Thesis for the Degree of Master of Fisheries Science

Comparison of the dietary organic and inorganic mercury threshold levels for the mercury toxicity in olive flounder, *Paralichthys olivaceus*

by

Said Majdood Raihan

KOICA-PKNU International Graduate Program of Fisheries Science

Graduate School of Global Fisheries

Pukyong National University

February 2014

Comparison of the dietary organic and inorganic mercury threshold levels for the mercury toxicity in olive flounder, *Paralichthys olivaceus*

넙치의 수은독성에 대한 유기와 무기수은의 사료내 한계농도 비교

Advisor: Prof. Sungchul C. Bai

by

Said Majdood Raihan

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Fisheries Science

in KOICA-PKNU International Graduate Program of Fisheries Science

Graduate School of Global Fisheries

Pukyong National University

February, 2014

Comparison of the dietary organic and inorganic mercury threshold levels for the mercury toxicity in olive flounder, *Paralichthys olivaceus*

A dissertation

by

Said Majdood Raihan

Approved by :

(Chairman) Prof. Min Do Huh

(Member) Prof Hyun-Woo Kim

(Member) Prof. Sungchul C. Bai

February 21, 2014

Tables of contents

List of Figures

List of Tables

Comparison of the dietary organic and inorganic mercury threshold levels for the mercury toxicity in olive flounder, *Paralichthys olivaceus*

Said Majdood Raihan

KOICA-PKNU International Graduate Program of Fisheries Science

Graduate School of Global Fisheries

Pukyong National University

Abstract

This study was carried out to compare the dietary organic and inorganic mercury threshold levels for the mercury toxicity in juvenile olive flounder, *Paralichthys olivaceus*. Twenty-eight fish averaging 3.1 ± 0.05 g (mean ± SD) were randomly distributed into each of twenty-seven tanks. Each tank was randomly assigned to one of three replicates of nine experimental diets for eight weeks. The experimental diets were formulated to contain 0 (Control), 10 (IHg10, OHg10), 20 (IHg20, OHg20), 40 (IHg40, OHg40) and 160 (IHg160, OHg160) mg organic or inorganic Hg/kg diet. At the end of eight weeks of feeding trial, weight gain (WG) from fish fed control and 10 (IHg10, OHg10) diets were significantly higher than those from fish fed 20 (IHg20, OHg20), 40 (IHg40, OHg40) and 160 (IHg160, OHg160) ($P < 0.05$). Specific growth rate (SGR) and feed efficiency (FE) from fish fed control and 10 (IHg10, OHg10) were significantly higher than those from fish fed 40

(IHg40, OHg40) and 160 (IHg160, OHg160). Tissue mercury concentrations increased with dietary organic mercury content of diets. Mercury accumulated the most in kidney, followed by liver and gill. Broken-line regression analysis of WG indicated that the dietary Hg threshold toxicity level for juvenile olive flounder could be 13.5 mg Hg/kg diet in the form of $HgCl_2$ and 8.7 mg Hg/kg diet in the form of MeHg.

Keywords: Comparison, Organic, Inorganic, Mercury, Olive flounder

Introduction

Mercury is a metallic element that occurs naturally in the environment. There are three primary categories of mercury and its compounds: elemental mercury, which may occur in both liquid and gaseous states; inorganic mercury compounds, including mercurous chloride, mercuric chloride and mercuric sulfide; and organic mercury compounds (WHO Geneva, 2003). The primary process involved in the transformation of mercury in aqueous environments is biological conversion to organomercury compounds by a variety of microorganisms, mainly sulfur-reducing forms of anaerobic bacteria (Gilmour & Henry, 1991). The formation of methylmercury is enhanced at low pH and higher mercury concentrations in the sediment (Gilmore and Henry, 1991). Some yeast species (e.g., *Candida albicans* and *Saccharomyces cerevisiae*) are also capable of methylating mercury at lower pH and can reduce ionic mercury species to elemental mercury as well. Lakes that have been acidified by acid rain or industrial runoff favor the methylation of mercury, although such conditions also decrease the abundance of fish species, which biomagnify mercury in the food-chain. Anaerobic conditions (Regnell & Tunlid, 1991) and increasing dissolved

organic carbon levels (Gilmour and Henry, 1991) both tend to substantially increase the methylation of mercury.

The conversion of inorganic mercury to methyl mercury is important for two reasons: (1) methyl mercury is much more toxic than inorganic mercury, and (2) Organisms require considerably longer time to eliminate methyl mercury. At this point, the methyl mercury-containing bacteria may be consumed by the next higher level in the food chain, or the bacteria may release the methyl mercury to the water where it can quickly adsorb to plankton, which are also consumed by the next level in the food chain (Krabbenhoft and Rickert, 2009).

Both inorganic and organic mercury are well known to cause damage to the central nervous system (CNS) of teleost fish. The organic, methylated, mercury compound readily passes the blood brain barrier and is considered by far the most neurotoxic form (Baatrup, 1991). Methylmercury easily accumulates along the aquatic food chain and dietary exposure is a major route of uptake in fish (Harris and Bodaly, 1998). Inorganic mercury may be methylated in the gut lumen prior to absorption (Rudd et al., 1980), or absorbed directly to produce toxic effects (Handy & Penrice, 1993).

