A THESIS

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Study on Dietary Myoinositol Requirement for Olive Flounder (*Pralichthys Olivaceus*)

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GRADUATE SCHOOL

CHEJU NATIONAL UNIVERSITY

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Study on Dietary Myoinositol Requirement for Olive Flounder (*Paralichthys Olivaceus*)

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국문초록

Inositol은 자연계에 9개의 이성질체가 존재하며 그 가운데 myoinositol로 불려지는 sis-1,2,3,5-trans-4,6-cyclohexanehexol은 세포막의 구조적 구성성분으로 유기체 내에 9개 이성질체 중 가장 높은 생리적 활성을 갖는 수용성 비타민으로 알려졌다(Cody, 1984). Myoinositol은 식품이나 사료원 중 피틴산(phytic acid)과 이노시톨인지질(phosphatidyliositol) 및 유리상태(free myoinositol)의 3가지로 존재하며, 인지질의 구성 성분으로서 인과 에테르결합 형태로 모든 유기체에 존재한다(Combs, 1992). 대부분의 연구들이 포유동물을 대상으로 수행되었으며 세포막의 구성 성분인 phosphatidylinositol의 생리적인 기능과 지단백질 합성 측면을 다루었다(Appel and Briggs, 1980). 최근에 세포 내 칼슘 조절과 2차 신경전달 기능에 초점을 맞춘 연구가 이루어 졌다(Irvine, 1992; Hughes and Michell, 1993; Colodny et al., 1998).

양식 어류에 있어서 사료 내 myoinositol 연구는 미비할 뿐 아니라, 넙치를 대상으로 한 연구는 거의 없는 실정이다. 이전에 붉은조기와 뱀장어 및 돌돔과 방어가 대상인 연구결과는 실험사료에서 myoinositol이 결핍되었을 때 다양한 결핍증상들을 보여 식욕부진, 무기력증, 성장지연, 지느러미 부식, 채색흑화, 위 공복지연, 콜린 가수분해 효소, 그리고 특정 아미노전이효소 활성 감소 등이 보고되었다(McLaren et al., 1947; Kitamura et al., 1967b; Yone et al., 1971; Arai et al., 1972; Ikeda et al., 1988; Hosokawa, 1989). 최근 결과는 몇몇 어종에서 새로 합성된 myoinositol 양으로 결핍증을 예방할 수 있어 사료 내 myoinositol의 첨가가 필요 없는 것으로 보고되었다(Burtle and Lovell, 1989; Waagbø et al., 1997; Deng et al., 2002; Peres et al., 2004). Myoinositol 요구량은 어종에 따라 큰 차이를 나타내며 틸라피아를 대상으로 수행한 연구는 같은 어종일지라도 어류의 성장 단계에 따라 그 필요성이 다르다고 보고하였다(Peres et al., 2004; Shiau and Su, 2005).

넙치는 우리나라를 비롯하여 중국, 일본에서 주요 해산 양식어종이며 지난 10년간 우리나라에서 가장 많이 생산되고 있다. 이 연구는 넙치의 myoinositol 요

구량이 아직까지 밝혀진 바가 없으므로, 넙치치어에 있어서 사료 내 myoinositol의 적정 요구량과 결핍 시 어류에 미치는 생리적 영향을 조사하려고 수행 되었다.

실험에 사용된 어류는 평균무게가 10 g인 넙치치어를 사용하였으며, 총 18개의 35 ℓ 원형수조에 각 수조당 12 마리씩(3반복구) 무작위 배치하였다. 사육실험 11주째에는 실험어의 사육밀도를 조절하려고 100 ℓ 원형수조로 재 배치하였다. 반 정제 사료원이 기초인 총 6개의 실험사료는 52 %의 조단백질과 18.3 MJ/kg diet의 에너지함량을 갖도록 조성하여 myoinositol 함량을 각각 0, 0, 200, 400, 800, 1600 mg/kg diet으로 기초사료에 첨가하였다(M0, M0+, M200, M400, M800, M1600). 실험사료 중 M0+ 사료는 항생제인 tetracycline hydrochloride (SIGMA, USA)를 0.4%로 첨가함으로써, myoinositol이 넙치 장내 미생물들에 의하여 합성되는지를 알기 위해 설계되었다.

