.36	0.51	0.71	0.88	0.96
C20:5n-3	0.57	0.46	0.58	0.54	0.49	0.40
C22:0	0.04	0.05	0.06	0.07	0.07	0.07
C22:6n-3	0.62	0.50	0.57	0.55	0.53	0.59
∑saturates	4.11	4.00	3.81	3.67	3.80	3.85
∑MUFA[1]	4.20	4.06	3.77	3.58	3.59	3.46
∑PUFA[2]	3.33	3.33	3.63	3.78	3.82	4.06
∑n-3	1.22	1.00	1.19	1.15	1.07	1.10
∑n-6	2.02	2.26	2.35	2.52	2.67	2.88
DHA/EPA	1.07	1.09	0.99	1.01	1.09	1.48
∑n-3/∑n-6	0.60	0.44	0.51	0.46	0.40	0.38

[1] MUFA: monounsaturated fatty acid.
[2] PUFA: polyunsaturated fatty acid.

Experimental procedure. The 8 week experiment was carried out in a pond in Shenzhen, China. Juvenile golden pompano (*T. ovatus*) were obtained from Shenzhen Base of South China Sea Fisheries Research Institute (Shenzhen, China). Prior to onset of the trial, the fish were stocked in a large cage (4 m × 4 m × 2 m), and fed a commercial diet for 2 weeks to acclimate them to the culture conditions. All fish were fasted for 24 h prior to the start of the 8 week trial. They were then anesthetized with eugenol (1:10000) (Yike Da Chengdu Chemical Reagent Ltd., Chengdu, China) and weighed. Juvenile golden pompano of similar size (initial body weight 15.20 ± 0.12 g, mean ± SE) were randomly distributed into floating cages (1 m × 1 m × 1.5 m) @ 20 fish per cage. Each experimental diet was randomly allotted to triplicate cages. In total, 18 cages were used in the present experiment. All fish were hand-fed experimental diets to apparent satiation twice daily (6:00 and 17:00). Dead fish were immediately removed from the cage and weighed. Feed intake of each cage was recorded daily. Daily fluctuation of water temperature ranged between 28°C and 35°C. Dissolved oxygen was about 5 mg/L and salinity from 15‰ to 18‰.

Sample collection and analysis. At harvest, all fish from each cage were anesthetized with eugenol (1:10000), then counted and group weighed per cage after fasting for 24 h. Survival rate (SR), weight gain (WG), specific growth rate (SGR), and feed conversion rate (FCR), were determined. Three fish from each cage were euthanized and sealed in bags at -20°C for proximate analysis. Another four fish per cage were randomly selected to determine hepatosomatic index (HSI), viscerosomatic index (VSI), and condition factor (CF) after dissection. Concurrently, liver samples, gut and stomach were collected and kept at -80°C for enzyme activity determination. Pepsin and lipase activity in stomach tissue and in gut tissue respectively, were measured with commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) in accordance with the instructions of the manufacturer.

Segments of intestines, approximately 2 cm in length were collected for histomorphological analysis. All segments were washed in saline solution, and fixed in Bouin solution until paraffin embedding. Paraffin section preparation, image collection, and sample measurements were determined according to the method described by Krogdahl et al. (2003).

Chemical composition of experimental diets and fish were determined by standard procedures using proximate composition analysis (AOAC, 1990). Samples were dried to a constant weight at 105°C to determine moisture content and were subsequently, pulverized and stored in a glass desiccator. Crude protein was determined by Kjeldahl method (N×6.25) using a Kjeltec system (FOSS 2300, Hoganas, Sweden) after acid digestion. Crude lipid was analyzed with petroleum ether extraction method using a Soxtec™ 205 (FOSS, Hoganas, Sweden), and crude ash by incineration at 550°C in a muffle furnace (FO610C, Yamato Scientific Ltd., Tokyo, Japan). The fatty acid profile of diets was analyzed by a slightly modified method described by You et al. (2015). The resultant fatty acid methyl esters were analyzed using Gas Chromatography Mass Spectrometry (GCMS-QP2010 plus, Shimadzu, Japan).