Olive flounder, *Paralichthys olivaceus*, is one of the most economically important fish species farmed in eastern Asia including Korea, Japan and China. The Korean aquaculture production of olive flounder reached 54.675 metric tons in 2009 (National Statistical Office 2010). Due to its high nutritional value and profitable economic returns, olive flounder has become an important aquaculture species in Asia. According to FAO (2008) olive flounder, *P. olivaceus*, contributed over 42% (41.171 metric tons) of the 97,663 metric tons of marine finfish production in Korea in 2007, a huge increase from the meager 20 metric tons in 1987 and 26,274 metric tons in 1997 (Lee, 2013).

In the republic of Korea, fish consumption, including consumption of olive flounder, has increased over the past few years, despite the increased awareness of the risks posed by harmful contaminants. Daily seafood consumption reached 50.6 g per person, which accounted for 3.8% of the total food ingested. The concentration of methyl mercury and total mercury in seafood ranged from 1.02 to 780 (mean: 55.6) ng/g wet weight and 4.89 to 1008 (mean: 100) ng/g wet weight, respectively (Lee 2013). Since olive flounder, a marine species, is one of the major food fish in Korea, there is the likelihood of this species being a source of exposure to mercury. Most

studies have been on organic mercury toxicity because inorganic mercury generally is converted to organic mercury along the food chain in nature. However, inorganic mercury has been detected in seafood, too. Currently, no studies have been carried out on toxicity levels of dietary mercury. Therefore, this study aims to compare the dietary organic and inorganic mercury threshold levels for the mercury toxicity in olive flounder, *Paralichthys olivaceus*, by using MeHg and $HgCl_2$ as dietary organic (OHg) and inorganic (IHg) sources of mercury, respectively.

Materials and Methods

Experimental diets

Feed formulation and proximate composition of the basal diet is shown in Table 1. Nine semi-purified experimental diets were formulated to contain 50.0% crude protein and 10.0% crude lipid. Four of the diets were formulated to contain 10 (IHg10), 20 (IHg20), 40 (IHg40) or 160 (IHg160) mg organic mercury (OHg)/kg diet in the form of methyl mercury (MeHg); four were to contain 10 (OHg10), 20 (OHg20), 40 (OHg40) or 160 (OHg160) mg inorganic (IHg)/kg diet in the form of mercuric chloride ($HgCl_2$) while one diet (control) was formulated to contain no mercury. Casein and fish meal were used as the protein sources in the diets; wheat flour, dextrin, and corn starch as the carbohydrate sources; and fish oil as the lipid source.

Dry ingredients were mixed, followed by the addition of water and oil into the mixture. Mercury was dissolved in 200 mL distilled water and mixed well with the other feed ingredients prior to pelleting. The mixture was formed into dough and dry pellets were made by passing the dough through a screw type pelleting machine (3 mm diameter) and air drying the formed

pellets for approximately 48 hours. After processing and drying, the diets were broken up and sieved into the right pellet size, packed into small bags, and all the experimental diets were stored at −20 C until they were fed to fish.

Experimental fish and feeding trial

A feeding trial was carried out at the Feeds and Foods Nutrition Research Center, Pukyong National University, Busan, Republic of Korea. Fish were transported to the experimental station and acclimated to the experimental conditions for two weeks before the feeding trial began. During this period, fish were fed a mercury-free diet to deplete the body mercury reserve. The feeding trial was conducted by using the recirculating system with twenty-seven 30-L tanks receiving filtered seawater at the rate of 0.8 L/min from the center tank. Seawater temperature was maintained at 18±2°C by heaters in the center tank during the whole experimental period. Supplemental aerations were provided to maintain dissolved oxygen levels near saturation (6.5±0.5 ppm) and the salinity of the seawater was 33±1 g/L. Twenty-eight fish of initial average weight 3.1±0.05 g (mean±SD) were randomly distributed into each of twenty-seven tanks. Each tank was then randomly

assigned to one of three replicates of nine diets. Triplicate groups of fish were fed each experimental diet to satiation (approximately 2% of wet body weight per day at the beginning and 1% of wet body weight per day at the end of the feeding trial). Fish were fed twice a day at 800 and 1800 h for eight weeks. During the feeding, the water circulation was stopped for 30 min to enhance ingestion and to reduce carryover of mercury from one tank to the other. Feeding was done slowly and carefully to ensure that no uneaten food remained in the tanks during feeding, thus leaching of Hg into water was very low and negligible. Before restarting water circulation, tanks were siphoned and water in the center tank was completely replaced in the evening. Total body weight in each tank was determined every two weeks and the amount of diet fed to the fish was adjusted accordingly.

Sample collection and analyses

At the end of the feeding trial the total number and weight of fish in each tank were counted and measured for weight gain (WG), specific growth rate (SGR), feed efficiency (FE), protein efficiency ratio (PER) and survival. Samples of gill, liver and kidney tissues were taken for determination of mercury concentration in the respective tissues; except of tanks assigned to

OHg160 containing diets, because only 2% of the fish were survived at the end of feeding trail. Gill, liver, and kidney tissues were taken from four randomly selected fish per tank. The obtained tissues were fixed in the 10% neutral buffered formalin, dehydrated in a graded ethanol series and embedded in paraffin. Tissue blocks were sectioned and stained with hematoxylin and eosin (H and E). Tissue sections were examined under an AX70 Olympus (Japan) microscope for common and/or significant lesions. Three other fish were randomly sampled from each tank and pooled into treatment groups for whole-body Hg concentration analysis. Hematocrit was determined by the micro hematocrit method (Brown 1980), and hemoglobin (Hb) was measured by the cyanmethemoglobin procedure using Drabkin's solution. Mercury contents of diet, tissue, whole-body excluding the intestine were determined spectrophotometrically following digestion of samples in nitric acid (AOAC, 2000). The concentrations of Hg in the diluted digest solutions were determined by using an Inductively Coupled Plasma Mass Spectrometer (Perkin-Elmer 3300, Waltham, MA, USA)

Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA) test using SAS Version 9.0 (SAS Institute, Cary, NC, USA). When a significant treatment effect was observed, a least significant difference (LSD) test was used to compare the means. Treatment effects were considered significant at $P < 0.05$.

