Asian-Aust. J. Anim. Sci.
Vol. 23, No. 4 : 508 - 514
April 2010

www.ajas.info

# Effects of Dietary Arachidonic Acid (20:4n-6) Levels on Growth Performance and Fatty Acid Composition of Juvenile Eel, *Anguilla japonica*

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**ABSTRACT :** This study was conducted to evaluate the effects of dietary arachidonic acid (AA, 20:4*n*-6) levels on growth performance and body composition in juvenile eel, *Anguilla japonica*. Six semi-purified experimental diets were formulated to be iso-nitrogenous and iso-caloric containing 55.0% crude protein and 15% crude lipid (18.3 kJ of available energy $g^{-1}$). Six different levels of AA were added to the basal diet, with 0, 0.2, 0.4, 0.6, 0.8 or 1.2% on a dry matter (DM) basis, respectively ($AA_{0.07}$, $AA_{0.22}$, $AA_{0.43}$, $AA_{0.57}$, $AA_{0.78}$ or $AA_{1.23}$). After a conditioning period, fish initially averaging 27±0.5 g (mean±SD) were randomly distributed into each aquarium as triplicate groups of 20 fish each. One of six experimental diets was fed on a DM basis to fish in three randomly selected aquaria at a rate of 2-3% of total body weight twice a day. At the end of the 12-week feeding trial, weight gain (WG) and feed efficiency (FE) of fish fed $AA_{0.78}$ and $AA_{1.23}$ diets were significantly higher than of fish fed $AA_{0.07}$, $AA_{0.22}$ and $AA_{0.43}$ diets ($p<0.05$). Specific growth rate (SGR) of fish fed the $AA_{0.78}$ diet was significantly higher than of fish fed $AA_{0.07}$, $AA_{0.22}$ and $AA_{0.43}$ diets ($p<0.05$). However, there were no significant differences in WG, SGR and FE among fish fed $AA_{0.57}$, $AA_{0.78}$ and $AA_{1.23}$ diets ($p>0.05$). Whole body AA deposition of fish fed the $AA_{1.23}$ diet was significantly higher than for the other diets ($p<0.05$). Broken-line model analysis on the basis of WG and SGR indicated that the dietary AA requirement could be greater than 0.69% but less than 0.71% of the diet in juvenile eel. The growth-promoting activity of AA observed in the present study provides strong support for the contention that dietary AA is essential for juvenile eel. (**Key Words :** Arachidonic Acid (AA), AA requirement, Essential Fatty Acids, Eel, *Anguilla japonica*, Growth Performance)

## INTRODUCTION

It has been demonstrated that *n*-3 highly unsaturated fatty acids (*n*-3 HUFA), mainly eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA), are essential fatty acids (EFA) in the diet of marine fish species for normal growth and survival (Sargent et al., 1999; Furuita et al., 2002). Compared with marine fish, freshwater species require either linoleic acid (LA, 18:2*n*-6) or linolenic acid (LNA, 18:3*n*-3) or both of these fatty acids (Sargent et al., 1989). These fatty acids are important structural and physiological components of cell membranes and thought to play an important role in permeability, enzyme activity, eicosanoid metabolism and the maintenance of membranes (Bell et al., 1986; Henderson and Tocher, 1987). A dietary deficiency of EFA is observed as poor growth, increased water content of muscle, high liver lipid content, poor feed efficiency, shock syndrome, fin erosion, mitochondrial swelling and a decrease in haemoglobin (Stickney and Andrews, 1972; Castell et al., 1972a, b; Watanabe et al., 1974).

Among *n*-6 HUFA, several studies have indicated the importance of arachidonic acid (AA, 20:4*n*-6) in fish metabolism since it is known to be the main fatty acid precursor of eicosanoids in fish (Henderson and Sargent, 1985; Henderson et al., 1985; Bell et al., 1994). The AA in fish tissues has long been known to be located almost

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Received September 21, 2009; Accepted November 24, 2009

exclusively in the 2-position of the glycerol of the inositiol phospholipids which have critical roles in many areas of cellular signal transduction (Sargent et al., 1989). Moreover, series-2 prostaglandins derived from AA have long been used to induce spawning in fish (Sargent et al., 1995). A number of studies have been conducted on dietary AA in several fish species which have confirmed that elevated AA can improve growth and survival (Bessonart et al., 1999) and resistance to handling stress (Koven et al., 2001) in larval gilthead sea bream and juvenile turbot (Castell et al., 1994).

