Thesis for Degree of Master of fisheries science

Effects of the fermented plant-based protein concentrate as a fish meal replacer in juvenile Olive flounder, *Paralichthys olivaceus*

by

Minji Seong

Department of Fisheries Biology

The Graduate School

Pukyong National University

February 24, 2017

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치어기 넙치에 있어 어분대체재로써 식물성 발효 농축단백질의 효과

Advisors : Sungchul C. Bai

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Effects of the fermented plant-based protein concentrate as a fish meal replacer in juvenile Olive flounder, *Paralichthys olivaceus*

A dissertation

By

Minji Seong

Approved as to style and content by:

(Chairman) Jong-Myoung Kim

(Member) Seung Pyo Gong

(Member) Sungchul C. Bai

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성 민 지

부경대학교 대학원 수산생물학과

요약

본 논문은 양어용 배합사료에서 중요한 비중을 차지하고 있는 어분의 사용량을 줄이기 위한 어분대체에 관한 논문이다. 대두박과 산가수분해 한 콘글루텐밀을 (1:1 혼합) *Bacillus subtilis* (37℃, 95% humidity)을 이용하여 발효과정을 거친 식물성 발효 농축단백질 (Fermented plant-based protein concentrate, FPPC)을 만들었다. 실험 1 에 있어서 치어기 넙치의 식물성 발효 농축단백질 (FPPC) 소화율을 알아보기 위해 실험을 실시하였으며, 어분, 식물성 발효 농축단백질(FPPC), 대두박, 콘글루텐밀을 비교하였다. 외관상 소화율을 비교한 결과 식물성 발효 농축단백질(FPPC)은 어분과 유의적인 차이가 나타나지 않았다 ($P>0.05$). 실험 2 는 치어기 넙치에 있어서 식물성 발효 농축단백질(FPPC)의 어분 대체의 적정 수준을 평가하기 위해 사육실험을 실시하였다. 실험사료는 어분과, 어분의 7.5%, 15%, 22.5%, 30%, 40%를 식물성 발효 농축단백질로 대체하여 $FPPC_0$(대조구), $FPPC_{7.5}$, $FPPC_{15}$, $FPPC_{22.5}$, $FPPC_{30}$, $FPPC_{40}$ 로 제작하였으며 각 메티오닌과 라이신을 첨가하여 대조구와 같은 수준으로 맞추었다. 실험 종료 후 증체율과 일간성장률, 사료효율에 있어서 $FPPC_0$(대조구), $FPPC_{7.5}$, $FPPC_{15}$, $FPPC_{22.5}$, $FPPC_{30}$은 $FPPC_{40}$보다 유의적으로 높았다 ($P<0.05$). 따라서 위 실험결과를 통해 치어기 넙치 사료에 있어서 식물성 발효 농축 단백질 (FPPC)은 단백질 소화율에 있어 어분과 차이가 없으며, 라이신과 메티오닌을 첨가하였을 때 식물성 발효 농축단백질(FPPC)을 30%까지 어분 대체가 가능하다고 볼 수 있다.

Effects of the fermented plant-based protein concentrate as a fish meal replacer in juvenile Olive flounder, *Paralichthys olivaceus*

Minji Seong

Department of Fisheries Biology Graduate School Pukyong National University

Abstract

Two feeding trials were conducted to evaluate the optimum dietary fermented plan-based protein concentrate (FPPC) and apparent digestibility coefficient (ADC) of crude protein in juvenile olive flounder, *Paralichthys olivaceus*. FPPC consists of soybean meal and corn gluten meal pre-treated by acid hydrolysis (mixing ratio 1:1). These plant sources were fermented by *Bacillus subtilis* at 37℃ and 95% humidity. In the experiment 1, apparent digestibility of ingredients (ADIs) for crude protein in fish fed FPPC was no significantly different from fish fed fish meal (FM) diets (P>0.05). In the experiment 2, based on weight gain, specific growth rata and feed efficiency, FPPC could replace up to 30% of fish meal with lysine and methionine supplementation in juvenile olive flounder diet. In conclusion, FPPC could be considered as a feed ingredient that has the potential to replace FM in the diet of juvenile olive flounder without growth deterioration and digestibility decline.