Results

Growth performances

Weight gain (WG), specific growth rate (SGR), feed efficiency (FE), Protein efficiency ratio (PER) and survival of juvenile olive flounder, *P.olivaceus*, fed diets containing different levels of organic and inorganic mercury are shown in Table 2.

At the end of 8 weeks of feeding trial, (WG) from fish fed Hg0, IHg10 and OHg10 diets were significantly higher than those from fish fed IHg20, IHg40, IHg160, OHg20, OHg40 and OHg160 diets ($p \leq 0.05$). Also, WG from fish fed IHg20 diet was significantly higher than those from fish fed IHg40, IHg160, OHg40 and OHg160 diets. Furthermore, WG from fish fed IHg40 diet was significantly higher than those from fish fed OHg40 and OHg160 diets. However, there were no significant differences in WG among fish fed Hg0, IHg10 and OHg10 diets, between fish fed IHg20 and OHg20 diets, between fish fed IHg40 and IHg160 diets or between those fed IHg160 and OHg40. Specific growth rate from fish fed Hg0, IHg10 and OHg10 diets were significantly higher than those from fish fed IHg40, IHg160, and OHg40 and OHg160 diets. Also, SGR from fish fed OHg40 diet was significantly higher than those from fish fed OHg160 diet.

However, there were no significant differences in SGR among fish fed Hg0, IHg10, IHg20, OHg10 and OHg20 diets, among fish fed IHg20, IHg40 and OHg20 diets or among those fed IHg40, IHg160 and OHg40 diets. Feed efficiency from fish fed Hg0, IHg10 and OHg10 diets were significantly higher than those from fish fed IHg160, OHg40 and OHg160 diets. Also, FE from fish fed IHg160 diet was significantly higher than that from fish fed OHg160 diet. However, there were no significant differences in FE among fish fed Hg0, IHg10, IHg20, IHg40, OHg10 and OHg20 diets, among fish fed IHg20, IHg40, OHg20 and OHg40 diets or among those fed IHg160 and OHg40 diets. Protein efficiency ratio from fish fed OHg10 diet was significantly higher than those from fish fed IHg160, OHg20, OHg40 and OHg160 diets. Also, PER from fish fed IHg160 diet was significantly higher than that from fish fed OHg160 diet. However, there were no significant differences in PER among fish fed Hg0, IHg10, IHg20, IHg40 and OHg10 diets, among fish fed Hg0, IHg10, IHg20, IHg40 and OHg20 diets, among fish fed IHg20, IHg40, OHg20 and OHg40 diets or among those fed IHg160 and OHg40 diets. There were no significant differences in survival from fish fed all the experimental diets, except fish fed OHg160 diet.

Proximate Composition

Proximate composition of whole-body and muscle of juvenile olive flounder, fed the experimental diets containing nine different mercury levels for 8 weeks is summarized in table 3. There were no significant differences in whole-body moisture, crude protein, crude lipids and ash from fish fed all experimental diets, except for the whole-body crude lipid from fish fed OHg40, which was significantly lower than that from fish fed Hg0. Also there were no clear trends that could suggest the effects of the different dietary mercury levels on proximate composition of fish fed all the experimental diets.

Mercury concentration

Total mercury accumulations in liver, kidney and gill tissues of fish fed all the experimental diets for 8 weeks are shown in Table 5. Whole body Hg burden increased in a dose-dependent manner, mercury concentration in liver, kidney and gill tissues of fish fed OHg40 diets were significantly higher than those of fish fed containing less than Hg0, IHg10, IHg20, IHg40, IHg160, OHg10 and OHg20 diets ($p<0.05$), and there were no significant differences on total mercury concentrations in liver, kidney and gill from

fish fed Hg0, IHg10 and IHg20 diets ($p \geq 0.05$). Meanwhile, kidney showed the highest total mercury concentration followed by liver and gill.

Serological characteristics

Serological characteristics of juvenile olive flounder, *P.olivaceus*, fed diets containing different levels of organic and inorganic mercury are shown in Table 4. There were no significant differences in total protein, aspartate transaminase, alanine transaminase and cholesterol from fish fed all the experimental diets.

Broken line analysis

Broken-line analysis of WG indicate that the dietary organic and inorganic mercury threshold levels for the mercury could be 13.5 mg Hg/kg diet in the form of HgCl2 and 8.7 mg Hg/kg diet in the form of MeHg in juvenile olive flounder.

Table 1. Formulation and proximate analysis of the basal diet (% of DM basis).