Eel, *Anguilla japonica*, is a very popular freshwater fish, being one of the traditional foods in Asia, and is widely distributed in East Asia. This species is constantly increasing in popularity as a food fish with a corresponding increase in production from the meager value of 77,339 mt in 1984 through 179,777 mt in 1994 to 257,818 mt in 2007 (FAO, 2008). Previous studies showed that EFA requirements for optimal growth of the eel, *A. japonica*, were satisfied by both *n*-3 and *n*-6 PUFA (Takeuchi et al., 1980). Additionally, we investigated the optimum levels of dietary *n*-3 or *n*-6 fatty acids and the availability of AA in elver and juvenile stages of eel, *A. japonica* (Bae, 2003; Bae et al., 2004). Despite the important functions described above, quantitative AA requirement in juvenile eel have not been determined. The objective of this study was to determine the dietary AA requirement for juvenile eel, *A. japonica*, based on growth performance and body fatty acid composition.

## MATERIALS AND METHODS

### Experimental diets

Composition of the semi-purified basal diet is shown in Table 1. Six different diets were prepared containing the equivalent of 0, 0.2, 0.4, 0.6, 0.8 or 1.2% arachidonic acid (AA, 41.3% in fatty acid, DSM Nutrition Korea Co. Ltd., Seoul, Korea) on a dry matter basis. Fatty acid compositions of the experimental diets are shown in Table 2. The actual AA concentrations of six experimental diets determined by gas chromatograph (GC) were 0.07, 0.22, 0.43, 0.57, 0.78 or 1.23% of the diets. So the diets were designated as $AA_{0.07}$, $AA_{0.22}$, $AA_{0.43}$, $AA_{0.57}$, $AA_{0.78}$ or $AA_{1.23}$ for diets containing 0.07, 0.22, 0.43, 0.57, 0.78 or 1.23%, respectively. In diets supplemented with AA, equivalent amounts of ethyl oleate were removed (Furuita et al., 2003).

The experimental diets were formulated to be iso-nitrogenous and iso-caloric and to contain 55.0% crude protein and 15% crude lipid (18.3 kJ of available energy $g^{-1}$) based on previous studies (Bae et al., 2004, 2008). Defatted fish meal and casein were used as the main protein sources. Fish meal was extracted four times using hot (75-80°C) ethanol (fish meal/ethanol = 1:2, W/V) before incorporation into the diets (Kosutarak et al., 1995; Wang et al., 2003). The experimental diets were prepared by mixing the dry ingredients in an electric mixer, followed by the addition of oils and water. This mixture was formed into dough, and dry pellets were made by passing the dough through a screw-type pelleting machine and air drying the formed pellets for approximately 48 h. After drying, the pellets were broken up, sieved into the proper pellet size, sealed, and stored at -20°C until use.

**Table 1.** Composition and proximate analysis of the basal diet for juvenile eel

| Basal diet | % dry matter |
|---|---|
| Ingredients | |
| Defatted fish meal[1] | 51.6 |
| Casein[2] | 11.4 |
| Gelatine[2] | 3.0 |
| Corn starch[1] | 16.0 |
| Fish oil[3] | 3.0 |
| Linseed oil[4] | 6.0 |
| Arachidonic acid[5] | 0.0 |
| Ethyl oleate[6] | 6.0 |
| Vitamin pre-mixture[7] | 1.0 |
| Mineral premix[8] | 1.0 |
| Proximate analysis | |
| Moisture | 12.5 |
| Crude protein | 54.7 |
| Crude lipid | 14.5 |
| Crude ash | 9.9 |