Experiment 1 : Apparent digestibility of the fermented plant-based protein concentrate (FPPC) in diet of juvenile Olive flounder, *Paralichthys olivaceus*

A 6-weeks feeding trial was conducted to evaluate the apparent digestibility coefficient (ADC) of crude protein in fish meal (FM), fermented plant-based protein concentrate (FPPC), soybean meal (SBM) and corn gluten meal (CGM) for juvenile olive flounder *Paralichthys olivaceus*. In this study, chromic oxide (Cr_2O_3) marker was used and each of four experimental diets consisted of 70% of reference diet and 30% test ingredient. Twelve fish with an average initial weight of 36.6 ± 0.25g (mean ± SD) were randomly distributed in each of 15 semi-circulated tanks in triplicates. Fish were fed the experimental diets at apparent satiation level twice in a day. The feces collection was carried out once a day, for 42 days, by siphoning. The results revealed that apparent digestibility of ingredients (ADIs) for crude protein (CP) were significantly higher in fish fed FM and FPPC compared to SBM and CGM diets (P<0.05). However, there were no significant differences among fish fed FM and FPPC (P>0.05). In conclusion, FPPC could be considered as a feed ingredient that has the potential to replace FM in the diet of juvenile olive flounder without digestibility decline.

Experiment 2 : Evaluation of the optimum dietary fermented plant-based protein concentrate (FPPC) level in juvenile Olive flounder, *Paralichthys olivaceus*

An 8-week feeding trial was conducted to investigate the effects of the fermented plant-based protein concentrate (FPPC) as fish meal (FM) replacer in the diet of juvenile olive flounder *Paralichthys olivaceus*. FPPC consists of soybean meal and corn gluten meal pre-treated by acid hydrolysis (mixing ratio 1:1). These plant sources were fermented by *Bacillus subtilis* at 37℃ and 95% humidity. Triplicate groups of 20 fish averaging 8.36 ± 0.02 g (mean ± SD) were fed one of the seven experimental diets. The amount of 0%, 7.5%, 15%, 22.5%, 30% and 40% of FM substitution with FPPC was performed as the $FPPC_0$ (control), $FPPC_{7.5}$, $FPPC_{15}$, $FPPC_{22.5}$, $FPPC_{30}$ and $FPPC_{40}$ diets, respectively. The five FPPC diets were supplemented with lysine and methionine to balance the amino acids levels with control diet ($FPPC_0$). After the feeding trial, weight gain (WG), specific growth rate (SGR) and feed efficiency (FE) of fish fed the $FPPC_0$ diet was significantly higher than $FPPC_{40}$ diets ($P<0.05$). However, WG, SGR and FE of fish fed $FPPC_0$, $FPPC_{7.5}$, $FPPC_{15}$, $FPPC_{22.5}$ and $FPPC_{30}$ were not significantly different ($P>0.05$). Whole body proximate composition, hematological parameters such as aspartate

amino transferase, alanine amino transferase, glucose and total protein in serum, non-specific immune response such as lysozyme activity and superoxide dismutase (SOD) activity showed no significant differences among all treatments ($P>0.05$). These results indicated that FPPC, as a fish meal replacer, could replace up to 30% of fish meal in juvenile olive flounder diet without growth deterioration.

Ⅰ. Introduction

Olive flounder *Paralichthys olivaceus* is important marine fish for food on economical aspect in Northeast Asia such as Korea, China and Japan (Fuji et al., 2006). Olive flounder is carnivorous and the most popular fish species in Korea. The production of olive flounder is 45,749 ton which it top fish and total finfish production is 109.232ton (KOSTAT, 2016). Feed cost accounts for 30~50% in olive flounder culture (NIFS, 2006), high cost of culture result from fish meal. Carnivorous fish require high protein and thus fish meal accounts for 50~70% of olive flounder feed in Korea (Tacon et al., 2008).

Fish meal is recognized as an important protein source because of high protein concentration, balanced amino acid composition, high quality of phospholipids, source of essential fatty acid, abundant nutrient factors (Miles and Chapman, 2006). Advantages of fish meal contribute to main source for aquafeed. However, global production of fish meal seems to be difficult to increase in the future (Boyd, 2015). On the contrary, demand of fish meal is increasing despite of limitation for fish meal supply. This situation brings about that it is inevitable to find fish meal replacer. Consequently, Studies about fish meal replacer by animal protein source such as meat and bone meal (Bharadwaj et al., 2002), poultry by-product meal (Markey et al., 2010), blood meal (Kang et al., 1999) and plant protein source such as soybean meal (McGoogan et al., 1997), corn gluten meal

(Kikuchi, 1999b), canola meal (Luo et al., 2012) and rice distillers dried grain (Bae et al., 2015) have been researched for a long time.

