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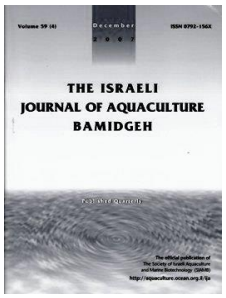

Effects of Different Lipid Sources on the Growth Performance and Muscle Fatty Acid Composition of Juvenile *Anguilla marmorata*

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Keywords: lipid sources; Anguilla marmorata; growth performance; fatty acid

Abstract

The aim of this study was to investigate the effects of different oils on growth performance of juvenile *Anguilla marmorata*. Five isonitrogenous and isoenergetic experimental diets were formulated to contain fish oil (FO), sunflower oil (SFO), peanut oil (PO), corn oil (CO) and soybean oil (SBO), respectively, as lipid sources each at an inclusion level of 50 g/kg. The diets were fed to apparent satiation for 10 weeks, twice a day to triplicate tanks of 30 fish each with an initial body weight 6.00±0.06 g. Weight gain (WG) and specific growth rate (SGR) of fish fed the FO and SBO diets were markedly higher than those fed the CO diet, while no other difference in WG and SGR were observed among other treatments. Fish fed FO and SBO diets had better feed utilization than the other diet fish groups. Muscle of fish fed the FO diet had significantly higher levels of C_20:5n-3 and n-3/n-6 ratio compared with fish fed vegetable oil (VO) diets, while muscle n-6 PUFA and C18:2n-6 content showed an opposite trend. The present results suggest that soybean oil is a suitable replacement for fish oil for *Anguilla marmorata*.

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Introduction

River eels, such as the Japanese eel (*Anguilla japonica*), European eel (*Anguilla anguilla*) and American eel (*Anguilla rostrata*), have been cultured under extensive or intensive aquaculture systems mainly in Asia. (Lee et al., 2003). In 2011 global river eel production was 255,284 tons (FAO, 2010). In recent years, the catch of glass eel of European, American, and Japanese eels has markedly decreased due to environmental destruction and overfishing (Geejm et al., 2009, Tsukamoto et al., 2009). Some farmers have tried to culture other inexpensive eel species (Luo et al., 2013). *Anguilla marmorata,* one of the most widely distributed species among the anguillids, are found throughout most of the tropical and sub-tropical western Pacific and Indian Oceans. Anguilla marmorata is an important species that can replace Japanese and European eel in China, due its low price and fast growth (Luo et al., 2013).

Lipids are essential as a source of energy, as structural components of biomembranes, eicosanoid percursors, carriers of fat-soluble vitamins, hormones, vitamin D, and as enzyme co-factors (Higgs and Dong, 2000). Lipid used for energy can also spare dietary proteins and improve growth performance (Ghanawi et al., 2011). Fish oil (FO) is traditionally used as the principal lipid source in commercial fish feeds, as it contains abundant quantities of mono-unsaturated fatty acids (MUFA) used by fish as a metabolic energy source and n-3 highly unsaturated fatty acids (n-3 HUFA) that are necessary for stress resistance and development of immunity in fish (Montero et al., 2003). Annual FO production has not increased beyond 1.5 million tons per annum since 1980, while the global consumption of FO by the rapidly growing aquaculture industry has increased significantly over the past few decades,. FO supply may not be able to meet demands in the near future (Turchini et al., 2009). The use of alternative lipid sources to replace FO has become a priority in the aquaculture industry. Vegetable oils are suitable alternatives to fish oil in fish diet due to their abundance and relatively stable prices (Asdari et al., 2011). Intensive research activities are being conducted globally to evaluate alternative lipid sources for fish (Turchini et al., 2009).

Information associated with the use of vegetable oils in *Anguilla marmorata* juvenile diets is scarce. The principal objective of this study was to investigate the effects of different vegetable oils on the growth performance, liver histology, and muscle fatty acid composition of *Anguilla marmorata* juveniles.

Materials and Methods

Experimental diet and diet preparation. Five isonitrogenous and isolipidic experimental diets, varying only in the dietary lipid source, were formulated to contain 48% protein. Fish oil (FO) was substituted by sunflower oil (SFO), peanut oil (PO), corn oil (CO), or soybean oil (SBO) respectively (Table 1). Diets were prepared and stored as previously reported (Engin and Carter, 2001). Fatty acid composition of diets are shown in Table 2.