20주간의 사육실험 결과, myoinositol이 결핍된 M0 실험구와 첨가된 실험구 사이에서 어류의 성장에 차이가 나타나지 않았다. 이전의 초기넙치 치어(Initial body weight, 1.2 g/fish)를 대상으로 한 myoinositol 요구량 실험에서는 성장지연과 같은 결핍증상을 보였으나(Lee et al., 2006), 어체 무게 약 10g인 넙치치어에서는 사료 내 myoinositol이 어류성장에 영향을 미치지 않는 것으로 보였다. 이러한 결과는 사료 내 myoinositol 요구량이 어류 성장단계에 따라 그 요구량이 변화되는 것으로 판단된다. 넙치치어 간의 myoinositol 함량을 분석한 결과에서는 사료 내 myoinositol 함량이 높아짐에 따라서 축적된 myoinositol 함량 또한 높게 검출 되었다. 간과 근육내의 지방함량 분석에서는 모든 실험구에서 차이가 나타나지 않았다. 비록 유의적 차이를 밝힐 수는 없었지만, myoinositol이 결핍된 실험구에서 지방이 축적되는 경향을 보였다. 또한 간에서의 지방산 분석결과, 고농도의 myoinositol을 섭취한 실험구에서 높은 고도불포화 지방산 함량을 나타내었다. 초기 넙치치어의 장내 미생물에 의한 myoinositol 신생합성 능력 평가를 위하여 M0와 M0+ 실험구를 비교한 결과, 장내 미생물에 의한 inositol의 신생합성은 넙치치어에서 중요한 inositol 공급원이 아닌 것으로 판단되었다.

넙치는 성장함에 따라 사료 내 myoinositol 의존성이 감소하는 것으로 보이

며, 약 10g 이상의 넙치 치어에서는 조직에서 합성되는 myoinositol 양으로도 성장 지연과 같은 결핍증상을 예방하기에 충분한 것으로 판단된다. 넙치 치어에서 myoinositol은 지방대사와 깊이 연관되어 있는 것으로 보이며, 특히 고도불포화 지방산 대사에 있어 중요한 작용을 하는 것으로 판단된다.

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1. Introduction

Myoinositol (cis-1,2,3,5-trans-4,6-cyclohexanehexol) is the most biologically active isomer in tissues as a structural component of cell membrane (Cody, 1984). Myoinositol which exists in combined forms of ethers, phosphates or as a component of phospholipids is found in all living cells (Combs, 1992). Myoinositol (1,2,3,4,5,6)-hexakisphosphate known as phytic acid is the most abundant source of myoinositol as a storage form of phosphorus in plant seeds. It exists around 70% of seed total phosphorus. However, it is difficult to use in most animals, because there is little or no intestinal phytase activity in their body. Therefore, most animals are dependent on the intestinal microflora that produces those enzymes. Dietary phytic aicd can be excreted through the feces in humans and other non-ruminant animals, because it makes salts by forming mineral cations, such as calcium, iron, and zinc. It causes some problems that reduce the utilization of phosphorus and divalent cation. To deal with the problems, low phytic aicd mutations in corn and barley cause the seed to store most of the phosphorus as inorganic phosphorus instead of as phytate phosphorus (Raboy, 2001). Sugiura et al. (1999) investigated the biological availability of phosphorus in the low phytate mutants of barley, dent corn and flint corn for rainbow trout. As the results of the study, apparent availability of phosphrus in low phytate was increased than that in ordinary grains when they were combined with low-ash ingredients. Fecal phosphorus content was also decreased by 50.2 % (in phytate-phosphorus) or 42.9 % (in total phosphorus) by replacing ordinary grains with low-phytate grains in the low-ash diets.

Many studies on myoinositol have been conducted in lipoprotein synthesis and in roles of phosphatidylinositol in cell membrane of mammals (Appel and Briggs, 1980). Katayama (1997) suggested that dietary myoinositol and phytate can protect an accumulation of hepatic lipids in sucrose-fed rats. In the previous study, the excessive mobilization of free fatty acid from adipose depots was considered to be the direct cause of fatty liver syndrome in myoinositol deficiency (Hayashi et al., 1974). Other studies also reported that

myoinositol plays a major role in central nervous system with relationship between second messenger and control of cytosolic calcium (Hughes and Michell, 1993; Colodny, 1998; Irvine, 2002).