Soybean meal is one of the plant protein sources as a fish meal replacement due to low cost and stable supply. Several studies about soybean replacement have been also investigated (El-Saidy et al., 2002; Shiau et al., 1989). However, soybean meal face a challenge since it has anti nutrient factors such as trypsin inhibitor, beta conglycinin, glycinin and high non-digestible carbohydrates. Various processes have been developed to remove anti nutrient factors and enhance feed utilization. Physical, chemical and biochemical treatment such as hydrolysis, fermentation, heat-treated reduce anti nutrient factors in soybean meal (Heikkinen et al., 2006; Storebakken et al., 2000).

Another alternative source of fish meal is corn gluten meal. Corn gluten meal is a by-product as the wet milling from corn (Mente et al., 2003). It has more than 60% protein contents, low fiber and no anti nutrient factors but imbalance of amino acid composition like the lack of arginine, lysine and methionine (Pereira et al., 2003). Traditionally, it has been source of livestock feed and pet food ingredient (Robinson et al., 2001). However, Kikuchi et al., (1999b) ; Lewis et al., (2008); Regost et al., (1999); Wu et al., (1995) presented the availability of feed ingredient in fish as a fish meal replacer.

The blend of the different protein source as a fish meal replacer is common to enhance feed utilization or growth performance. Yamamoto et al., (1995)

showed that the combined use of soybean meal and corn gluten meal improved growth performance as well as protein utilization compare with the use of soybean meal alone.

The main source of the fermented plant-based protein concentrate (FPPC) is soybean meal and corn gluten meal. Corn gluten meal was treated acid hydrolysis for increase of solubility. Two hundred gram of two plant protein sources (1:1 w/w ratio) were mixed with distilled water (200mL). The mixture was heated to 100℃ for 30 minutes in an autoclave and cooled to room temperature. It was inoculated with 20mL of *B. subtilis* U304, isolated from the Korean traditional fermented soybean, and incubated for 24 h at 37℃ and 95% humidity in a constant temperature humidity chamber. After solid-fermentation, it was air dried at 60℃ for 8 h and stored at 4℃.

The term “Apparent digestibility coefficient (ADC)” is used to explain the fact that values obtained using either the direct or indirect method are not corrected for endogenous gut losses (NRC, 2011). Feces don’t mean “true” digestibility due to feces composition. Feces are composed of the undigested food and residues such as mucosal cells, digestive enzymes, mucoproteins. To determine ADC, nontoxic, inert, indigestible indicator such as chromium oxide or yttrium oxide is added to feed and then feces are collected. The apparent ADC of nutrients in feeds is calculated using the concentration of the indicator in feed and feces using the equation (Bureau et al., 2006).

The nutritional values of feed ingredients depend on their digestibility and bioavailability to the fish species being fed (Chi et al., 2016). The digestibility coefficient values of diets and ingredients are highly important to the growth by delivering appropriate amount of nutrients.

Therefore, this study was conducted to investigate effects of the fermented plant-based protein concentrate (FPPC) as a fish meal (FM) replacer and to compare ADC of FM and FPPC in juvenile olive flounder *Paralichthys olivaceus*.

Ⅱ. Apparent digestibility of the fermented plant-based protein concentrate (FPPC) in diet of juvenile Olive flounder, *Paralichthys olivaceus*

Abstract

A 6-weeks feeding trial was conducted to evaluate the apparent digestibility coefficient (ADC) of crude protein in fish meal (FM), fermented plant-based protein concentrate (FPPC), soybean meal (SBM) and corn gluten meal (CGM) for juvenile olive flounder *Paralichthys olivaceus*. In this study, chromic oxide (Cr_2O_3) marker was used and each of four experimental diets consisted of 70% of reference diet and 30% test ingredient. Twelve fish with an average initial weight of 36.6 ± 0.25g (mean ± SD) were randomly distributed in each of 15 semi-circulated tanks in triplicates. Fish were fed the experimental diets at apparent satiation level twice in a day. The feces collection was carried out once a day, for 42 days, by siphoning. The results revealed that apparent digestibility of ingredients (ADIs) for crude protein (CP) were significantly higher in fish fed FM and FPPC compared to SBM and CGM diets (P<0.05). However, there were no significant differences among fish fed FM and FPPC (P>0.05). In conclusion, FPPC could be considered as a

feed ingredient that has the potential to replace FM in the diet of juvenile olive flounder without digestibility decline.