Ingredients	% in diet
Casein[1]	27.0
Fish meal[2]	32.0
Wheat flour[3]	18.0
Dextrin[1]	4.20
Corn starch[2]	3.50
Fish oil[4]	6.10
Vitamin premix[5]	3.00
Mineral premix[6]	3.00
Cellulose[1]	3.20
Hg-premix(5000 ppm)	0.00

[1] Sigma aldrich , St. Louis MQ 63103.
[2] Su Hyup Feed Co., Uiryeong, Republic of Korea.
[3] Young Nam Flour Mills Co., Busan, Republic of Korea.
[4] E-Wha Oil Co. Ltd., Busan, Republic of Korea.
[5] contains (as mg/kg in diets) : Ascorbic acid,300: Inositol, 150; Menadione, 6;Niacin, 150; Pyridoxine.HCl, Riboflavin, 30; Thiamine mononitrate, 15; dl-a-Tocopherol acetate, 201;Retinyl acetate, 6; Biotin, 1.5;B12 ,0.06
[6] contains(as mg/kg in diets) : Nacl,437; MgSo4.7H2O,1379.8; ZnSo4.7H2O,226.4;Fe-Citrate,299;MnSo4,0.016; FeSo4, 0,378;CuSo4,0.00033; Calcium Iodate, 0.0006; MgO,0.00135;NaSeO3,0.00025.

Table 2. Growth performances of juvenile olive flounder, *Paralichthys olivaceus* fed nine experimental diets for 8 weeks[1]

Diets[2]	WG[3]	SGR[4]	FE[5]	PER[6]	SR[7]
Hg0	288.4^{a}	2.77^{a}	109.8^{a}	2.33^{ab}	81.7
IHg10	285.3^{a}	2.75^{a}	106.4^{a}	2.29^{ab}	83.3
IHg20	266.9^{b}	2.65^{ab}	102.1^{ab}	2.16^{abc}	85.0
IHg40	232.1^{cd}	2.45^{bcd}	95.8^{ab}	2.06^{abc}	78.3
IHg160	207.6^{de}	2.29^{cd}	77.4^{c}	1.65^{d}	85.0
OHg10	282.9^{a}	2.74^{a}	110.2^{a}	2.34^{a}	81.7
OHg20	243.8^{bc}	2.52^{abc}	94.4^{ab}	1.99^{bc}	86.7
OHg40	200.4^{e}	2.24^{d}	87.0^{bc}	1.87^{cd}	73.3
OHg160	7.3^{f}	0.12^{e}	4.1^{d}	0.07^{e}	13.3
Pooled SEM[8]	15.5	0.15	6.0	0.13	4.2

[1]Values are mean from triplicate groups of flounder where the values in each row with different superscripts are significantly different ($P<0.05$).

[2]Refer to the table1

[3]WG Weight Gain (%) = (final weight-initial weight)*100/initial weight.

[4] SGR (%/day) = (Ln final weight – Ln initial weight)× 100 /day

[5] FE (%) = (wet weight gain (g) ×100 /dry feed intake)

[6] PER = Wet weight gain (g) / Protein intake (g)

[7]SR (%): (total stocked fish-dead fish at the end)*100/total stocked fish

[8]Pooled SEM: pooled Standard Error of Means: SD /√n.

Table 3. Whole-body proximate composition of juvenile olive flounder, *Paralichthys olivaceus* fed nine experimental diets for 8 weeks

Diets[1]	Moisture	Crude Protein	Crude Lipid	Ash
Hg0	76.3[a]	70.5[a]	11.7[a]	12.7[a]
IHg10	77.7[a]	70.2[a]	11.1[ab]	13.3[a]
IHg20	77.5[a]	69.4[a]	11.2[ab]	14.1[a]
IHg40	78.3[a]	69.1[a]	10.7[ab]	14.1[a]
IHg160	77.7[a]	69.0[a]	9.9[ab]	14.4[a]
OHg10	77.8[a]	70.0[a]	11.0[ab]	13.4[a]
OHg20	77.5[a]	69.7[a]	9.1[ab]	14.2[a]
OHg40	77.3[a]	69.1[a]	8.8[b]	15.2[a]
OHg160	ND	ND	ND	ND
Pooled SEM[8]	0.2	0.3	0.3	0.3

[1] Refer to the table1

[2]Pooled SEM: pooled standard Error of Means: SD/√n

Table 4. Serological characteristics of juvenile olive flounder, *Paralichthys olivaceus* fed nine experimental diets for 8 weeks[1]

Diets[1]	Total Protein[2]	AST[3]	ALT[4]	Cholesterol[5]
Hg0	1.8	242.5	15	141
IHg10	2.2	297.7	11.7	160.3
IHg20	2.2	224.3	9.0	155
IHg40	2.0	268.7	12.3	150.3
IHg160	1.7	225.3	10.7	140.5
OHg10	1.9	224	11.0	167.7
OHg20	1.7	254.3	11.7	142.3
OHg40	1.7	232	11.3	152
OHg160	ND	ND	ND	ND
Pooled SEM[8]	0.1	10.4	0.7	3.3

[1]Values are mean from triplicate groups of flounder where the values in each row with different superscripts are significantly different ($P<0.05$)

[2]Refer to the table1

[3]Total protein (mg/dL)

[4]AST Aspartate transaminase (Glutamic oxaloacetic transaminase)

[5]ALT Alanine transaminase (Glutamic pyruvic transaminase)

[6]Cholesterol (mg/dL)

[7]Pooled standard error of means: $SD/\sqrt{n}$

Table 5. Tissues mercury content of juvenile olive flounder, *Paralichthys olivaceus* fed nine experimental diets for 8 weeks[1]

Diets	Gill	Kidney	Liver
Hg0	1.83^{d}	2.02^{e}	1.38^{e}
IHg10	1.63^{d}	2.30^{e}	1.52^{e}
IHg20	1.77^{d}	2.88^{e}	3.12^{de}
IHg40	2.55^{d}	5.09^{de}	7.55^{c}
IHg160	3.37^{cd}	13.79^{c}	17.08^{b}
OHg10	5.32^{bc}	8.44^{d}	6.69^{cd}
OHg20	7.58^{b}	17.74^{b}	14.53^{b}
OHg40	13.82^{a}	28.29^{a}	24.20^{a}
OHg160	ND	ND	ND
Pooled SEM[8]	0.77	1.66	1.49