Myoinositol is classified as a vitamin-like nutrient for most animals. However, it has not been clearly demonstrated whether the vitamin should be supplemented in diets for fishes because of its *de novo* synthesis. Mammals can make myoinositol in their liver, kidney, brain from glucose by *de novo* synthesis. The biosynthesis involves the cyclization of glucose-6-phosphate to inositol-1-phosphate by inositol-1-phosphate synthase followed by a dephosphorylation by inositol-1-phosphatase (Combs, 1992). Myoinositol synthetase activity was presented in liver and brain of channel catfish and sunshine bass, and in intestine of common carp (Burtle and Lovell, 1989; Waagbø et al., 1998; Deng et al., 2002; Peres et al., 2004). Mai et al. (2001) reported that the visceral tissue of abalone showed high levels of myoinositol synthetase activity (combined activities of myoinositol-1-phosphate synthetase and inositol-1-phosphatase), ranging from 74.0 to 98.2 μmol/h/g protein showing a negative correlation with dietary myoinositol level. The intestinal microbial synthesis, however, was not a significant source of inositol for tilapia (Shiau and Su, 2005). Deficiency symptoms of myoinositol were reported in rainbow trout, Chinook salmon, red sea bream, Japanese eel, parrotfish, and yellow tail indicating poor appetite, anemia, poor growth, fin erosion, dark skin coloration, slow gastric emptying, decreased cholinesterase and certain aminotransferase activities (McLaren et al., 1947; Kitamura et al., 1967; Yone et al., 1971; Arai et al., 1972; Ikeda et al., 1988; Hosokawa, 1989).

No information on myoinositol is available in olive flounder *Paralichthys olivaceus* which is one of the most important marine fish species in Korea, China, and Japan. The purpose of this study, therefore, was to determine the essentiality and requirement of myoinositol in diets for olive flounder.

2. Materials and Methods

2.1. Experimental diets

Six semi-purified diets were formulated to be isonitrogenous and isocaloric with 52% crude protein and 18.3 MJ/kg diet by graded levels of myoinositol (Table 1). Ethanol-extracted fish meal was employed in the diets as an attractant to enhance palatability in semi-purified diets (Lee and Dabrowski, 2004). To remove myoinositol from the basal diet, fish meal was extracted two times by 70% aqueous ethanol solution (ethanol/water, 7/3, v/v) for 48 h, and then the extracted fish meal was dried using an electric fan at room temperature. Six casein-gelatin based semi-purified diets were formulated to contain five different levels of myoinositol (0, 0+antibiotic, 200, 400, 800, and 1600 mg/kg designated as M0, M0+, M200, M400, M800, and M1600, respectively) at the expense of cellulose. One experimental diet contained the antibiotic (0.4%, w/w) tetracycline hydrochloride (SIGMA, USA) without myoinositol supplementation (Mai et al., 2001). Supplementation of the antibiotic was designed to prevent the fish from its intestinal synthesis of myoinositol by microflora. Inositol content in the experimental diets was measured according to the enzymatic assay described by Ashizawa et al. (2000). The concentration of inositol was 0 (M0), 0 (M0+), 159 (M200), 354 (M400), 793 (M800), and 1,585 (M1600) mg/kg diet, respectively.

The experimental diets were prepared by thoroughly mixing ingredients with oil and 35% distilled cold water in a mixer (NVM-14-2P, Korea) and pelleting the wet dough by a chopper machine (SMC-12, Korea) at 3 mm of diameter. The diets were then freeze dried at -40 ° C, crushed into desirable particle sizes (0.4 – 2.0 mm) and stored at –20 ° C until use.

Table 1. Composition of the basal diet (dry weight)[1]

Ingredients	%
Casein (vitamin free)	38
Gelatin	9
Fish meal (defatted)[2]	10
Dextrin	15
Starch	10
Squid liver oil	11
CMC	0.5
Vitamin Mix.[3]	3
Mineral Mix.[4]	3
Cellulose	0.5

[1] Calculated based on the compositions of the ingredients used (NRC 1993).

[2] Fat and vitamin of fish meal were extracted by 70% aqueous ethanol solution(water : ethanol = 3 : 7) for 48h.

[3] Vitamin premix (g/kg of mixture): retinyl acetate, 0.667; cholecalciferol, 0.033; menadione, 0.133; thiamine hydrochloride, 2.667; (-)-riboflavin, 2.933; d-pantothenic acid hemicalcium, 9.667; pyridoxine hydrochloride, 2.667; cyanocobalamin, 0.007; niacinamide, 20.000; folic acid, 0.320; d-biotin, 0.133; ascorbic acid, 30.000; α-tocopherol, 6.667.