Materials and Methods

1. Experimental diets

To determine apparent digestibility coefficient (ADC) of protein as feed ingredients, five experimental diets were formulated. One experimental diet is basal diet as reference die. Four experimental diets were designed to compare apparent protein digestibility of fish meal (FM), fermented plant-based protein concentrate (FPPC), soybean meal (SBM) and corn gluten meal (CGM). Table 1 shows proximate composition of four feed ingredients Feed formulation and proximate composition of the basal diet are shown Table 2. Fish meal was used for protein source and fish oil was used for lipid source. 0.5% chromic oxide (Cr2O3) was added by indigestible digestion indicator. 70% reference diet was combined with 30% each feed ingredients, therefore, feed ingredient and proximate composition of experimental diets are presented Table 3.

2. Experimental fish and feeding trial

Juvenile olive flounder were obtained from a private hatchery (Tongyeong-si, Gyeongsangnam-do, South Korea). The feeding trial was conducted at Pukyong National University, Busan, South Korea. Before starting of feeding trial, experimental fish were fed the FM diet for 2 weeks to become acclimatized to the experimental conditions. 12 fish with an initial weight averaging 36.57±0.25g (mean ± SD) were randomly allocated into individual aquarium in triplicate of

five dietary treatments. Fish were fed two times a day (09:00 and 18:00 hours) for 6 weeks until satiation by hand-fed. The feeding trial was conducted by using the indoor semi-recirculating system with eighteen 50L tank receiving filtered seawater at the rate of 0.8~1.0 L/ min. Supplemental aeration was provided to maintain the dissolved oxygen near saturation, and the water temperature (22.0 ± 1.2°C) and pH (7.5 ± 0.3) were maintained during the experiment. The photoperiod of 12h light : 12h dark was used throughout the experimental period.

3. Sample collection and analysis

The feces collection was carried out once a day (16:00) by siphoning. To prevent interruption from uneaten feed, feed residue was removed before feces collection. Crude protein content in feeds and feces were determined by the (AOAC, 1995). In brief, crude protein content was analysed by Kjeldahl method (N × 6.25) after acid digestion.

4. Apparent digestibility determination

The apparent digestibility of protein of was determined by the chromic oxide (Cr_2O_3) method (Hanley, 1987). Cr_2O_3 concentrations were determined by flame atomic absorption spectrophotometers following combustion of the sample in a muffle furnace, before and after digestion in nitric acid (AOAC, 1995). Apparent

digestibility values were calculated as previously described by (Bureau et al., 2006).

The apparent protein digestibility in fish was calculated by:

ADC of protein (%) =(1-(Cr in diet*Protein in feces)/(Cr in feces*Protein in diet))*100

The apparent ingredient digestibility in fish was calculated by:

ADC Ingredient (%) = ADC Feed+[(ADC Feed-ADC reference diet)*(0.7*Protein in Ref/0.3*Protein in Ingredient)]

5. Statistical analysis

All data were analyzed by one-way ANOVA using SAS version 9.4 (SAS Institute, Cary, NC, USA). Duncan's multiple range test (Duncan, 1955) was used to compare significant differences among the treatment diets.

Results

Apparent digestibility of ingredients (ADI) for crude protein (CP) of juvenile olive flounder fed experimental diets is shown in Table 4. The results show that fish fed FM diet and FPPC was significantly higher than those of fish fed SBM, CGM diets ($P<0.05$). However, there was no significant difference in ADI of fish fed FM and FPPC diets ($P>0.05$).

Discussion

The results show that fish fed FM diet was significantly higher than those of fish fed SBM, CGM diets ($P<0.05$). However, there was no significant difference in ADI of fish fed FM and FPPC diets ($P>0.05$). ADI of fish fed SBM and CGM were significantly lower than those of other experimental diets ($P<0.05$).

Protein digestibility is the most important factor rather than lipid and carbohydrate because aquatic animal use protein as energy source. Olive flounder can rarely utilize dietary carbohydrate or lipid. (Kikuchi, 1999a) concluded that protein is the most critical ingredient in olive flounder. Plant proteins have availability problems in the way that they prevent the activation of digestive enzymes (NRC, 2011).