[1] Provided by A-Bank Co., Ltd. Seoul, Korea.
[2] United States Biochemical (USB), Cleveland, Ohio.
[3] Provided Ehwa fat industry Co. Ltd., Busan, Korea.
[4] Dong Suh Oil & Fats, Changwon, Korea.
[5] Provided by DSM Nutrition Korea Ltd., Seoul, Korea.
[6] Sigma, St. Louis, USA.
[7] Vitamin premix (mg/kg feed unless indicated otherwise): vitamin A, 3,000 IU; vitamin $D_3$, 2,400 IU; vitamin E, 120 IU; menadione sodium bisulfate, 6; vitamin $B_1$-HCl, 15; vitamin $B_2$, 30; vitamin $B_6$-HCl, 15; vitamin $B_{12}$, 0.06; vitamin C, 300; calcium pantothenate, 150; nicotinamide, 150; inositol, 150; d-biotin, 1.5; choline chloride, 3,000; pancreatin, 12.5.
[8] Mineral premix (mg/kg feed): $MnSO_4$, 320; $ZnSO_4$, 270; $FeSO_4$, 750; $CuSO_4$, 60; $CoSO_4$, 7; $MgSO_4$, 17.25; $K_2SO_4$, 212.24; NaCl, 51.88; $K_2HPO_4$, 136.09; $NaSeO_3$, 0.013; KI, 0.15.

### Experimental fish and feeding trial

Prior to the start of the feeding trial, juvenile eel were fed with the basal diet for 4 weeks to adjust to the semi-purified diet and to deplete possible body reserves of arachidonic acid. At the start of the experiment, fish were starved for 24 h and weighed after being anesthetized with ethylene glycol phenyl ether. The feeding trial was conducted in a re-circulating system with a bio-filter installed in a concrete water reservoir at Inland Aquaculture Research Institute, National Fisheries Research and Development Institute (NFRDI), Jinhae, Korea. All aquaria

**Table 2.** Fatty acid composition of the six experimental diets[1]

| Fatty acids | Experimental diets (% of total fatty acids) | | | | | | Pooled |
|---|---|---|---|---|---|---|---|
| | $AA_{0.07}$ | $AA_{0.22}$ | $AA_{0.43}$ | $AA_{0.57}$ | $AA_{0.78}$ | $AA_{1.23}$ | SEM[2] |
| 14:0 | 1.03 | 1.14 | 1.03 | 1.06 | 1.12 | 1.02 | 0.02 |
| 15:0 | 0.11 | 0.12 | 0.12 | 0.12 | 0.13 | 0.12 | 0.00 |
| 16:0 | 13.48 | 12.76 | 13.15 | 13.21 | 13.29 | 13.37 | 0.10 |
| 16:1 | 1.55 | 1.57 | 1.52 | 1.48 | 1.57 | 1.24 | 0.05 |
| 17:0 | 0.37 | 0.32 | 0.37 | 0.37 | 0.38 | 0.32 | 0.01 |
| 17:1 | 0.13 | 0.12 | 0.14 | 0.12 | 0.14 | 0.11 | 0.00 |
| 18:0 | 4.40 | 4.41 | 4.69 | 4.81 | 4.96 | 5.37 | 0.15 |
| 18:1n-9 | 25.31 | 25.33 | 24.26 | 24.37 | 24.06 | 22.83 | 0.38 |
| 18:2n-6 | 4.14 | 4.27 | 3.82 | 3.77 | 3.58 | 3.64 | 0.11 |
| 18:3n-3 | 38.38 | 39.72 | 37.29 | 37.20 | 35.21 | 35.33 | 0.71 |
| 18:3n-6 | 0.00 | 0.11 | 0.20 | 0.26 | 0.35 | 0.58 | 0.08 |
| 20:0 | 0.35 | 0.37 | 0.37 | 0.39 | 0.40 | 0.45 | 0.02 |
| 20:1n-9 | 0.90 | 0.88 | 0.91 | 0.86 | 0.92 | 0.80 | 0.02 |
| 20:2n-6 | 0.14 | 0.15 | 0.17 | 0.17 | 0.19 | 0.20 | 0.01 |
| 20:3n-6 | 0.00 | 0.14 | 0.26 | 0.35 | 0.48 | 0.77 | 0.11 |
| 20:4n-6 | 0.42 | 1.39 | 2.72 | 3.59 | 4.89 | 7.71 | 1.07 |
| 20:5n-3 | 3.26 | 2.61 | 3.18 | 2.77 | 2.86 | 2.04 | 0.18 |
| 22:0 | 0.39 | 0.43 | 0.46 | 0.50 | 0.52 | 0.62 | 0.03 |
| 22:1 | 0.36 | 0.14 | 0.36 | 0.37 | 0.37 | 0.29 | 0.04 |
| 23:0 | 0.13 | 0.12 | 0.13 | 0.12 | 0.13 | 0.08 | 0.01 |
| 24:0 | 0.77 | 0.73 | 0.75 | 0.72 | 0.78 | 0.57 | 0.03 |
| 22:6n-3 | 4.40 | 3.18 | 4.11 | 3.39 | 3.65 | 2.52 | 0.27 |
| ΣSaturates | 21.02 | 20.40 | 21.07 | 21.30 | 21.70 | 21.92 | 0.22 |
| ΣMonoenes | 28.25 | 28.04 | 27.19 | 27.20 | 27.07 | 25.27 | 0.43 |
| ΣPUFA | 42.52 | 44.10 | 41.31 | 41.23 | 39.15 | 39.55 | 0.75 |
| ΣHUFA | 8.21 | 7.46 | 10.44 | 10.27 | 12.08 | 13.25 | 0.90 |
| Total | 100 | 100 | 100 | 100 | 100 | 100 | |