[1]Values are mean from triplicate groups of flounder where the values in each row with different superscripts are significantly different ($P<0.05$)

[2] Refer to the table1

[3]Pooled SEM: pooled standard Error of Means: SD/√n

Weight Gain

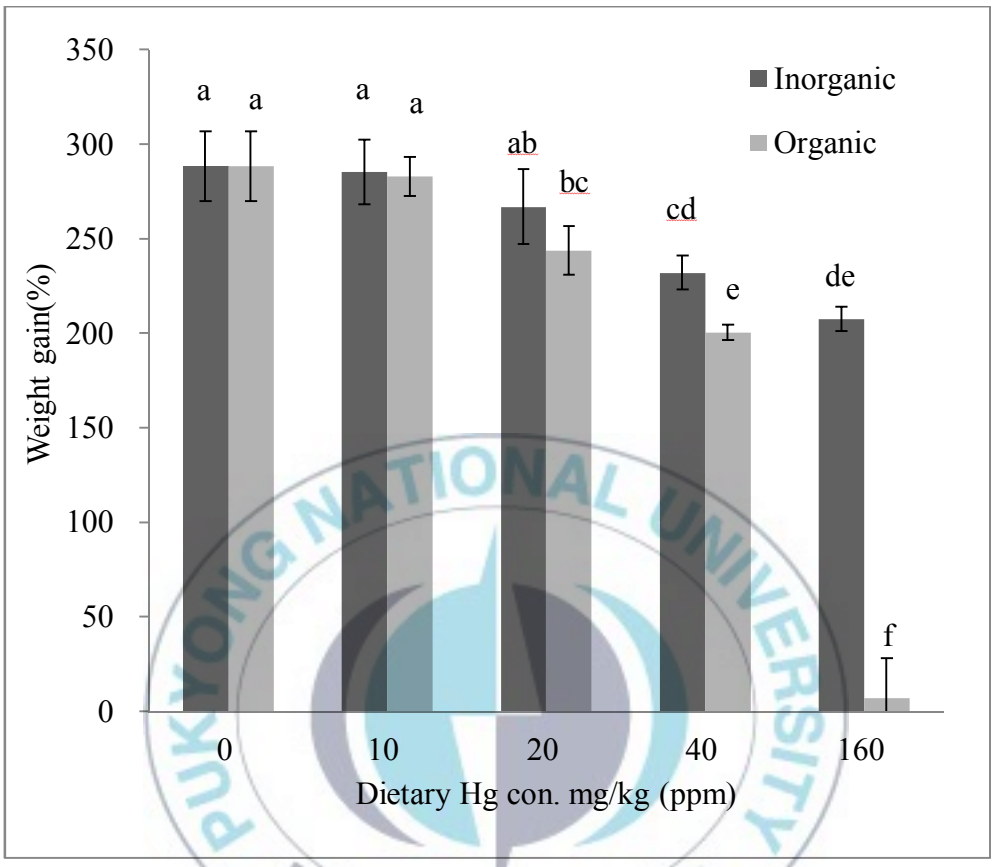

Fig.1.Average weight gain of juvenile olive flounder, *Paralichthys olivaceus*, fed diets containing different levels of dietary mercury for 8 weeks. Values are means (± SD) of triplicate groups, where bars with different letters are significantly different ($P < 0.05$).

Feed efficiency

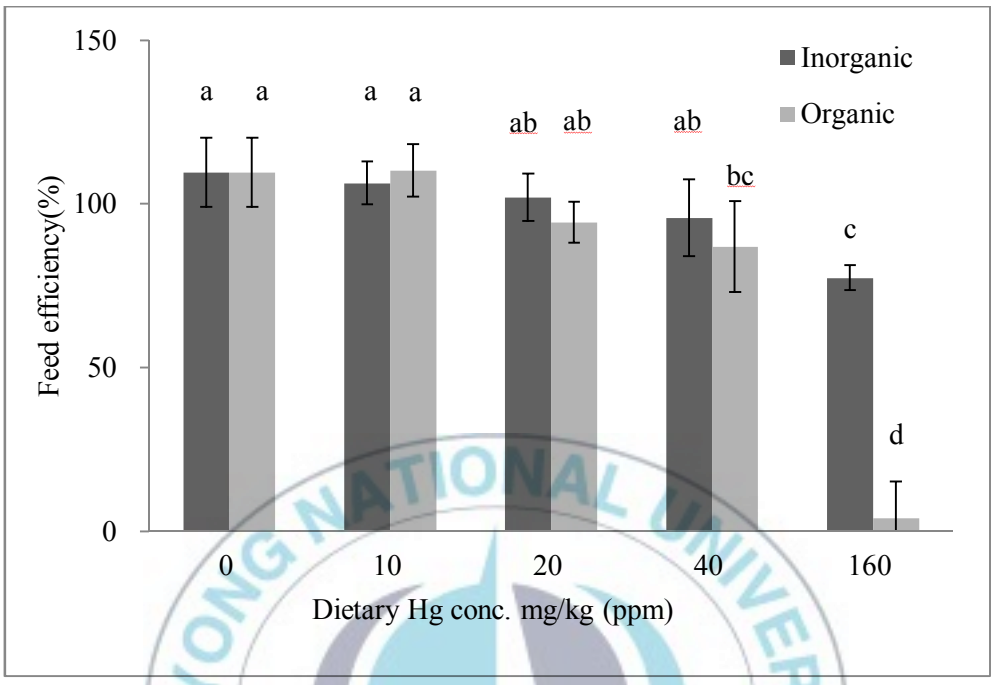

Fig.2.Average feed efficiency of juvenile olive flounder, *Paralichthys olivaceus*, fed diets containing different levels of dietary mercury for 8 weeks. Values are means (± SD) of triplicate groups, where bars with different letters are significantly different ($P < 0.05$).