Proper processing techniques enhance nutrient availability due to reduced anti nutrient factors (ANFs). In this case, further treatment by more methods such as acid hydolysis or addition of enzymes can effectively reduce the ANFs in the aquafeeds (Siddhuraju and Becker 2005). Kim et al., (2014) presented that there was no significant difference FM and acid-concentrated soybean meal in ADIs for crude protein. These result concluded that treated soybean meal enhance the digestibility despite of plant protein sources. Refstie et al., (2004) reported no significant effect of fish protein hydrolysate on digestibility of protein for Atlantic salmon, although the lowest digestibility was observed in fish-fed diet with no protein hydrolysate supplementation. Aksnes et al., (2006) found no

significant differences in digestibility of dry matter and protein for rainbow trout fed FM or fish hydrolysate–containing diets.

The studies about apparent digestibility of protein in soybean meal and corn gluten meal for several fish species have been investigated. Cobia (Zhou et al., 2004), gilthead sea bream (Nengas et al.,1995) and rockfish (Lee, 2002) showed 94%, 50%, 92% protein digestibility in corn gluten, respectively. Protein digestibility of soybean meal is 91% in cobia (Zhou et al., 2004), 94% in largemouth bass (Portz and Cyrino, 2004) and 84% in rockfish (Lee, 2002).

Kim et al., (2010) conducted ADC of different feed ingredients for growing olive flounder. ADC value of soybean meal is 78% and corn gluten meal is 79%. Although corn gluten meal digestibility is higher than soybean meal, there were no significant differences. Fish meal ingredients such as white fish meal, herring meal, anchovy meal, salmon meal, sardine meal, mackerel meal is significantly higher than two plant protein ingredients. Similar results were shown in this experiment. Corn gluten meal digestibility is higher than soybean meal whereas there was no significantly different. Fish meal is significantly higher than soybean meal and corn gluten meal. Even though FPPC has low digestibility than fish meal, FPPC is no significant difference compare to fish meal.

This experiment showed higher digestibility than (Kim et al., 2010). Feed intake, fish size, and water temperature are experimental variables that may affect digestibility. Feed processing conditions also affect ADC values for some feed

constituents (Sulivan and Reigh, 1995). ACD values always same results with different experiments even same species (McGoogan and Reigh, 1996).

The reason might be due to the use of different feces collection methodologies. Feces collection methods are dissection, stripping, decantation. Stripping is ideal method, and it could prohibit feces contact with water which may give more accurate digestibility data. As this experiment conducted siphoning methods for feces collection, overestimation may occur due to high solubility of feces leading to loss of soluble nutrients (Glencross et al. 2007). Despite the potential problems associated with leaching of nutrients from fecal material, published values for in vitro digestibility coefficients of common feed ingredients determined for shrimp do not differ greatly from those measured using fish (NRC, 2011). The tendency of protein digestibility could not different in juvenile olive flounder.

In conclusion, FPPC could be considered as a feed ingredient that has the potential to replace FM in the diet of juvenile olive flounder without digestibility decline. Thus it can be concluded that FPPC could be recommended as a fish meal replacer due to high digestibility and lower price comparing to FM.

Tables and Figures

Table 1. Nutrient contents of the feed ingredients used to test diets[1].

	Diets			
	FM	FPPC	SBM	CGM
Moisture (%)	5.86	4.90	7.46	5.35
Crude Protein (%)	67.7	67.3	48.2	67.07
Crude Lipid (%)	7.03	3.38	1.61	3.84
Crude Ash (%)	15.01	6.63	7.17	1.94

[1] Values are mean of duplicate samples.

Table 2. Feed formulation and proximate composition of the basal diets (% of DM basis)

Ingredients	%
Fish meal[1]	70.0
Wheat gluten meal	3.00
Wheat flour	17.5
Fish oil	5.00
Vitamin mix[2]	2.00
Mineral mix[3]	2.00
Chromium(III) oxide (Cr_2O_3)[4]	0.50
Proximate analysis (% of DM basis)	
Moisture	8.18
Crude protein	52.5
Crude lipid	9.72
Crude ash	11.6

[1] Feed stuffs not mentioned here are the same feed stuffs as the domestic aquaculture feed companies are using currently.

[2] Contains (as mg/kg in diets) : Ascorbic acid, 300; dl-Calcium panthothenate, 150; Choline bitatrate, 3000; Inositol, 150; Menadione, 6; Niacin, 150; Pyridoxine · HCl, 15; Riboflavin, 30; Thiamine mononitrate, 15; dl-α-tocopherol acetate, 201; Retinyl acetate, 6; Biotin, 1.5; Folic acid, 5.4; B_{12}, 0.06

[3] Contains (as mg/kg in diets) : NaCl, 437; $MgSO_4 \cdot 7H_2O$, 1,380; $NaH_2P_4 \cdot 2H_2O$, 878; $Ca(H_2PO_4) \cdot 2H_2O$, 1,367; KH_2PO_4, 2,414; $ZnSO_4 \cdot 7H_2O$, 226; Fe-Citrate, 299; Ca-lactate, 3,004; $MnSO_4$, 0.016; $FeSO_4$, 0.0378; $CuSO_4$, 0.00033; Calcium iodate, 0.0006; MgO, 0.00135; $NaSeO_3$, 0.00025.