Experimental procedures. Anguilla marmorata juveniles (initial wet weight 6.00±0.06g) from the Philippines were used in this experiment. The eels were acclimated to experimental conditions for 2 weeks prior to onset of the experiment. Thirty healthy fish were randomly distributed into each of the 15 experimental fiberglass tanks (300 L) connected to a recirculation system.

Table 1. Formulation and proximate composition of the experimental diets (%)

	Diets				
Ingredients	*FO*	*SFO*	*PO*	*CO*	*SBO*
Fish meal	68	68	68	68	68
Brewers' yeast	2.5	2.5	2.5	2.5	2.5
α-starch	22.6	22.6	22.6	22.6	22.6
Mineral premix	0.5	0.5	0.5	0.5	0.5
Vitamin premix	0.5	0.5	0.5	0.5	0.5
Choline chlorine (50%)	0.2	0.2	0.2	0.2	0.2
Monocalcium phosphate	0.5	0.5	0.5	0.5	0.5
VC Ascorbic acid	0.1	0.1	0.1	0.1	0.1
Betaine	0.1	0.1	0.1	0.1	0.1
Fish oil	5				
Sunflower oil		5			
Peanut oil			5		
Corn oil				5	
Soybean oil					5
Total	100	100	100	100	100
Nutrient contents (%, *dry weight basis*)					
Moisture	6.44	6.87	7.5	7.48	6.5
Crude protein	47.50	47.77	47.90	48.25	48.12
Crude lipid	8.09	7.69	7.88	7.88	8.39

Vitamin premix (mg/kg or IU): vitamin A, 6000 IU; vitamin D, 4000 IU; vitamin E, 250 IU; vitamin K (menadione sodium bisulphite), 30 mg/kg; thiamin, 40 mg/kg; riboflavin, 50 mg/kg; d-calcium pantothenate, 150 mg/kg; biotin (1%), 0.8 mg/kg; folic acid, 15 mg/kg; vitamin B12 (0.1%), 0.05 mg/kg; niacin, 200 mg/kg; pyridoxine, 30 mg/kg; ascorbic acid (phosphate, 15%), 200 mg/kg; inositol, 400 mg/kg.
Mineral premix (mg/kg of diet): $MnSO_4 \cdot H_2O$ (32.5% Mn), 40.0; $CuSO_4 \cdot 5H_2O$ (25.4% Cu), 10.0; $ZnSO_4 \cdot 7H_2O$ (22.7% Zn), 50.0; $MgSO_4 \cdot 7H_2O$ (9.95% Mg), 0.04%; KI (76.4% I), 5.0; Na_2SeO_3 (45.6% Se), 1.0; $CoCl_2 \cdot 6H_2O$ (24.8% Co), 10.0.

Table 2 Fatty acid composition of experiment diets(%)

Fatty acid	*Diets*				
	FO	*SFO*	*PO*	*CO*	*SBO*
C14:0	3.36	1.59	1.31	1.38	1.71
C16:0	19.44	14.43	16.76	19.53	16.82
C18:0	4.67	5.42	4.46	3.26	5.09
C20:0	/	/	0.80	/	/
C22:0	/	/	1.22	/	/
C24:0	/	/	0.59	/	/
ΣSFA	27.47	21.44	25.14	24.17	23.62
C16:1	4.74	2.37	2.06	2.30	2.06
C18:1	27.36	24.60	32.43	27.61	25.10
C20:1	/	/	2.35	2.06	/
∑MUFA	32.10	26.97	36.84	31.97	27.16
C18:3n-3	2.00	/	/	0.74	3.42
C20:5n-3	8.28	5.13	4.8	5.43	4.85
C22:6n-3	9.86	7.43	7.22	7.84	7.29
Σn-3PUFA	20.14	12.56	12.02	14.01	15.56
C18:2n-6	12.07	34.31	21.38	28.83	29.35
C20:2n-6	0.34	/	/	/	/
C20:4n-6	0.90	/	0.69	/	/
Σn-6PUFA	13.31	34.31	22.07	28.83	29.35
n-3/n-6PUFA	1.51	0.37	0.54	0.49	0.53

The water was oxygenated, passed through an artificial sponge (3 cm thickness) and through coral-sand (25 cm thickness) to remove chlorine. During the trial period, the diurnal cycle was 12h light/12h dark. Water quality was monitored weekly and maintained as follows: temperature, 27.5±1.6^{0}C; pH, 7.1±0.2, respectively; ammonia nitrogen was lower than 0.05 mg/L ; dissolved oxygen was about 6.2 mg/L. The fish were manually fed to satiation twice per day for 8 weeks. To calculate feed intake, uneaten feed was collected and dried 1h after feeding.