Broken line analysis

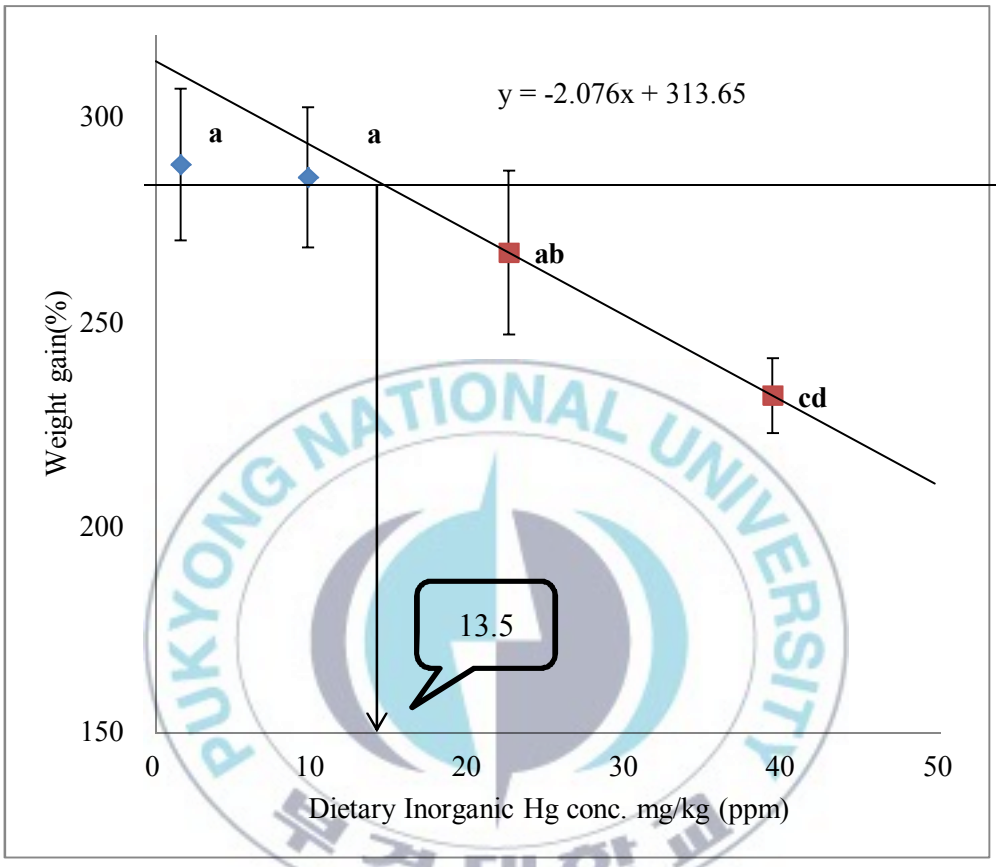

Fig.3. Broken-line analysis of weight gain (%); each point represents the mean of three replicate groups. The dietary threshold level for inorganic Hg toxicity could be 13.5 mg Hg/kg diet in the form of $HgCl_2$