Calculations and statistical methods. The parameters of this study were calculated as follows:

Weight gain (WG, %) = $(W_t - W_0) \times 100 / W_0$;

Specific growth rate (SGR, % day^{-1}) = (Ln W_t – Ln W_0) × 100 / T;

Protein efficiency ratio (PER) = weight gain / protein intake;

Feed intake (FI, g fish^{-1}) = (total feed intake – feed intake of dead fish) / final number of fish;

Feed conversion ratio (FCR) = feed intake / $(W_t - W_0 + W_d)$;

Hepatosomatic index (HSI, %) = liver weight / body weight;

Viscerosomatic index (VSI, %) = visceral weight / body weight;

Condition factor (CF) = body weight × 100 / body length^3;

Survival rate (SR, %) = 100 × final number of fish / initial number of fish;

Where W_t, W_0 and W_d were final fish weight, initial fish weight, and dead fish weight respectively. T was the duration of the trials.

All data were subjected to one-way analysis of variance (ANOVA), and when the mean differences of each treatment were significant, Duncan's multiple range test was

used to compare means among the treatments. Statistical analysis was performed using the SPSS 16.0 for Windows (SPSS, Michigan Avenue, Chicago, IL, USA). Broken-line analysis was used to estimate the optimum dietary ARA level.

Results

Growth performance and survival rate. Dietary ARA levels significantly influenced WG, SGR, PER, FCR and VSI of juvenile golden pompano *T. ovatus* after 8-weeks feeding trial (Table 3).

Table 3 Growth performance and survival of juvenile golden pompano (*Trachinotus ovatus*) fed the diets containing ARA levels for 8 weeks[1]

	Diet 1	*Diet 2*	*Diet 3*	*Diet 4*	*Diet 5*	*Diet 6*	
	0.15%	*0.36%*	*0.51%*	*0.71%*	*0.88%*	*0.96%*	*Pooled SEM*[13]
IW[2]	15.22	15.17	15.20	15.24	15.20	15.17	0.01
FW[3]	61.68^{a}	66.47^{ab}	74.49^{c}	72.99^{bc}	70.48^{bc}	70.45^{bc}	1.26
WG[4]	305.13^{a}	338.43^{ab}	390.17^{c}	378.79^{bc}	363.77^{bc}	364.23^{bc}	8.25
SGR[5]	2.49^{a}	2.64^{ab}	2.84^{c}	2.79^{bc}	2.74^{bc}	2.74^{bc}	0.03
PER[6]	1.58^{a}	1.62^{ab}	1.81^{c}	1.77^{bc}	1.76^{bc}	1.66^{abc}	0.03
FI[7]	68.24	73.85	76.79	76.00	73.26	77.79	1.31
FCR[8]	1.47^{c}	1.44^{bc}	1.30^{a}	1.32^{a}	1.33^{ab}	1.41^{abc}	0.02
HSI[9]	0.83	0.82	0.73	0.68	0.69	0.69	0.03
VSI[10]	5.97^{d}	5.59^{bc}	5.31^{ab}	5.15^{a}	5.45^{abc}	5.78^{cd}	0.08
CF[11]	3.06	3.16	3.21	3.13	3.17	3.07	0.02
SR[12]	98.33	96.67	100.00	93.33	91.67	100.00	2.56

[1]Data are means of triplicate. Means in the same row with different superscript letters are significantly different (p<0.05).
[2]IW: initial weight.
[3]FW: final weight.
[4]WG: weight gain.
[5]SGR: specific growth rate.
[6]PER: protein efficiency ratio.
[7]FI: feed intake.
[8]FCR: feed conversion ratio.
[9]HSI: hepatosomatic index.
[10]VSI: viscerosomatic index.
[11]CF: condition factor.
[12]SR: survival rate.
[13] Pooled SEM : Pooled standard error of mean.

As dietary ARA levels increased from 0.15% to 0.51%, WG increased significantly (p<0.05), and when dietary ARA level was greater than 0.51%, WG declined slightly compared to fish fed the diet with 0.51% ARA. WG of fish fed the diet containing 0.51% ARA was significantly higher than those fed diets with 0.15% and 0.36% ARA (p<0.05). Difference among fish fed diets with 0.36%, 0.71%, 0.88% and 0.96% ARA (p>0.05) was not significant. SGR and PER trend was similar to WG. FI, HSI, CF and SR were not significantly different among fish fed graded levels of dietary ARA (p>0.05). Trend in FCR and VSI of fish was opposite to WG, SGR, or PER. FCR and VSI were the lowest in fish fed the diet containing 0.51% ARA and the diet containing 0.71% ARA, respectively. Broken-line regression analysis of SGR against dietary ARA level indicated that the optimal dietary ARA requirement for juvenile golden pompano *T. ovatus* was 0.53% dry matter (Figure 1).