Sampling and analytical methods. At the onset of the feeding trial, 18 fish were randomly sampled from the untreated fish, for analysis of whole body composition. After the 8 week experiment, 9 fish from each tank were randomly collected for proximate analysis, 3 for analysis of whole-body composition, and 6 were anesthetized with ice water to obtain weight of individual whole body, viscera, liver and intraperitoneal fat. White muscle from both sides of the fillets without the skin and liver were dissected, frozen immediately in liquid nitrogen, and stored at -70^{0}C until use.

Diets and fish samples (including white muscle and liver) were analyzed in triplicate for proximate composition. Crude protein, crude lipid, moisture, crude ash and gross energy (GE) were determined following standard methods (AOAC, 1984). Lipids for fatty acid analysis were extracted from diet ingredients, experimental diets, and muscle tissue with chloroform and methanol using the procedure of Bligh and Dyer (1959). Fatty acids were analyzed by gas chromatography–mass spectrometry (GC–MS) as previously described (Wei et al., 2009). The GC–MS analysis was carried out using an Agilent 6890 GC system equipped with an Agilent 5975 inert mass selective detector and an Agilent DB-23 capillary column (30 m length, 0.25 mm inner diameter, and 0.25 μm film thickness). Ultra-high purity (99.99%) helium was used as the carrier gas at a flow-rate of 1.0 ml/min. Sample volumes of 0.2 μl were injected with a split ratio of 10:1. The injector and detector temperatures were 250°C, and the solvent delay time was 4 min. Oven temperature was kept at 110°C for 2 min and programmed to 220°C at a rate of 5°C/min, then held at 220°C for 5 min. Fatty acids were identified by retention indices and by comparing their mass spectra with the NIST 05 spectral database. The relative percentages of individual fatty acids were calculated and expressed as mass percentages of total fatty acids.

Statistical analysis. All data are presented as mean ± S.E, which were subjected to one-way ANOVA. When differences were significant, the treatment means were further compared with Duncan's multiple-range tests. All statistical analyses were performed using the SPSS software Ver. 20.0 for Windows (SPSS Inc., Chicago, IL, USA Ver 20.0, USA). Differences were considered significant at $P< 0.05$.

Results

After the 8 week feeding trial, there were no significant differences in survival among fish fed all the diets (Table 3). Different dietary lipid sources were found to have significantly ($P<0.05$) influenced the growth performance in *Anguilla marmorata* juveniles. Weight gain (WG) and specific growth rate (SGR) of fish reared on FO and SBO diets were markedly higher than those fish fed the CO diet, while no other differences in WG and SGR were observed among other treatments. Fish fed FO and SBO diets had better feed utilization (feed conversion ratio, FCR) than those fed other diets, while fish fed PO diet showed the lowest feed utilization.

Table 3 Effects of different lipid sources on growth performance and feed utilization of *Anguilla marmorata*[1]

Parameters	*Diets*				
	FO	*SFO*	*PO*	*CO*	*SBO*
Initial weight (g)	6.04±0.01	6.01±0.06	5.94±0.02	6.03±0.05	6.02±0.02
Final weight (g)	12.9±0.22^{ab}	11.5±0.12^{abc}	11.1±0.50^{bc}	10.5±1.42^{c}	13.5±0.24^{a}
WG[2] (%)	114.2±4.05^{a}	90.5±3.08^{ab}	87.2±8.21^{ab}	74.3±23.12^{b}	123.8±4.00^{a}
SGR[3] (% day^{-1})	1.27±0.03^{a}	1.07±0.03^{ab}	1.04±0.07^{ab}	0.90±0.22^{b}	1.34±0.03^{a}
FE[4] (%)	2.08±0.07^{a}	1.66±0.04^{bc}	1.53±0.06^{b}	1.74±0.02^{c}	2.00±0.04^{a}
FCR[5]	1.53±0.05^{c}	1.93±0.05^{b}	2.09±0.08^{a}	1.85±0.02^{b}	1.61±0.03^{c}
Feed intake[6] g	8.11±0.67	9.29±0.92	7.41±1.61	7.16±1.96	8.93±1.26
Survival rate[7] (%)	84.5±2.2	93.3±3.85	82.2±4.45	93.3±3.85	82.2±4.45
VSI[8] (%)	5.67±0.18^{b}	6.49±0.19^{a}	5.92±0.24^{ab}	5.81±0.24^{ab}	6.20±0.30^{ab}
HSI[9] (%)	1.34±0.09^{bc}	1.65±0.10^{a}	1.40±0.06^{bc}	1.54±0.07^{ab}	1.29±0.08^{c}
IPF[10] (%)	1.17±0.16	1.19±0.19	1.17±0.19	1.10±0.13	1.31±0.18
CF[11] (%)	2.42±0.33^{a}	2.45±0.45^{a}	2.21±0.34^{a}	1.79±0.45^{b}	2.32±0.21^{a}