[4] Mineral mixtures were based on the composition of Lee et al., 2003.

2.2. Fish and feeding trial

Juvenile olive flounder were transported from a private hatchery (Chang-Hae Fisheries Co.) in Jeju Island to Marine and Environmental Research Institute, Cheju National University. All the transported fish were fed a commercial diet for 6 weeks to be acclimated in the experimental facilities and conditions, and to be recovered from the stress of transportation. After the acclimation, the fish (initial body weight 10.0±0.1g) were randomly assigned to eighteen 35L plastic tank (triplicate groups per dietary treatment) at a density of 12 fish/tank. At the 11^{th} week, the fish were restocked into eighteen 100L polyvinyl tanks. The feeding trial was conducted for 20 weeks in the flow through system supplied with sand filtered seawater. Aeration was also provided to maintain dissolved oxygen levels near to the saturation. The photoperiod was scheduled by 11:13 h light/dark by fluorescent light. Water temperature ranged from 13 to 29 ° C according to the seasonal change. Salinity of the water was maintained at 34 ppt, dissolved oxygen was ranged from 7.80 to 8.05 mg/L, and pH was maintained at 8.02±0.01. The experimental diets were fed to the fish at a feeding rate of 4 % body weight at the beginning and 1% at the end of the feeding trial, twice a day (8:00 and 18:00 h), 7 days a week for 20 weeks. After feeding, uneaten feeds were removed by siphoning to calculate feed consumption and utilization. Inside of the tanks was routinely cleaned by a sponge to prevent the growth of microflora. The growth of fish was measured every two weeks and feeding rate was adjusted accordingly. Feeding was stopped 24 h prior to weighing.

2.3. Sample collection and analysis

At the end of 20 weeks of feeding trial, all fish were weighed and counted for the calculations of growth performances and feed utilizations. Three fish per each tank (9 fish per treatment) were randomly collected and anaesthetized with tricaine methanesulfonate (MS-222, 100 ppm). Blood was taken from the caudal vein for determination of hematocrit,

hemoglobin, aspartate aminotransferase (AST), alanine aminotransferase (ALT). Approximate compositions of the experimental diets were analyzed by the method of AOAC (1995). The lipid concentration was analyzed in the liver and muscle of the fish by the method of Folch et al. (1957). Fatty acids in the liver of the fish were analyzed by gas chromatography (HP-5890 II; Hewlett-Packard, Palo Alto, CA, USA) (Kim and Lee, 2004).

Inositol content in the experimental diets and liver samples were analyzed according to the enzymatic assay described by Ashizawa et al. (2000). Briefly, inositol was extracted from the sample by perchloric acid (16%, w/v) and centrifuged at 5,000 × g for 10 min at 4 ˚C. All supernatants were mixed with 2.0 M K_2CO_3 and centrifuged again. A 100 ml aliquot of the supernatant was added to 10 ml hexokinase reagent, which contained 200 mM Tris-HCl buffer, 400 mM adenosine triphosphate disodium, (the pH of the solution was adjusted to 8.6 by adding 10.0 M NaOH) and 115 U/ml hexokinase. The mixture was incubated at 37 ˚C for 90 min, and then heated for 3 min in a boiling water bath to stop the reaction and the adding 20 ml of 4.5 M HCl. After 10 min at 25 ˚C, 22 ㎕ of 3.0 M K_2CO_3 was added. Measurements were done using a 96-well microplate. One hundred ml of sample extract obtained as described above were mixed with 100 ml of myoinositol reagent, which contained 210 mM triethanolamine hydrochloride-32 mM K_2HPO_4-KOH buffer (pH 8.6), 1.2 % (v/v) Triton X-100, 10 mM β-NAD, 1.0 U/ml diaphorase, 0.1 % (w/v) bovine serum albumin and 60 mg/ml INT. After the absorbance of the solution was measured at 492 nm with a microplate reader (model Multiskan EX, Thermo Electron, USA), the reaction was initiated by addition of 10 ml 2.1 U/ml myoinositol dehydrogenase dissolved in 20 mM potassium phosphate buffer (pH 7.0) to each well. The mixture was allowed to stand for 30 min at room temperature, and then the absorbance (A) at 492 nm was measured again. From ΔA, an increase in absorbance during the reaction, myoinositol content was calculated.