[4] DAEJUNG, South Korea

Table 3. Feed formulation and proximate composition of experimental diets.

Ingredients[1] *(g/100g diet)*	Diets				
	Reference Diet	FM	FPPC	SBM	CGM
Fish meal	70.0	79.0	49.0	49.0	49.0
Fermented plant-based protein concentrate (FPPC)			30.0		
Soybean meal				30.0	
Corn gluten meal					30.0
Wheat gluten meal	3.0	2.1	2.1	2.1	2.1
Wheat flour	17.5	12.3	12.3	12.3	12.3
Fish oil	5.0	3.5	3.5	3.5	3.5
Vitamin premix	2.0	1.4	1.4	1.4	1.4
Mineral premix	2.0	1.4	1.4	1.4	1.4
Chromium(III) oxide (Cr_2O_3)[4]	0.5	0.35	0.35	0.35	0.35
Proximate analyses					
Moisture	8.18	8.27	9.56	9.53	8.92
Crude protein	52.5	57.7	56.2	51.8	56.7
Crude lipid	9.72	9.19	7.94	8.09	8.63
Crude ash	11.6	12.1	10.5	9.69	7.84

[1] Feed stuffs not mentioned here are the same feed stuffs as the domestic aquaculture feed companies are using currently.

[2] Contains (as mg/kg in diets) : Ascorbic acid, 300; dl-Calcium panthothenate, 150; Choline bitatrate, 3000; Inositol, 150; Menadione, 6; Niacin, 150; Pyridoxine · HCl, 15; Riboflavin, 30; Thiamine mononitrate, 15; dl-α-tocopherol acetate, 201; Retinyl acetate,

6; Biotin, 1.5; Folic acid, 5.4; B_{12}, 0.06

[3] Contains (as mg/kg in diets) : NaCl, 437; $MgSO_4 \cdot 7H_2O$, 1,380; $NaH_2P_4 \cdot 2H_2O$, 878; $Ca(H_2PO_4) \cdot 2H_2O$, 1,367; KH_2PO_4, 2,414; $ZnSO_4 \cdot 7H_2O$, 226; Fe-Citrate, 299; Ca-lactate, 3,004; $MnSO_4$, 0.016; $FeSO_4$, 0.0378; $CuSO_4$, 0.00033; Calcium iodate, 0.0006; MgO, 0.00135; $NaSeO_3$, 0.00025.

[4] DAEJUNG, South Korea

Table 4. Apparent digestibility coefficient (%) of crude protein in test feed ingredients for juvenile olive flounder [1].

	Diets			
	FM	FPPC	SBM	CGM
Crude protein (%)	95.8 [a] ± 2.76	91.5 [a] ± 2.38	82.1 [b] ± 0.85	83.7 [b] ± 3.16