[1]Values (mean ± S.E. of three replications) in the same row with different superscript letters are significantly different (P<0.05).
[2]Weight gain (WG, %) = 100 × (final weight–initial weight)/(initial weight).
[3]Specific growth rate (SGR, % day^{-1}) =100 × [Ln(final weight)–Ln(initial weight)]/(days of feeding trial).
[4]Feed efficiency (FE, %) =100 × (final weight–initial weight)/(total dry weight of feed fed)
[5] FCR, feed conversion ratio = Feed consumed / Body weight gain
[6]Feed intake.
[7]Survival rate (SR, %) = 100 × (final fish number)/(initial fish number).
[8] Viscerosomatic index (VSI) = 100 × (visceral weight)/(body weight).
[9] Hepatosomatic index (HSI) = 100 × (liver weight)/(body weight).
[10] Intraperitoneal fat ratio (IPF) =100 × (intraperitoneal weight)/(body weight).
[11] Condition factor (CF) =100 × (body weight)/(body length^{3}).

The Viscerosomatic index (VSI) and Hepatosomatic index (HSI) of fish fed FO diet were significantly lower than fish fed SFO diet. There were no other differences in VSI and HSI compared to other treatments. Condition factor (CF) of fish fed an FO diet was markedly higher than fish fed a CO diet.

The proximate composition of whole body and muscle of the *Anguilla marmorata* are shown in Table 4. The whole body protein content of fish fed FO was significantly higher than that of the fish fed SBO diet; the whole body lipid content moisture showed an opposite trend. For white muscle composition, no significant differences were found between treatments.

Table 4 Effect of different lipid sources on whole body and muscle composition of *Anguilla marmorata* (%)

Parameters	*Diets*				
	FO	*SFO*	*PO*	*CO*	*SBO*
Whole body composition					
Moisture	70.29±0.05	68.18±1.15	67.89±0.50	68.30±1.46	66.81±0.67
Protein	16.14±0.17^{a}	15.84±0.10^{ab}	15.84±0.14^{ab}	15.96±0.14^{ab}	15.67±0.08^{b}
Lipid	10.88±0.28^{b}	12.15±0.66^{ab}	12.34±0.58^{ab}	12.12±0.96^{ab}	13.91±0.39^{a}
Muscle composition					
Moisture	65.68±0.57	64.80±1.56	63.90±1.36	66.14±1.18	67.38±0.75
Protein	17.34±0.15	18.58±0.78	18.62±1.01	16.83±0.43	17.59±0.13
Lipid	14.50±0.33	13.46±0.30	14.63±0.56	14.47±0.94	12.94±0.46

Values (mean ± S.E. of three replications) in the same row with different superscript letters are significantly different (P<0.05).

Muscle fatty acid composition is shown in Table 5. Muscle from fish fed the FO diet had significantly higher levels of C20:5n-3 and n-3/n-6 ratio compared to that of fish fed vegetable oil (VO) diets, while muscle n-6 PUFA and C18:2n-6 content showed an

opposite trend. Muscle C22:6n-3 content of fish fed FO diet was significantly higher than that fed PO diet. The content of n-3PUFA of fish fed FO diet was markedly higher than that of fish fed VO diets (except the SBO diet).

Table 5 Effects of different lipid sources on fatty acid composition in muscle of *Anguilla marmorata*(%)