2.4. Statistics

All experimental diets were distributed by a completely randomized design. Data were subjected to one-way ANOVA test in SPSS (version 12.0). The significant differences between group means were compared using Duncan's multiple tests ($P<0.05$). Data are presented as mean±SD. Percentage data were arcsine transformed before analysis.

3. Results

3.1. Growth performance

Weight gain, feed intake (FI), feed efficiency ratio (FER), specific growth rate (SGR), protein efficiency ratio (PER) and survival of flounder fed the experimental diets are presented in Table 2. WG, SGR, and PER of the fish fed diets containing different levels of myoinositol were not significantly different except for those of the fish fed M800 diet. The fish fed M800 diet was significantly higher than fish fed M0+ and M200 diets in WG, SGR, and PER. Also, the fish fed M800 diet was significantly higher than the fish fed M200 diet in FI. FER of the fish fed M800 diet was significantly higher than that of fish fed M0+ diet. The survival of fish fed M800 and M1600 diets was significantly higher than that of fish fed M0+ diet. However, all the results of growth performances were not significantly different between M0 treatment and myoinositol supplemented groups. Growth performances, feed utilization and survival of fish fed M0 diet were not significantly different compared to the fish fed M0+ diet which contained antibiotic to suppress *de novo* synthesis of myoinositol by intestinal microflora.

Table 2. Mean body weight (MBW), weight gain (WG), feed intake (FI), feed efficiency ratio (FER), feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER), and survival (SUV) of olive flounder fed six experimental diets with five different levels of ornithine for 20 weeks[1]

Diets	M0	M0+	M200	M400	M800	M1600
MBW	69.1±13.83^{ab}	64.9±9.06^{a}	64.6±3.89^{a}	77.3±9.84^{ab}	88.2±11.29^{b}	77.2±3.16^{ab}
WG (%)[2]	591±144.9^{ab}	546±90.1^{a}	542±45.1^{a}	674±105.3^{ab}	773±113.0^{b}	669±33.5^{ab}
FI (DM,	61.8±8.17^{ab}	63.3±2.83^{ab}	59.7±1.98^{a}	67.5±5.06^{ab}	71.7±5.99^{b}	68.2±2.40^{ab}
FER[4]	0.95±0.12^{ab}	0.87±0.17^{a}	0.91±0.04^{ab}	0.99±0.07^{ab}	1.08±0.06^{b}	0.99±0.03^{ab}
SGR (%)[5]	0.59±0.07^{ab}	0.58±0.05^{a}	0.58±0.02^{a}	0.63±0.04^{ab}	0.67±0.04^{b}	0.63±0.01^{ab}
PER (%)[6]	1.74±0.22^{ab}	1.59±0.31^{a}	1.64±0.07^{a}	1.82±0.13^{ab}	2.06±0.12^{b}	1.81±0.06^{ab}
SUV (%)	88.9±9.62^{abc}	72.2±17.35^{a}	72.2±9.62^{ab}	75.0±8.33^{ab}	94.4±9.62^{bc}	97.2±4.81^{c}

[3] FI (DM, g / fish): total feed fed (g)/fish

[4] FER: wet weighted gain/dry feed intake

[5] SGR (%): 100×(loge final mean body weight-loge initial mean body weight)/days

[6] PER (%): wet weighted gain (g)/total protein given (g)

3.2. Hematological parameters and analysis

Hemoglobin (Hb) concentration was significantly higher in the fish fed M400 diet than in the fish fed M0+ diet (Table 3). Hematocrit and AST activity were not significantly different among all the dietary groups. However, ALT activity was significantly lower in the fish fed M800 diet than in the fish fed M0 and M0+ diets (Table 3).

Fish fed the diets containing myoinositol showed a decreasing trend in the concentration of liver and muscle lipids compared to the fish fed myoinositol free diets (M0 and M0+ diets). However, the lipid concentration was not significantly different among all the dietary treatments (Table 4). Analyzed inositol concentration in the liver of the fish fed the experimental diets for 20 weeks was significantly increased by supplementation of dietary myoinositol ($P<0.05$). There is a correlation between inositol concentration and lipid in the liver of the fish fed the experimental diets for 20 weeks (Fig. 1). The fish group fed the diets containing high levels of myoinositol showed significantly increased proportion of polyunsaturated fatty acid in their liver (Table 5). Clinical deficiency symptom such as poor appetite in the fish fed the diets containing lower dietary myoinositol was observed during 20 weeks of feeding trial.