Broken line analysis

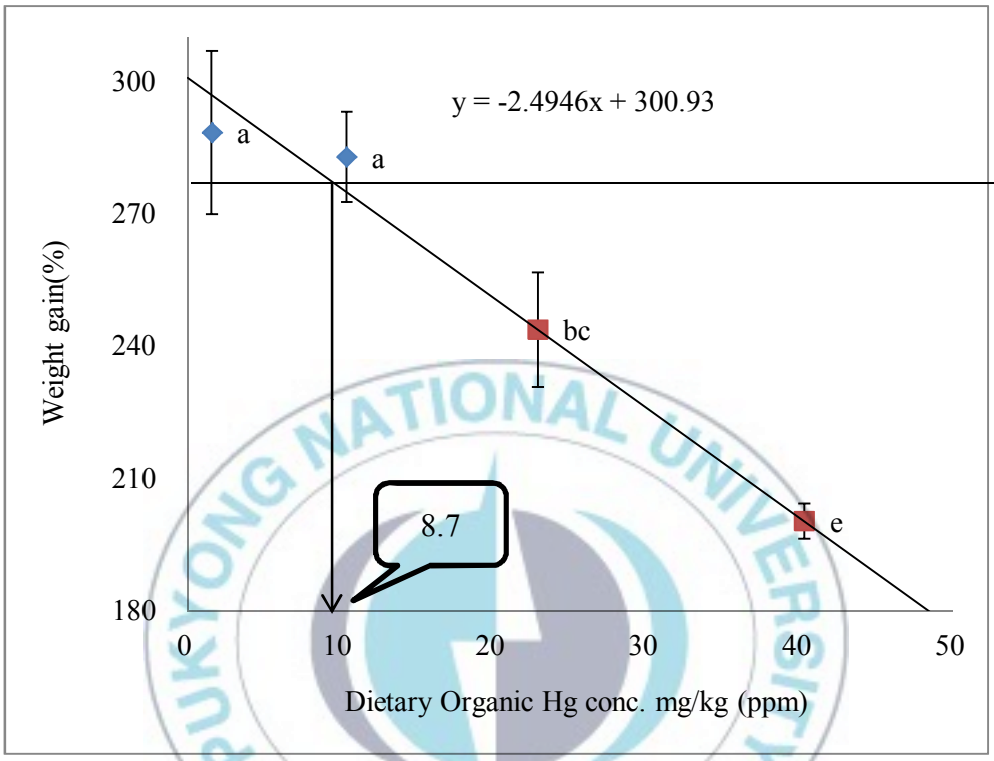

Fig.4. Broken-line analysis of weight gain (%); each point represents the mean of three replicate groups. The dietary threshold level for organic Hg toxicity could be 8.7 mg Hg/kg diet in the form of MeHg

Discussion

In the present study, organic and inorganic mercury concentrations ranging from 10(OHg, IHg) mg to 160 (OHg, IHg) mg/kg diet were tested. The diet containing 160 mg organic mercury/kg diet (OHg160) had the most toxic effect on growth performance of fish compare with those of diets containing less than Organic and inorganic mercury concentration and at the end of feeding trail almost 97% of the fish were died. In previous studies with teleosts, Rodgers and Beamish (1982) found no mortality in rainbow trout exposed to 25, 45, or 95 mg MeHg/kg diets for 12 weeks. Similarly, Houck and Cech (2004) reported no significant mortality in Sacramento blackfish fed 22.2 or 55.5 mg MeHg/kg diets over 10 weeks. Significant mortality was observed in Sacramento blackfish fed diets with 55.5 mg MeHg/kg only after 35 weeks. However, juvenile beluga sturgeon (Huso huso) have been shown to be more sensitive than green sturgeon to dietary MeHg, in beluga sturgeon 100% mortality was observed when they were fed a 16.22 mg MeHg/kg diet for 6 weeks (Gharaei et al., 2008). Generally, organic mercury proved to be more toxic than the inorganic form, with fish fed

organic mercury having significantly, or at least, numerically poorer growth than those fed inorganic mercury at the same dietary inclusion levels. Broken-line regression analysis of weight gain (WG) indicated that the dietary Hg threshold toxicity level for juvenile olive flounder could be 13.5 mg Hg/kg diet in the form of $HgCl_2$ and 8.7 mg Hg/kg diet in the form of MeHg. (Lee 2013) reported that the dietary threshold level for mercury toxicity in juvenile olive flounder, *Paralichthys olivaceus* could be 55.9 mg$HgCl_2$/kg diet based on broken line analysis of weight gain for the external growth level and less than 4.64 mg $HgCl_2$/kg diet based on broken line analysis of metallothionein expression for the molecular level.

Previous studies that include the biological consequences of dietary mercury exposures are contradictory as to the dietary threshold level that threatens fish health. Wobeser (1975) found no adverse effects on growth in rainbow trout (Oncorhynchus mykiss) fed mercury concentrations up to 16 mg/kg dry feed. In contrast, Friedmann et al. (1996) reported induction of reduced growth in juvenile walleye (*Stizostedion vitreum*) fed fish muscle contaminated with mercury at concentrations as low as 0.1 mg/kg wet weigh. However, the organism's physiology could be clearly affected by mercury toxicity. High-affinity binding of divalent mercuric ions to thiol or

sulfhydryl groups of proteins is believed to be the major mechanism of mercury toxicity. Binding to hydroxyl, carboxyl, and phosphoric groups may also contribute to toxicity (ATSDR, 1999). Sulfhydryl groups play an integral part in the structure and function of most proteins, and binding by mercury results in decreased enzyme activities, impaired structural functionality, and disruption of transport process (Zalups and Lash, 1994).

Except for the whole-body crude lipid from fish fed OHg40, there were neither significant differences nor clear trends that could suggest the effects of the different dietary mercury levels on proximate composition of fish fed all the experimental diets. Previous study indicated that, the whole body moisture content in green sturgeon was significantly higher than that in white sturgeon in each treatment group, whereas whole body crude protein, lipid, and energy con-tents in white sturgeon were significantly higher than those in green sturgeon in each treatment group (Jang and Bia, 2011), therefore, dietary MeHg had no significant effect on whole body moisture, crude protein, lipid, or energy content in either species.

Fish fed containing OHg40 mg/kg diet showed the highest total mercury concentrations in liver, kidney, and gill tissues than those fish fed diets containing less than organic and inorganic mercury concentration.

Meanwhile, the kidney showed the highest total mercury concentration followed by liver and gill. The increased mercury load in the experimental diets is resulted in a great accumulation in fish tissues. According to Van der Oost *et al*. (2003), bioaccumulation should be addressed including toxicokinetics, metabolism, and organ-specific bioaccumulation.

Similar to whole-body proximate composition, serological parameters characteristics of juvenile olive flounder, *P. olivaceus*, were not significantly affected by dietary mercury in the two forms at any of the tested levels, due to variations in the strength and duration of the serological response and lack of validated and standardized procedures, detection of fish antibodies has not yet been accepted as a routine diagnostic method for assessing mercury toxicity level in fish. These results show that whole-body proximate composition and serological characteristics may not be good early indicators of mercury toxicity in juvenile olive flounder.