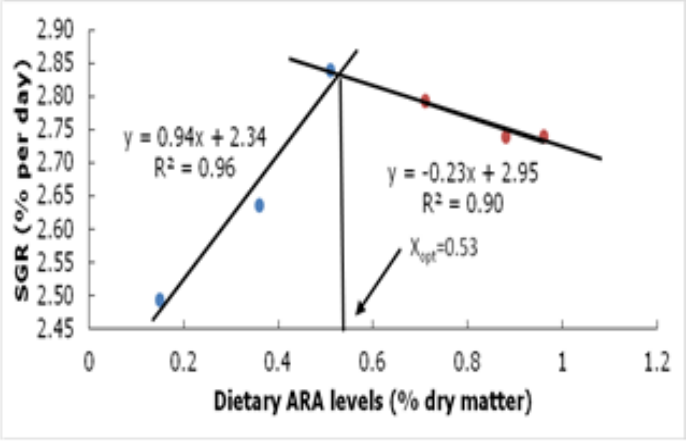

Figure 1 Broken-line analysis of specific growth rate of juvenile golden pompano (*Trachinotus ovatus*) fed diets containing different arachidonic acid levels.

Whole-body proximate composition. Results of whole-body proximate composition analysis are shown in table 4. No significant difference (p>0.05) was found in whole body moisture content among fish fed graded levels of dietary ARA. When dietary ARA levels increased from 0.15% to 0.88%, whole body protein content declined significantly (p<0.05), and when dietary ARA level was more than 0.88%, whole body protein content slightly increased (p>0.05) compared with fish fed the diet with 0.88% ARA. Whole body protein content in fish fed the diet with 0.15% ARA was significantly higher (p<0.05) than in fish fed diets with 0.36%, 0.51%, 0.71%, 0.88% and 0.96% ARA. No significant differences (p>0.05) were seen among fish fed diets with 0.51%, 0.71%, 0.88% and 0.96% ARA. In contrast, whole body lipid content increased when dietary ARA levels increased from 0.15% to 0.51%. Fish fed the diet with 0.15% ARA content had the lowest whole body lipid content. Dietary ARA levels significantly affected the whole body ash content (p<0.05).

Table 4 Effects of dietary ARA level on whole-body proximate composition of juvenile golden pompano (*T. ovatus*)

	Diet 1	*Diet 2*	*Diet 3*	*Diet 4*	*Diet 5*	*Diet 6*	
	0.15%	*0.36%*	*0.51%*	*0.71%*	*0.88%*	*0.96%*	*Pooled SEM*[2]
Moisture	69.43	69.14	68.17	68.59	67.80	68.69	0.00
CP[1]	60.64^{c}	59.01^{b}	58.26^{ab}	58.24^{ab}	56.79^{a}	58.05^{ab}	0.33
Ash	13.48^{e}	12.38^{c}	12.02^{b}	12.68^{d}	11.69^{a}	12.27^{c}	0.14
Lipid	23.08^{a}	25.66^{b}	26.70^{b}	25.87^{b}	27.27^{b}	25.81^{b}	0.44

[1] CP: Crude protein.
[2] Pooled SEM: Pooled standard error of mean.

Digestive parameters. In the present study, there was no significant difference (p>0.05) in pepsin activity between treatments (Table 5). Dietary ARA levels had a significant influence (p<0.05) on lipase activity which increased with the increasing dietary ARA levels from 0.15% to 0.51% and then decreased from 0.51% to 0.88%. Highest lipase activity was observed in fish fed the diet with 0.96% ARA.