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

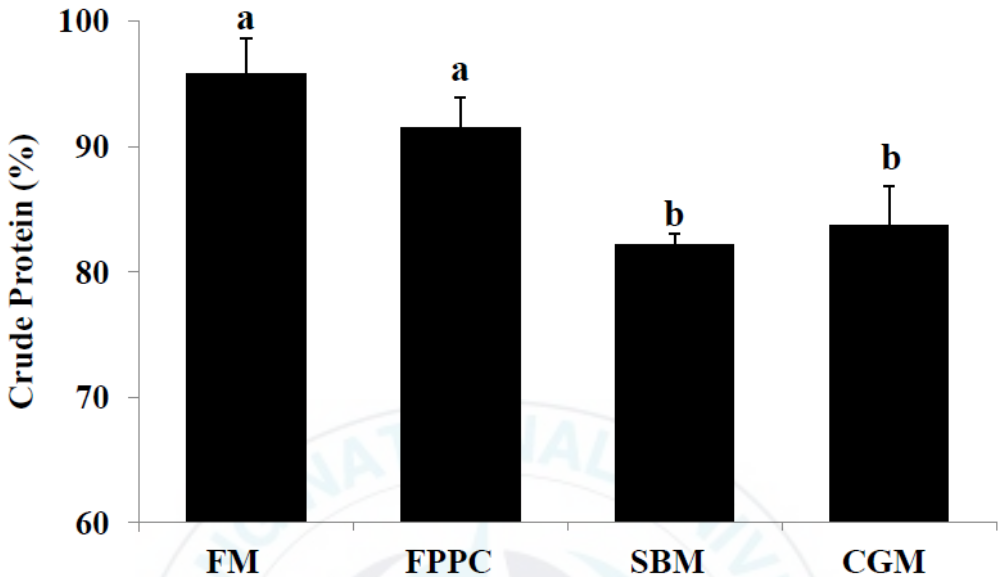

Fig 1. Apparent digestibility coefficient (%) of crude protein in test ingredients for juvenile olive flounder.

Ⅲ. Evaluation of the optimum dietary fermented plant-based protein concentrate (FPPC) level in juvenile Olive flounder, *Paralichthys olivaceus*

Abstract

An 8-week feeding trial was conducted to investigate the effects of the fermented plant-based protein concentrate (FPPC) as fish meal (FM) replacer in the diet of juvenile olive flounder *Paralichthys olivaceus*. FPPC consists of soybean meal and corn gluten meal pre-treated by acid hydrolysis (mixing ratio 1:1). These plant sources were fermented by *Bacillus subtilis* at 37℃ and 95% humidity. Triplicate groups of 20 fish averaging 8.36±0.02 g (mean ± SD) were fed one of the seven experimental diets. The amount of 0%, 7.5%, 15%, 22.5%, 30% and 40% of FM substitution with FPPC was performed as the $FPPC_0$ (control), $FPPC_{7.5}$, $FPPC_{15}$, $FPPC_{22.5}$, $FPPC_{30}$ and $FPPC_{40}$ diets, respectively. The five FPPC diets were supplemented with lysine and methionine to balance the amino acids levels with control diet ($FPPC_0$). After the feeding trial, weight gain (WG), specific growth rate (SGR) and feed efficiency (FE) of fish fed the $FPPC_0$ diet was significantly higher than $FPPC_{40}$ diets ($P<0.05$). However, WG, SGR and FE of fish fed $FPPC_0$, $FPPC_{7.5}$, $FPPC_{15}$, $FPPC_{22.5}$ and $FPPC_{30}$ were not significantly

different (P>0.05). Whole body proximate composition, hematological parameters such as aspartate amino transferase, alanine amino transferase, glucose and total protein in serum, non-specific immune response such as lysozyme activity and superoxide dismutase (SOD) activity showed no significant differences among all treatments (P>0.05). These results indicated that FPPC, as a fish meal replacer, could replace up to 30% of fish meal in juvenile olive flounder diet without growth deterioration.

Materials and Methods

1. Experimental diets

The fermented plant-based protein concentrate (FPPC) was made from soybean meal and corn gluten meal. Proximate composition and amino acid composition of FPPC are presented Table 5 and Table 6. Six diets were formulated to compare substitution levels of olive flounder *Paralichthys olivaceus* feed. The amount of 0%, 7.5%, 15%, 22.5%, 30% and 40% of FM substitution with FPPC was performed as the $FPPC_0$ (control), $FPPC_{7.5}$, $FPPC_{15}$, $FPPC_{22.5}$, $FPPC_{30}$ and $FPPC_{40}$ diets, respectively. The five FPPC diets were supplemented with lysine and methionine to balance the amino acids levels with control diet ($FPPC_0$). All experimental diets were formulated at isonitronic (54%, Crude Protein) and isolipidic (10%, Crude Lipid) diets. Feed formulation and proximate composition for experiment diets are shown in Table 7. Amino acid analysis of experimental diets is given in Table 8. Procedures for diet preparation and storage were followed as previously mentioned by (Bai and Kim, 1997). In brief, dry ingredients were mixed by electric mixer and then fish oil and tap water was added. After that, mixture was pelleted using the screw-type pelleting machine (Baokyong Commercial Co., Busan, Korea) and dried indoor for approximately 48h at room temperature. After drying, the pellets were broken up to similar size, sieved to remove powder, sealed and stored at -20ºC until use for feeding trial.