[1] Diets: Control group 0% ($AA_{0.07}$), 0.2% ($AA_{0.22}$), 0.4% ($AA_{0.43}$), 0.6% ($AA_{0.57}$), 0.8% ($AA_{0.78}$), 1.2% ($AA_{1.23}$) in diets.
[2] Pooled standard error of mean: $SD/\sqrt{n}$.

were equipped with an aeration system and water was heated by electric heaters in the concrete reservoir. Water temperature was maintained at 25±1.0°C and water flow rate was 1 L/min. Experimental fish averaging 27±0.5 g (mean±SD) were randomly distributed into each of 18 aquaria of 60 L capacity as groups of 20 fish. Each diet was fed to triplicate groups at a feeding rate of 2% to 3% of wet body weight, with the feed for each day divided into two parts and fed twice daily for 12 weeks. Total fish weight per aquarium was determined every 4 weeks, and the amount of diet fed was adjusted accordingly. Dead fish were immediately removed and weighed, and the amount of feed for the tanks was fixed at the proper percentage of the fish weight in the tanks. Uneaten feed was collected from each tank by siphoning and dried in an oven at 70°C to a constant weight. Actual feed consumption was estimated as the difference in weight between the feed supplied into the aquaria and the uneaten feed removed by siphon.

**Sample collection and analysis**

Weight gain (WG), specific growth rate (SRG) and feed efficiency (FE) were measured and calculated after each weighing. After the final weighing, six fish were randomly collected from each aquarium and frozen at -20°C for analysis. A proximate composition analysis of the experimental diets was performed by the standard methods of AOAC (1995). Samples were dried to a constant weight at 105°C to determine moisture content. Ash was determined by incineration at 550°C, crude lipid by soxhlet extraction using a Soxtec system 1046 (Tecator AB, Hoganas, Sweden), and crude protein by Kjeldahl method (N×6.25) after acid digestion. Lipid for fatty acid analysis was extracted by a mixture of chloroform and methanol (2:1 v/v) according to the method of Folch et al. (1957), and fatty acid methyl esters were prepared by transesterification with 14% $BF_3$-MeOH (Sigma, USA). The composition of fatty acid methyl esters was determined by gas

**Table 3.** Growth performance and survival of juvenile eel fed diets containing different AA levels for 12 weeks[1]

| | Experimental diets[2] | | | | | | Pooled SEM[6] |
|---|---|---|---|---|---|---|---|
| | $AA_{0.07}$ | $AA_{0.22}$ | $AA_{0.43}$ | $AA_{0.57}$ | $AA_{0.78}$ | $AA_{1.23}$ | |
| Initial wt. (g) | 27.3 | 27.5 | 27.4 | 27.6 | 27.4 | 27.6 | 0.05 |
| Final wt. (g) | 59.2 | 61.9 | 67.2 | 75.7 | 80.6 | 78.2 | 2.11 |
| WG (%)[3] | 117.0$^{c}$ | 125.1$^{c}$ | 145.2$^{bc}$ | 174.7$^{ab}$ | 194.1$^{a}$ | 183.4$^{a}$ | 7.58 |
| SGR (%)[4] | 1.55$^{c}$ | 1.62$^{c}$ | 1.79$^{bc}$ | 2.02$^{ab}$ | 2.16$^{a}$ | 2.08$^{ab}$ | 0.06 |
| FE (%)[5] | 46.8$^{c}$ | 50.1$^{c}$ | 58.1$^{bc}$ | 69.6$^{ab}$ | 77.6$^{a}$ | 73.4$^{a}$ | 3.03 |
| Survival (%) | 91.7 | 95.0 | 96.7 | 96.7 | 93.3 | 91.7 | 1.09 |