Table 5 Effects of dietary ARA levels on digestive enzymes of juvenile golden pompano (*T. ovatus*)

	Diet 1	*Diet 2*	*Diet 3*	*Diet 4*	*Diet 5*	*Diet 6*	
	0.15%	*0.36%*	*0.51%*	*0.71%*	*0.88%*	*0.96%*	*Pooled SEM*[1]
Pepsin	13.02	14.10	13.57	14.37	14.99	13.46	0.28
Lipase	20.72^{ab}	21.16^{ab}	26.57^{c}	25.88^{bc}	20.29^{a}	29.18^{c}	1.00

[1] Pooled SEM: Pooled standard error of mean.

Gut morphology. Number of goblet cells increased significantly (p<0.05) with increasing dietary ARA levels from 0.15% to 0.51%, (figure 2). When dietary ARA levels increased from 0.51% to 0.96% the number of goblet cells decreased Villus length was significantly greater in fish fed the 0.51% ARA diet compared with other groups, and no significant differences were observed in fish fed diets containing 0.15%, 0.36%, 0.71%, 0.88% and 0.96 ARA (figure 3).

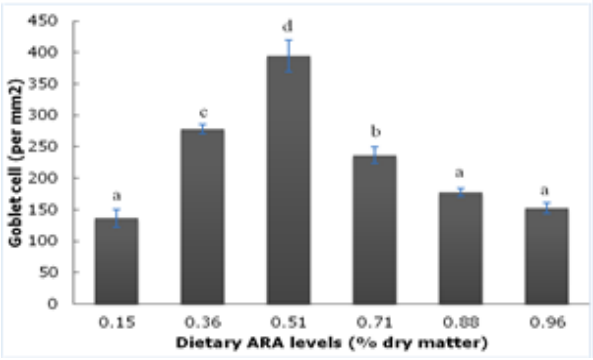

Figure 2 Effects of dietary ARA level on intestinal goblet cells of juvenile golden pompano (*T. ovatus*). Values of X-axis were dietary ARA level

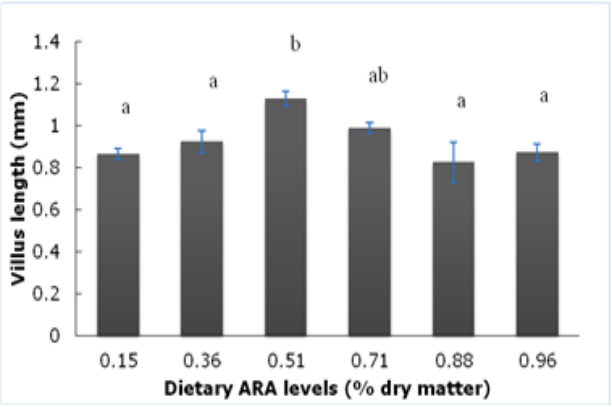

Figure 3 Effects of dietary ARA level on intestinal villus length of juvenile golden pompano (*T. ovatus*). Values of X-axis were dietary ARA level

Discussion

Previous reports have demonstrated that dietary ARA is an essential fatty acid for some juvenile fish species (Castell et al., 1994; Bae et al., 2010) and could promote growth of juvenile finfish. Results from our study have shown that dietary ARA levels significantly affected WG, SGR, and PER of golden pompano (*T. ovatus*). These results are similar to reports from studies on other species, such as Japanese seabass, *Lateolabrax japonicas,* (Xu et al., 2010), Eel *Anguilla japonica,* (Bae et al., 2010) and *Synechogobius hasta* (Luo et al., 2012). Some other studies have shown that dietary ARA levels did not significantly affect WG and SGR of gilthead bream fingerling *Sparus aurata* L., (Fountoulaki et al., 2003). It has also been shown that higher dietary ARA levels negatively affect growth and survival of fish (Furuita et al., 2003; Xu et al., 2010). Our study showed no effects of higher dietary ARA level on SGR and survival compared to fish fed the 0.51% ARA diet. Broken-line regression analysis of SGR and dietary ARA levels indicated that 0.53% dry matter was the optimal dietary ARA requirement for juvenile golden pompano (*T. ovatus*) was. This requirement was higher than 0.32% dry matter required by Japanese seabass (*L. japonicus*), (Xu et al., 2010), and lower than 0.69-0.71% for eel (*A. japonica*) (Bae et al., 2010). The differences might be due to differences in fish species, fish size and experimental formulation (Figure 4).