Table 3. Hemoglobin, hematocrit, alanin aminotransferase (ALT), and aspartate aminotransferase (AST) of olive flounder fed six experimental diets with five different levels of myoinositol for 20 weeks[1]

Diet	Hemoglobin	Hematocrit	ALT	AST
M0	3.36±0.23^{ab}	18.94±4.28	12.02±2.03^{c}	57.32±19.26
M0+	2.82±0.49^{a}	16.56±1.71	10.07±4.08^{bc}	46.22± 2.89
M200	3.32±0.03^{ab}	21.00±4.71	5.83±1.45^{ab}	42.34±17.24
M400	3.58±0.44^{b}	19.08±2.77	5.45±2.67^{ab}	52.92±13.80
M800	3.40±0.15^{ab}	21.39±6.61	3.23±0.85^{a}	43.18±21.28
M1600	3.38±0.11^{ab}	19.44±2.59	10.02±3.93^{bc}	36.91±12.15

[1] Values are presented as mean±SD. Value in the same column having different superscript letters is significantly different ($P<0.05$).

Table 4. Inositol concentration in the liver and lipid content in the liver and muscle of olive flounder fed six experimental diets with five different levels of myoinositol for 20 weeks[1]

Diet	Inositol (mg/kg wet tissue)	Lipid (%, wet tissue)	
		Liver	Muscle
M0	182.5±23.8^{ab}	27.56±12.50	0.90±0.15
M0+	162.5±5.7^{a}	23.70±6.99	0.88±0.06
M200	195.6±7.6^{ab}	18.11±7.39	0.88±0.04
M400	215.0±8.5^{cd}	16.47±2.29	0.77±0.05
M800	243.8±33.3^{d}	19.36±2.35	0.91±0.06
M1600	244.4±17.8^{d}	17.03±3.39	0.81±0.00

[1] Values are presented as mean±SD. Values in the same row having different superscripts are significantly different ($P<0.05$).

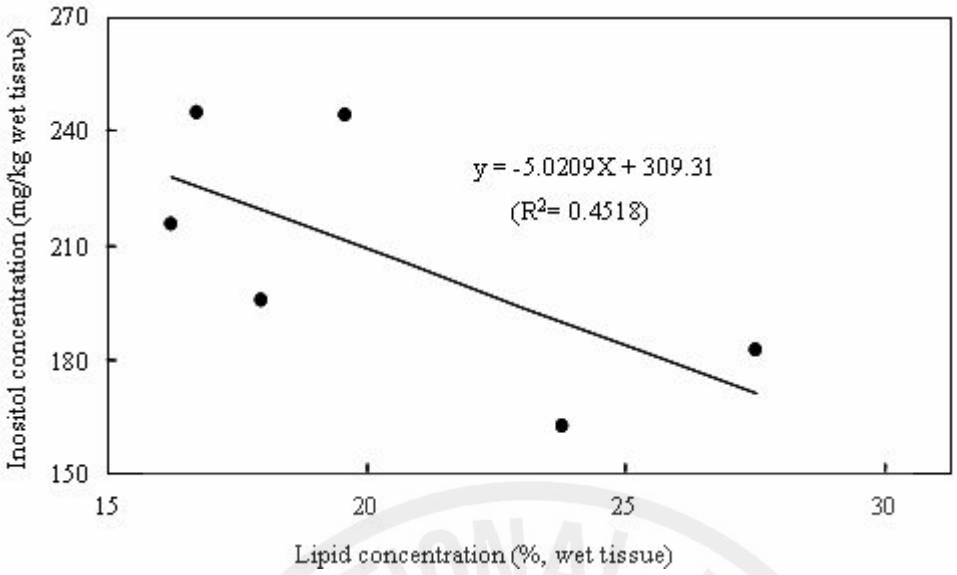

Fig. 1. Correlation between myoinositol and lipid concentration in the liver of olive flounder fed six experimental diets with five different levels of myoinositol for 20 weeks.