[1] Values are means of triplicate groups; values in the same row not sharing a common superscript are significantly different ($p<0.05$).
[2] Diets: Control group 0% ($AA_{0.07}$), 0.2% ($AA_{0.22}$), 0.4% ($AA_{0.43}$), 0.6% ($AA_{0.57}$), 0.8% ($AA_{0.78}$), 1.2% ($AA_{1.23}$) in diets.
[3] Weight gain = ((final body weight-initial body weight)/initial weight)×100. [4] Specific growth rate = (ln(final weight-initial weight)/days)×100.
[5] Feed efficiency = (wet weight gain/dry feed intake)×100. [6] Pooled standard error of mean: $SD/\sqrt{n}$.

chromatography (Trace GC, Theromo Finnigan, USA) with flame ionization detector, equipped with a Carbowax 007 capillary column (30 m×0.25 mm i.d., film thickness 0.25 μm, QUADREX, USA). Injector and detector temperatures were 250°C. The column temperature was programmed from 100°C to 220°C at a rate of 5°C/min and 220°C to 240°C at a rate of 3°C/min. Helium was used as the carrier gas. Fatty acids were identified by comparison with known standards.

**Statistical analysis**

All data were analyzed by one-way ANOVA to test for the effects of the dietary treatments. When a significant treatment effect was observed, a Least Significant Difference (LSD) test was used to compare means. Treatment effects were considered significant at $p<0.05$. Broken-line analysis (Robbins et al., 1979) was used to estimate the optimum dietary AA levels. All statistical analyses were carried out by SAS version 9.0 software (SAS Institute, Cary, NC, USA).

## RESULTS

At the end of the 12-week feeding trial, weight gain (WG), specific growth rate (SGR), feed efficiency (FE) and survival of juvenile eel fed the diets containing six graded levels of AA are summarized in Table 3. In the present study, WG, FE and SGR of fish were significantly influenced by the dietary AA graded levels. WG and FE of fish fed $AA_{0.78}$ and $AA_{1.23}$ diets were significantly higher than of fish fed $AA_{0.07}$, $AA_{0.22}$ and $AA_{0.43}$ diets ($p<0.05$); there were no significant differences among fish fed $AA_{0.6}$, $AA_{0.8}$ and $AA_{1.2}$ diets ($p>0.05$). There were no significant differences in WG and SGR among fish fed $AA_{0.57}$, $AA_{0.78}$ and $AA_{1.23}$ diets ($p>0.05$). SGR of fish fed $AA_{0.78}$ diet was significantly higher than of fish fed $AA_{0.07}$, $AA_{0.22}$ and $AA_{0.43}$ diets ($p<0.05$); there were no significant differences in SGR among fish fed $AA_{0.57}$, $AA_{0.78}$ and $AA_{1.23}$ diets ($p>0.05$). Survival rates of fish fed graded levels of dietary AA were not significantly different from each other ($p>0.05$). Broken-line model analyses on the basis of WG or SGR indicated that the dietary AA requirement of juvenile eel was 0.71 or 0.69% of diet, respectively (Figure 1 and 2).

After the 12 weeks feeding trial, the whole body fatty acid composition of juvenile eel fed the diets containing six supplementation levels of AA are summarized in Table 4. The AA concentration of fish fed the $AA_{1.23}$ diet was significantly higher than of fish fed the other diets ($p<0.05$). The concentration of unsaturated fatty acids, $\Sigma n$-6, of fish fed the $AA_{1.23}$ diet was significantly higher than of fish fed the other diets, while the $\Sigma n$-3 of fish fed the $AA_{0.07}$ diet was significantly higher than of fish fed the other diets ($p<0.05$). The eicosapentaenoic acid (EPA, 20:5$n$-3) concentrations of fish fed $AA_{0.07}$ and $AA_{0.22}$ diets were significantly higher than of fish fed the other diets; there were no significant differences among fish fed $AA_{0.07}$ and $AA_{0.22}$ diets ($p>0.05$). The docosahexaenoic acid (DHA, 22:6$n$-3) concentrations of fish fed $AA_{0.07}$ diet were significantly higher than of fish fed the other diets ($p>0.05$). The concentrations of