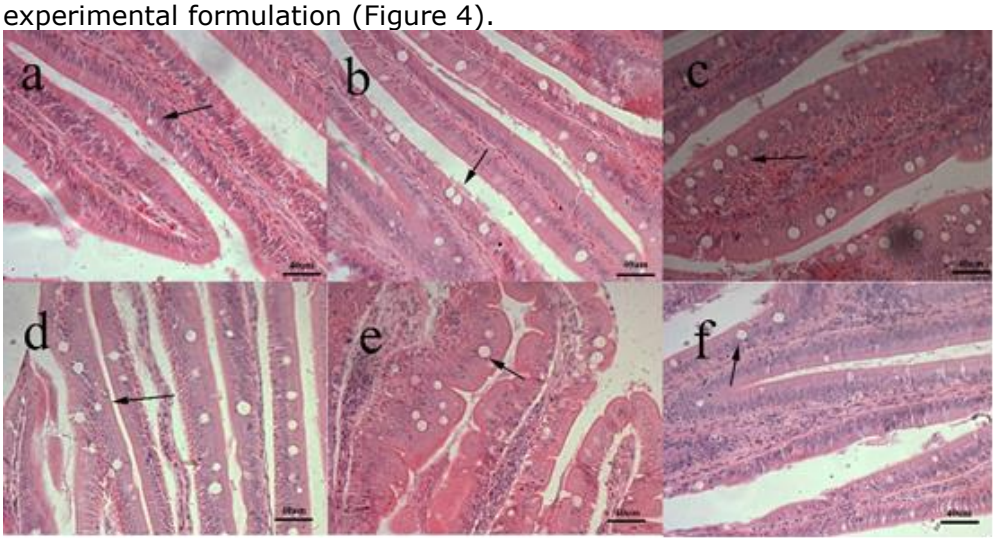

Figure 4 Histological sections of juvenile golden pompano (*T. ovatus*) fed different dietary ARA levels. a, b, c, d, e and f show the mid gut of fish fed diet with 0.15%, 0.36%, 0.51%, 0.71%, 0.88%, 0.96% ARA. Arrows indicate goblet cell of gut. Staining: H and E. Magnification ×20.

In the present study, dietary ARA levels had no significant effect on whole body moisture. This was similar to an experiment with Japanese seabass (*L. japonicus*) which showed that whole body protein content increased with the increasing dietary ARA levels from 0.08% to 0.56%, and lipid content showed an opposite trend (Xu et al., 2010). A consistent trend for lower lipid accumulation exists in relation to increased ARA dietary levels (Fountoulaki et al. 2003). Results of the present study showed that whole body protein content of golden pompano declined when dietary ARA levels increased from 0.15% to 0.88% while whole body lipid content increased. Additional research is needed to resolve some of these contradictory results.

Studies have indicated that digestive enzymatic activity could be affected by feed composition (Deng et al., 2010). In this study, dietary ARA levels influenced lipase activity but there were no differences in pepsin activity between treatments. No information is available regarding the effects of dietary ARA levels on these enzyme activities, making it difficult to make any comparative analysis. Our study was the first to show that dietary ARA levels could affect digestive enzyme activities in golden pompano.

Villus length is an important indicator of intestinal health, and absorption capacity. In the present study, villus length significantly increased as dietary ARA levels increased from 0.15% to 0.51%, and thereafter declined. ARA deficiency or excess in diets may affect the villus of the intestine thereby affecting intestinal function. There are no reports to show that polyunsaturated fatty acid could affect the intestinal structure of fish. Goblet cells play a crucial role in intestinal homeostasis, which could synthesize and secrete mucin glycoproteins covering the surface of gastrointestinal epithelium to protect

intestine from infection (Deplancke & Gaskins, 2001). In the present study, the number of goblet cells significantly increased with increasing levels of ARA up to 0.51 % and decreased thereafter. Our study indicates that polyunsaturated fatty acid could affect the number of goblet cells. To our knowledge, no previous studies are available to explain this phenomenon, and the effect on fish need further study.

Conclusion

The present study indicated that ARA is one of the essential fatty acids for juvenile golden pompano (*T. ovatus*), and the optimal dietary ARA requirement was 0.53% dry matter. Dietary deficiency of ARA could retard growth. The negative effect may be caused by the structure of the intestinal tract that influenced the activities of digestive enzymes. The mechanism of these effects for fish need further study.

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