Table 5. Total fatty acid composition in the liver of olive flounder fed six experimental diets with five different levels of myoinositol for 20 weeks[1]

Fatty acids	M0	M0+	M200	M400	M800	M1600	SEM[2]
14:0	5.53	5.00	5.20	5.00	4.90	4.80	0.10
15:0	0.50	0.43	0.50	0.50	0.47	0.50	0.01
16:0	17.00^{ab}	15.77^{ab}	17.80^{b}	15.00^{a}	14.80^{a}	14.80^{a}	0.36
17:0	0.63	0.67	0.65	0.60	0.67	0.67	0.02
18:0	2.60	2.57	2.75	2.63	2.57	2.43	0.08
18:1n-9	26.67^{ab}	27.90^{b}	25.10^{a}	25.97^{a}	26.53^{ab}	24.77^{a}	0.32
18:2n-6	2.37	2.10	2.45	2.47	2.30	2.47	0.05
18:3n-3	0.63	0.57	0.65	0.77	0.60	0.67	0.03
18:4n-3	0.83	0.77	0.65	0.87	0.80	0.83	0.03
20:4n-6	0.77^{ab}	0.63^{a}	0.80^{b}	0.80^{b}	0.73^{ab}	0.80^{b}	0.02
20:3n-3	0.43^{ab}	0.40^{a}	0.45^{ab}	0.47^{ab}	0.47^{ab}	0.50^{b}	0.01
20:4n-3	2.10	1.93	1.90	2.17	2.13	2.20	0.04
20:5n-3	6.40	5.87	5.75	6.57	6.07	6.47	0.18
22:1n-9	3.40^{a}	3.50^{a}	3.45^{a}	4.13^{ab}	4.27^{b}	3.87^{ab}	0.11
22:3n-6	0.23	0.30	0.30	0.20	0.10	0.23	0.04
22:5n-3	4.37	4.37	4.15	4.17	4.37	4.73	0.08
22:6n-3	11.87	12.73	13.35	12.07	12.53	14.10	0.29
Saturates	26.27^{bc}	24.43^{abc}	26.90^{c}	23.73^{ab}	23.40^{a}	23.20^{a}	0.44
Unsaturates	73.83^{ab}	75.60^{abc}	73.05^{a}	76.30^{bc}	76.60^{bc}	76.77^{c}	0.44
PUFA[3]	36.03^{a}	36.53^{a}	36.60^{a}	38.60^{ab}	38.97^{ab}	40.33^{b}	0.49

[1] Values are presented as mean. Values in the same row having different superscript letters are significantly different ($P<0.05$).

[2] Standard error of the treatment mean calculated from the residual mean square in the analysis of variance.

[3] Polyunsaturated fatty acid ($C \geq 20$).

4. Discussion

Growth retardation which is one of inositol deficiency symptoms was reported in trout, salmon, and carp (McLaren et al., 1947; Aoe and Masuda, 1967; Halver, 1972). In the present study, the fish fed myoinositol free diets showed a poor appetite during the feeding period. The findings in this study indicate that juvenile olive flounder might be independent on dietary myoinositol for normal growth performance and survival.

In our previous study with early stage juvenile olive flounder at the initial body weight of 1.22 g, the requirement of dietary myoinositol was 800 mg/kg diet based on weight gain in a broken-line regression model (Lee et al., 2006). However, olive flounder with its initial body weight over 10 g did not require dietary myoinositol in the present study. Similar results were reported in tilapia species by Shiau and Su (2005) and Peres et al. (2004). Shiau and Su (2005) suggested that 404 - 408 mg of myoinositol/kg diet was sufficient to maximize tissue concentration of myoinositol and growth rate of juvenile tilapia (*Oreochromis niloticus* × *Oreochromis aureus*) at initial body weight of 0.51 g. And, Peres et al. (2004) reported that dietary myoinositol had no effects on growth of juvenile Nile tilapia (*Oreochromis niloticus*) averaging 5.8 g. Based on these previous results, myoinositol synthesis rate in fish body and its dietary requirement could probably be increased and decreased as the fish grow. And, the essentiality of the myoinositol could be dependant on fish species.

Nielsen and Black (1994) reported that growth of intestinal bacteria can prevent the accumulation of hepatic lipid which is one of the myo-inositol deficiency symptoms in rats. In the present study, *de novo* synthesis of myoinositol by intestinal micro flora was not an important source in olive flounder. Similar results were reported in channel catfish, Atlantic salmon, and abalone (Burtle and Lovell, 1989; Waagbø et al., 1998; Mai et al., 2001). All of the above studies could not demonstrate that myoinositol synthesized by intestinal micro flora can be utilized to prevent the myoinositol deficiency symptoms in their host.