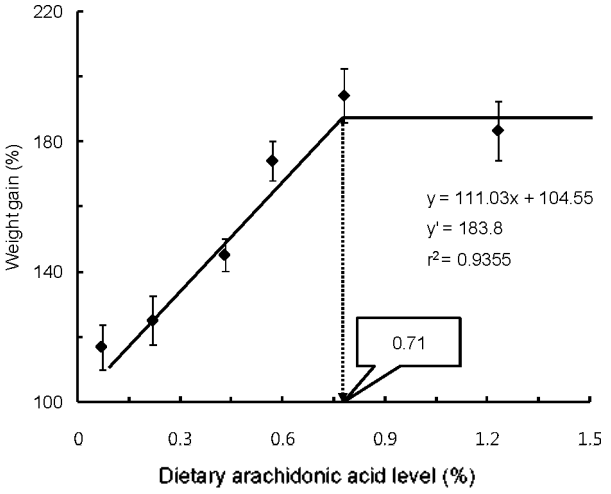


**Figure 1.** Broken line analysis on weight gain of juvenile eel fed the diets containing different arachidonic acid levels for 12 weeks. Values of the X-axis are the arachidonic acid levels in experimental diets. Values are means±SD of 3 replications.

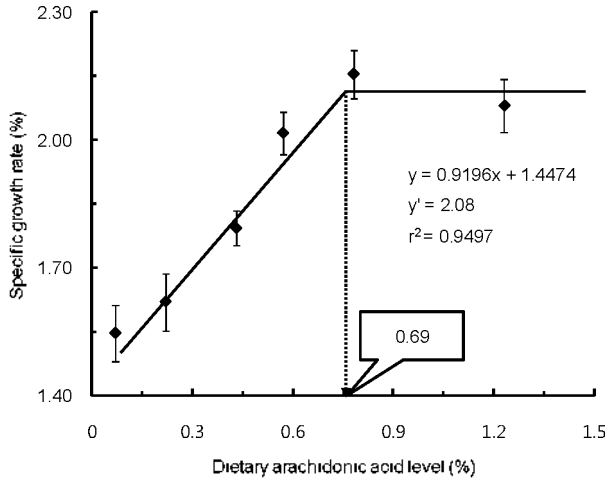


**Figure 2.** Broken line analysis on specific growth rate of juvenile eel fed the diets containing different arachidonic acid levels for 12 weeks. Values of the X-axis are the arachidonic acid levels in experimental diets. Values are means±SD of 3 replications.

ΣSaturates, Σmonoenes and Σ*n*-9 were not significantly affected by the dietary supplementation levels of AA ($p>0.05$).

## DISCUSSION

In the present study, the dietary arachidonic acid (AA) requirement for the juvenile eel, *A. japonica* based on WG and SGR was found to be 0.69-0.71% of the diet. The results of the present study were higher than those reported for Japanese flounder, *Paralichthys olivaceus* (0.6%, Furuita et al., 2003), and lower than those reported for juvenile turbot, *Scophthalmus maximus* (0.78%, Castell et al., 1994) and larval gilthead sea bream, *Sparus aurata* (1%, Bessonart et al., 1999).

In this study, best growth and feed utilization effects were observed in the fish fed an optimum AA level in the diet. In previous studies, Castell et al. (1994) and Bell et al. (1995) demonstrated that dietary AA promotes growth of juvenile turbot and concluded that AA is an essential fatty acid (EFA) for juvenile turbot. These studies provided the first evidence that AA is an EFA for normal growth, development and survival of juvenile marine fish. In addition, Koven et al. (2001) suggested the importance of