In conclusion, dietary mercury affects growth of juvenile olive flounder. Growth performance proved to be more reliable than whole-body proximate composition and serological characteristics in determining the dietary threshold levels for mercury toxicity in juvenile olive flounder. Based on broken-line analysis of WG, the dietary threshold levels for mercury toxicity in juvenile olive flounder could be 13.5mg Hg/kg diet in the form of $HgCl_2$ and 8.7mg Hg/kg diet in the form of MeHg.

References

ATSDR (Agency for toxic substances and Disease Registry). 1999. Toxic Substances portal-mercury

Baatrup, E. 1991. Structural and functional effects of heavy metals on the nervous system, including sense organs, offish. Comp. Biochemist. Physiol. C 100, 253_/257

Brad Gentner, James Kirkley, Paul R. Hindsley and Scott Steinback.,2010, Summer Flounder Allocation Analysis, NOAA Technical Memorandum NMFS-F/SPO-111.

Friedmann, A., M. Watzin, T. Brinckjohnsen and J. Leiter. 1996. Low levels of dietary methylmercury inhibit growth and gonadal development in juvenile walley (Stozpstedopm votreum). Aquat. Toxicol. 35, 265-728

Gary, J. J., Peter D., R. B. Johns, J. David Smith. 1987. The effect of mercury and cadmium on the fatty acid and sterol composition of the marine diatom *Asterionella glacialis*. Phytochemical. 26 (5), 1343-1348

Gilmour CC, Henry EA (1991) Mercury methylation in aquatic systems

affected by acid deposition. Environmental Pollution, 71(2–4):131–169.

Gharaei, A., Esmaili-Sari, A., Jafari-shamoshaki, V., Ghaffari, M., 2008. Beluga (Huso huso Brandet 1869) bioenergetics under dietary methylmercury. Fish Physiology and Biochemistry 34, 473–482.

Handy, R., Penrice, W., 1993. The influence of high oral doses of mercuric-chloride on organ toxicant concentrations and histopathology in rainbow-trout, Oncorhynchus-mykiss . Comp. Biochem. Physiol. 106 C, 717_/724.

Harris,R.C., Bodaly, R.A., 1998. Temperature, growth & dietary effects on fish mercury dynamics in two Ontario lakes. Biogeochemistry 40, 175_/187.

Houck, A., Cech, J.J., 2004. Effects of dietary methylmercury on juvenile Sacramento blackfish bioenergetics. Aquatic Toxicology 69, 107–123.

Krabbenhoft, D.P., and Rickert, D.A., 2009, Mercury contamination of aquatic ecosystems: U.S. Geological Survey Fact Sheet FS-216-95.

Lee, J.H. 2013. Detoxification effects of dietary antioxidants levels on mercury toxicity in juvenile olive flounder, *Paralichthys olivaceus*. PhD. thesis. Pukyong National University.Busan Republic of Korea.

Regnell O, Tunlid A (1991) Laboratory study of chemical speciation of mercury in lake sediment and water under aerobic and anaerobic conditions. Applied Environmental Microbiology.

Risher, J.F., 2003. Elemental mercury and inorganic mercury compounds: Human health aspects. Agency for Toxic Substances and Disease

Rodgers, D.W., Beamish, F.W.H., 1982. Dynamics of dietary methyl mercury in rain-bow trout, Salmo gairdneri. Aquatic Toxicology 2, 271–290.

.Rudd, J.W., Furutani, A., Turner, M.A., 1980. Mercury methylation by fish intestinal contents. Appl. Environ. Microbiol. 40, 777_ /782.

Van der Oost, R., j. Beyer and N.P.E. Vermeulen (2003): Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental.Toxicol. Pharmacol*., 13, 57-149

Wobeser, G. 1975. Prolonged oral administration of methyl mercury chloride to rainbow trout (Salmo gairdneri) fingerlings. J. Fish. Res. Board Can. 32, 20152032.

Zalups, R. K., L. H. Lash. 1994. Advances in understanding the renal transport and toxicity of mercury. J. Toxicol. & Environmental. Health 42, 1-44.

Acknowledgement

First and foremost my sincere thanks will go to Korea International Cooperation Agency (KOICA) for providing me this instrumental master's scholarship with a comfortable life in Korea by coming truth the dream of study abroad and pukyong national university (PKNU).

My greatest gratitude will be extended to my dearest and respectable supervisor; Professor Sungchul C. Bai, who gave me advices and proper supervision to complete this master's degree program very successfully. It would not have been extended, if I didn't have his outstanding guidance, patient, behaviour's and compassion.

My deepest appreciation will go to the members of thesis evaluation committee, professor. HUH Min Do, and professor. Hyun-Woo KIM for the guidance, advices and support.

Furthermore, I would like to acknowledge the untiring provision and leadership of professor. LEE Sang-GO chairperson and Dr. Kyoungmi Kang, coordinator of KOICA-PKNU internatiol graduate program of fisheries

science (GFS) who enabled me a memorable, enjoyable and fruitful life in Korea.

This work would not have been possible without the support of my lab mates; Hyeon-Ho Yun, Kumar katya, Mizan Rahman, Fernando Magalgaes Ferreira, Sera Choi, Muneer, Seung-Han Lee, young-chul Kim, Gwang-Yeol Yu, Jun- Young Bae, Nam-yong Hwang, Jin-hyeok Lee, Young-Kwang Lee, Young Jin Park , Yu-ri Jo, Kim Do Young, especially Gunhyun Park who coached me and fully joined during my research project as well as Dr Okorie Eme Okorie and Mr. Erfan shaker assisted me to write my thesis.

My deepest gratitude to my beloved mother, wife, and kids for their unflagging love, inspiration and moral supported during my stud, without them I would not be reached to this precious victory.