However, myoinositol synthesized by enzymes such as L-myoinositol-1-phosphate synthetase and L-myoinositol-1-phosphatase was sufficient to prevent the myoinositol deficiency signs in channel catfish, and abalone (Burtle and Lovell, 1989; Mai et al., 2001). In the present study, the myoinositol synthesis in tissue seems to sufficient to prevent the growth retardation in juvenile olive flounder.

Hemoglobin concentration of fish fed diet supplemented with different levels of myoinositol for 20 weeks was only different between M0+ and M400 diets. The reason for lower hemoglobin values in fish fed M0+ diet was due to the antibiotic. Turton et al. (2000) reported that the haematological response to chloramphenicol and thiamphenicol, administered at 2000 mg/kg for 17 days to the female BALB/c mouse, was a reduction in hemoglobin at day 1 post dosing. ALT activity is necessary to determine hepatic damage in certain animal species as a sensitive indicator (Bain, 2003). In this study, the fish fed M0, M0+, and M1600 diets had higher ALT values than fish fed M800 diet. These results indicate that fish fed a poor or excessive myoinositol could increase the ALT activity. The lipid concentrations in liver and whole body were not significantly different among all the dietary treatments (Table 4). However, lipid concentration in the liver of the fish showed a tendency of decreasing as dietary myoinositol concentration increased (Fig. 1). This result is similar to that of the study reported for tilapia (Peres et al., 2004; Shiau and Su, 2005).

In the results of total fatty acid composition in the liver, polyunsaturated fatty acids (PUFA) were higher in the fish groups fed myoinositol containing diets than in the fish fed myoinositol free diets (Table 5). Similar results were found in carp showing an increased 22:6n-3 value in liver of the fish fed myoinositol (Farkas et al., 1980). The reason for higher PUFA content in M1600 treatment might be attributed to the synthesis of phospholipids using myoinositol in the body.

Analyzed inositol content in the liver of the fish fed the experimental diets for 20 weeks was in the range from 162.5 to 244.4 mg/kg in this study. Inositol concentration in the present study was dramatically lower than tilapia.

In conclusion, the findings in this study indicates that the dietary requirement of

myoinositol for juvenile olive flounder could be decreased depending on fish size or growth stage. The biosynthesis of myoinositol in tissue seems to sufficient to prevent the growth retardation in juvenile olive flounder. However, we recommend the addition of myoinositol in juvenile olive flounder diet, because the change of environmental condition could require dietary myoinositol.

Summary

This study was conducted to examine the essentiality and requirement of inositol in diets for olive flounder *Paralichthys olivaceus* because no information is available in this species. Six casein-gelatin based semi-purified diets were formulated to contain five different levels of myoinositol (0, 0+antibiotic, 200, 400, 800, and 1600 mg/kg designated as M0, M0+, M200, M400, M800, and M1600, respectively). One experimental diet (M0+) contained the antibiotic without myo-inositol supplementation to prevent the fish from intestinal synthesis of myo-inositol by microflora. Juvenile olive flounder (initial body weight 10.0 ± 0.1 g) were randomly distributed into eighteen 35 L tanks (12 fish/tank) and fed with the experimental diets (3 replicates per diet) for 20 weeks. The fish were fed twice at a 4 - 1% of body weight per day. Weight gain, feed intake, specific growth rate, protein efficiency ratio, feed efficiency ratio, and survival of fish fed myo-inositol deficiency diet were not different compared to the fish fed dietary myoinositol. In this study, *de novo* synthesis of myoinositol by intestinal micro flora was not an important source in olive flounder. Myoinositol synthesis in tissue seems to sufficient to prevent the growth retardation in juvenile olive flounder. Liver lipid concentration exhibited a tendency of decreasing as dietary myoinositol increased, although there was no significant difference among all treatments. In addition, polyunsaturated fatty acid content in the liver of the fish fed the diets containing high levels of myoinositol was significantly increased. This study indicates that the dietary requirement of myoinositol for juvenile olive flounder could be decreased depending on fish size or growth stage. The biosynthesis of myoinositol in tissue seems to sufficient to prevent the growth retardation in juvenile olive flounder. However, we recommend the addition of myoinositol in juvenile olive flounder diet, because the change of environmental condition could require dietary myoinositol.

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