**Table 4.** Whole body fatty acid composition of the juvenile eel fed diets containing different AA levels for 12 weeks[1]

| Fatty acids | Experimental diets[2] (% of total fatty acids) | | | | | | Pooled SEM[3] |
|---|---|---|---|---|---|---|---|
| | $AA_{0.07}$ | $AA_{0.22}$ | $AA_{0.43}$ | $AA_{0.57}$ | $AA_{0.78}$ | $AA_{1.23}$ | |
| 14:0 | 3.34 | 3.14 | 2.99 | 2.73 | 3.04 | 2.81 | 0.09 |
| 15:0 | 0.36 | 0.31 | 0.32 | 0.30 | 0.32 | 0.29 | 0.01 |
| 16:0 | 18.75 | 19.67 | 18.65 | 18.41 | 19.31 | 18.66 | 0.19 |
| 16:1 | 5.70 | 5.86 | 5.83 | 4.92 | 5.43 | 5.18 | 0.16 |
| 17:0 | 0.34 | 0.32 | 0.35 | 0.33 | 0.34 | 0.33 | 0.00 |
| 17:1 | 0.34 | 0.28 | 0.34 | 0.27 | 0.28 | 0.26 | 0.01 |
| 18:0 | 3.60 | 3.71 | 3.50 | 4.07 | 4.02 | 3.85 | 0.09 |
| 18:1n-9 | 36.53 | 37.21 | 35.23 | 36.64 | 36.36 | 33.06 | 0.62 |
| 18:2n-6 | $1.16^{b}$ | $1.16^{b}$ | $1.46^{a}$ | $1.29^{ab}$ | $1.27^{ab}$ | $1.48^{a}$ | 0.06 |
| 18:3n-3 | $12.59^{c}$ | $11.86^{d}$ | $14.35^{b}$ | $14.30^{b}$ | $12.55^{c}$ | $15.15^{a}$ | 0.53 |
| 18:3n-6 | $0.91^{e}$ | $1.27^{b}$ | $1.01^{d}$ | $1.09^{c}$ | $0.78^{f}$ | $1.63^{a}$ | 0.12 |
| 20:0 | 0.14 | 0.16 | 0.17 | 0.15 | 0.18 | 0.18 | 0.01 |
| 20:1n-9 | 1.97 | 1.72 | 1.65 | 1.51 | 1.87 | 1.49 | 0.08 |
| 20:2n-6 | 0.66 | 0.62 | 0.74 | 0.77 | 0.80 | 0.73 | 0.03 |
| 20:3n-6 | 0.89 | 0.72 | 1.22 | 1.11 | 0.97 | 1.09 | 0.07 |
| 20:4n-6 | $1.24^{e}$ | $1.52^{d}$ | $1.79^{c}$ | $2.02^{b}$ | $1.97^{b}$ | $3.46^{a}$ | 0.32 |
| 20:5n-3 | $1.87^{a}$ | $1.86^{a}$ | $1.64^{b}$ | $1.55^{b}$ | $1.57^{b}$ | $1.66^{b}$ | 0.06 |
| 22:0 | 0.33 | 0.28 | 0.27 | 0.22 | 0.27 | 0.26 | 0.01 |
| 22:1 | 0.39 | 0.41 | 0.48 | 0.53 | 0.61 | 0.84 | 0.07 |
| 23:0 | 1.50 | 1.43 | 1.36 | 1.39 | 1.42 | 1.32 | 0.03 |
| 24:0 | $7.39^{a}$ | $6.50^{b}$ | $6.65^{b}$ | $6.42^{b}$ | $6.65^{b}$ | $6.30^{b}$ | 0.16 |
| 22:6n-3 | $28.43^{b}$ | $29.15^{a}$ | $27.82^{c}$ | $27.90^{bc}$ | $29.23^{a}$ | $28.27^{bc}$ | 0.25 |
| ΣSaturates | $44.53^{b}$ | $45.08^{a}$ | $42.98^{d}$ | $43.29^{d}$ | $43.94^{c}$ | $39.99^{e}$ | 0.73 |
| ΣMonoenes | $10.4^{a}$ | $9.51^{b}$ | $9.75^{b}$ | $9.25^{b}$ | $9.49^{b}$ | $9.43^{b}$ | 0.17 |
| ΣPUFA | $16.3^{d}$ | $16.0^{e}$ | $19.1^{b}$ | $19.3^{b}$ | $17.1^{c}$ | $22.1^{a}$ | 0.95 |
| ΣHUFA | $36.9^{b}$ | $37.5^{a}$ | $35.5^{c}$ | $36.9^{b}$ | $36.6^{b}$ | $33.3^{d}$ | 0.62 |
| Total | 100 | 100 | 100 | 100 | 100 | 100 | |

[1] Values are means of triplicate groups; values in the same row not sharing a common superscript are significantly different ($p<0.05$).