Fatty acid	*Diets*				
	FO	*SFO*	*PO*	*CO*	*SBO*
C14:0	3.01±0.03^{a}	2.63±0.11^{b}	2.65±0.10^{b}	2.49±0.14^{b}	2.39±0.12^{b}
C15:0	1.07±0.03	1.04±0.17	1.07±0.08	0.86±0.18	0.75±0.14
C16:0	1.07±0.03^{a}	1.04±0.17^{ab}	1.07±0.08^{a}	0.86±0.18^{ab}	0.75±0.14^{b}
C17:0	1.83±0.12^{a}	1.87±0.15^{a}	1.91±0.20^{a}	1.49±0.18^{ab}	1.19±0.03^{b}
C18:0	4.11±0.03^{ab}	4.47±0.11^{a}	4.10±0.04^{ab}	3.82±0.14^{b}	4.30±0.18^{a}
ΣSFA	29.9±0.51^{a}	28.7±0.94^{ab}	29.7±0.46^{a}	27.8±1.06^{ab}	26.9±0.20^{b}
C16:1	4.44±0.08^{ab}	5.53±0.47^{a}	4.72±0.55^{ab}	4.17±0.84^{ab}	3.28±0.28^{b}
C18:1	32.1±2.32	29.5±0.66	32.5±0.53	31.6±1.09	28.9±1.75
ΣMUFA	36.5±2.40	35.4±0.42	37.3±0.17	35.8±1.47	32.2±1.47
C18:3n-3	0.58±0.03^{b}	/	/	/	1.07±0.03^{a}
C20:5n-3	1.39±0.08^{a}	1.06±0.09^{b}	1.03±0.07^{b}	1.02±0.02^{b}	1.12±0.06^{b}
C22:6n-3	3.02±0.30^{a}	2.32±0.09^{ab}	2.11±0.22^{b}	2.47±0.13^{ab}	2.60±0.39^{ab}
Σn-3PUFA	4.99±0.40^{a}	3.39±0.04^{bc}	3.14±0.28^{b}	3.49±0.12^{bc}	4.42±0.50^{ac}
C18:2n-6	5.05±0.12^{c}	9.40±0.85^{a}	6.17±0.48^{bc}	8.34±0.61^{ab}	10.4±1.11^{a}
C20:2n-6	/	1.12±0.16	1.03±0.20	0.72±0.07	0.69±0.03
C20:4n-6	1.65±0.26	1.91±0.10	1.98±0.18	1.59±0.16	1.71±0.51
Σn-6PUFA	6.69±0.24^{d}	12.4±0.60^{ab}	9.18±0.22^{c}	11.1±0.81^{ab}	12.8±0.24^{a}
n-3/n-6PUFA	0.75±0.08^{a}	0.27±0.01^{b}	0.34±0.02^{b}	0.33±0.02^{b}	0.37±0.06^{b}

Values (mean ± S.E. of three replications) in the same row with different superscript letters are significantly different (P<0.05).

Discussion

In this study, all the experimental diets were readily accepted by the fish. No significant differences in survival were found between fish fed the FO and VO diets. Weight gain of fish fed different dietary lipid sources are in general accordance with earlier observations (Reyes et L., 2016; Luo et al., 2013). Similar reports have been published for seabream and seabass (Izquierdo et al., 2003), grouper *Epinephelus coioides* (Lin et al., 2007), and turbot (Regost et al., 2003). Fish fed the CO, PO, and SFO diets showed significantly lower feed utilization compared to those fed the FO and SBO diets. This suggests that CO, PO, and SBO may be unsuitable as complete fish oil substitution for *Anguilla marmorata*.

Fatty acid composition of fish muscle is directly related to dietary fatty acid composition (Dernekbaşi et al. 2011, Bell et al., 2004, Caballero et al., 2002, Izquierdo et al., 2003). In contrast to FO, VO lacks n-3 HUFA, such as EPA and DHA, and is characterised by a low n-3 / n-6 ratio. Muscle of fish fed VO diets showed a lower content of EPA, DHA and n-3PUFA compared to that of fish fed an FO diet. Similar results have been shown in almost all finfish species, from carnivorous to herbivorous species and from marine cold water to tropical freshwater species fed with vegetable oil (Lin et al., 2007, Izquierdo et al., 2003, Caballero et al., 2002, Du et al., 2008). The present results confirm this relationship between dietary fatty acid and muscle fatty acid content. More evidence suggests that n-3 HUFA is extremely beneficial to human health, for prevention of cardiovascular and auto-immune diseases (Ruxton et al., 2005, Sinclair et al., 2007). The reduction of n-3 HUFA, such as EPA and DHA, in fish muscle should be taken into account in human nutrition. Present results indicate that the nutritional value of fish fed SFO, PO, and CO diets is significantly reduced compared to that of fish fed an FO diet.

Conclusion

In conclusion, our results confirm that soybean oil may be appropriate as an alternative to fish oil with full substitution in fish meal-based diets for eel without a negative effect on growth performance and feed utilization. The inclusion of corn oil, peanut oil, and sunflower oil would result in poor feed utilization by eel.

Acknowledgements

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