[2] Diets: Control group 0% ($AA_{0.07}$), 0.2% ($AA_{0.22}$), 0.4% ($AA_{0.43}$), 0.6% ($AA_{0.57}$), 0.8% ($AA_{0.78}$), 1.2% ($AA_{1.23}$) in diets.

[3] Pooled standard error of mean: $SD/\sqrt{n}$.

dietary AA for improving resistance to handling stress in gilthead seabream larvae. The authors proposed that increased prostaglandin $E_2$ ($PGE_2$) production in the AA-supplemented fish was responsible for up-regulation of cortisol synthesis through the hypothalamus-pituitary-interregnal axis, resulting in improved response to acute stress (Bell and Sargent, 2003). On the other han

d, it has been reported that high levels of AA in the diet negatively affect growth and survival of larvae of cod (Zheng et al., 1996), Japanese flounder (Furuita et al., 1998) and yellowtail (Ishizaki et al., 1998). However, no effect of AA supplementation on survival of fish was observed among all treatments in this study. Growth parameters increased with increase in AA supplementation up to the requirement level and then decreased. These results indicate that an optimum amount of dietary AA is necessary for normal growth, development and survival but an overdose of AA has negative effects on fish, probably through the eicosanoid production mechanism mentioned above.

In Atlantic salmon, acclimations from freshwater to seawater AA levels and AA/EPA ratios have been shown to increase in the weeks before transfer to seawater (Bell et al., 1997; Tocher et al., 2000). Prostaglandins, especially prostaglandin F2$\alpha$ ($PGF_{2\alpha}$), are known to modulate adaptation to salinity alteration (Mustafa and Srivastava, 1989). $PGF_{2\alpha}$ production by isolated gill cells from Atlantic salmon post-smolts was increased in fish with increased gill polar lipid AA. In addition, gill cells with higher AA levels tended to adapt better to seawater challenge in terms of reduced plasma chloride levels, compared to those with lower gill AA levels (Bell et al., 1997; Tocher et al., 2000). These results indicate that AA, and series-2 prostaglandin production, are important in eliciting an appropriate response to environmental stress such as salinity change. For this reason, additional supplementation with AA may improve seawater adaptation (Bell and Sargent, 2003). Eel is a catadromous species that grows in freshwater and migrates back to the sea for spawning. Consequently, it is expected that more AA would be required for maintenance and osmoregulation during the transition from freshwater to seawater for artificial sexual maturation in eel.

The AA concentrations in the whole body increased with the increase in AA supplementation levels in diets. Similarly, Lee et al. (2003) reported that the percentage of AA in polar lipid from starry flounder, *Platichthys stellatus*, increased with increasing squid liver oil in the diet, probably due to AA from squid liver oil. Several studies have demonstrated the importance of AA in fish metabolism since it is known to be the main precursor fatty acid of eicosanoids in fish (Henderson and Sargent, 1985; Henderson et al., 1985; Bell et al., 1994) and it is also one of the main components of phosphatidyl-inositol (Bell et al., 1983; Bell and Dick, 1990).

In general, freshwater fish are able to produce DHA and EPA from linolenic acid (LNA, 18:3*n*-3) and AA from linoleic acid (LA, 18:2*n*-6). So, essential fatty acid (EFA) requirements of freshwater fish have been classified into three types such as rainbow trout type, tilapia type and carp type (Teshima, 1985). Rainbow trout require n-3 polyunsaturated fatty acids (PUFA) like 18:3*n*-3, while tilapia require *n*-6 PUFA as EFA. Carp require both *n*-3 and *n*-6 PUFA. Similarly to carp, EFA requirements of the eel, *Anguilla japonica*, are satisfied by both *n*-3 and *n*-6 PUFA (Takeuchi et al., 1980).

In conclusion, broken-line model analysis on the basis of WG and SGR indicated that the dietary AA requirement could be greater than 0.69% but less than 0.71% of the diet in juvenile eel. This study suggests that dietary AA is essential for normal growth in juvenile eel.

## ACKNOWLEDGMENTS

This study was partially funded by a grant (RP-2009-AQ-068) from the National Fisheries Research and Development Institute (NFRDI) and by the Korea Research Foundation Grant funded by the Korean Government (KRF-2008-313-F00085), and supported by the Feeds and Foods Nutrition Research Center (FFNRC), Pukyoung National University, Busan, Korea.

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