2. Experimental fish and feeding trial

Juvenile olive flounder were obtained from a private hatchery (Geoje-si, Gyeongsangnam-do, South Korea). The feeding trial was conducted at Pukyong National University, Busan, South Korea. Before starting of feeding trial, experimental fish were fed the FM diet for 2 weeks to become acclimatized to the experimental conditions. 20 fish with an initial weight averaging 8.36±0.02 g (mean ± SD) were randomly allocated into individual aquarium in triplicate of six dietary treatments. Fish were fed two times a day (09:00 and 18:00 hours) for 8 weeks at the rate of 2~3% of wet BW/day. The feeding trial was conducted by using the indoor semi-recirculating system with eighteen 50L tank receiving filtered seawater at the rate of 0.8~1.0 L/ min. Supplemental aeration was provided to maintain the dissolved oxygen near saturation, and the water temperature (18 ± 1.0°C) and pH (7.5 ± 0.3) were maintained during the experiment. The photoperiod of 12h light : 12h dark was used throughout the experimental period.

3. Sample collection and analysis

At the end of the 8 weeks experiment, the total number and weight of fish in each aquaria were counted for the calculation of growth parameters such as weight gain (WG), specific growth rate (SGR), feed efficiency (FE) and survival rate. Hepatosomatic index (HSI), visceralsomatic index (VSI) and condition

factor (CF) are also measured and calculated following these formula:

Weight gain (WG, %) = (final wt. - initial wt.) × 100 / initial wt

Specific growth rate (SGR, %/day) = ($\log_e$ final wt. - $\log_e$ initial wt.) × 100 / days

Feed Efficiency (FE, %) = (wet weight gain / dry feed intake) × 100

Survival rate (%) = (total fish - dead fish) × 100 / total fish

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

Hepatosomatic index (HSI, %) = liver wt. × 100 / body wt.

Visceralsomatic index (VSI, %) = viscera wt. x 100 / body wt.

Condition factor = (wet weight / total length^3) × 10

Three fish from each tank were selected for the analysis of whole-body proximate composition and amino acid. The analysis of the experimental diets and the fish whole bodies were performed by the standard methods of AOAC (1995). Samples of the whole-bodies were freeze-dried for 24 hours. Moisture contents were determined by dry oven at 105 ℃ and ash contents was determined by combustion at 550℃. Crude protein was analyzed by the Kjedahl method and crude lipid was analyzed by soxhlet extraction using the soxhlet system 1046 (Tacator AB, Sweden).

Blood samples were obtained by the 1.0ml disposable syringes without anticoagulant from the three fish randomly selected in each tank. The blood samples were kept until coagulation in room temperature for 30 minutes and the serums were separated from the blood by centrifugation at 5000 × g for 10 min.

Then, the serum were stored at −70°C for the analysis of non-specific immune response such as lysozyme and SOD activity and biochemical parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein (TP) and glucose. The serum levels of AST, ALT, total protein (TP) and glucose were determined by a chemical analyzer (Fuji DRI-CHEM 3500i, Fuji Photo Film Ltd. Tokyo, Japan).

Superoxide dismutase (SOD) and lysozyme activity in serums were analyzed as previously mentioned by (Lee et al., 2016). The analysis of SOD activity was conducted using the SOD Assay Kit (Sigma-Aldrich, Cat. no. 19160) in accordance with the product's protocols. SOD was measured by the superoxide radical dependent reaction inhibition rate of enzyme with WST-1 (Water Soluble Tetrazolium dye) substrate and xanthine oxidase (XO). Each endpoint assay was detected using the microplate reader (Infinite® m200 PRO, Tecan Trading AG, Switzerland) by the absorbance at 450nm after incubating at 37°C for 20 minutes. The percent of inhibition rate was calculated by mg protein as SOD unit mg^{-1}.

The lysozyme activity was recorded applying Lysozyme Detection Kit (Sigma-Aldrich, Cat. no. LY0100) according to the manufacturer's instructions. The results of lysozyme activity were defined by the lysis of the *Micrococcus lysodeikticus* cells. The results were expressed as the amount of enzyme producing a decrease in absorbance of 0.001/min. 0.1ml of serum and 2ml of a suspension of *Micrococcus lysodeikticus* (0.2 mg/ml) were in a 0.05 M sodium

phosphate buffer (pH 6.2). The reactions were conducted at 25℃ and absorbance at 530 nm was measured between 0.5 min and 4.5 min on the spectrophotometer (Infinite® m200 PRO, Tecan Trading AG, Switzerland).

4. Statistical analysis

All data were analyzed by one-way ANOVA using SAS version 9.4 (SAS Institute, Cary, NC, USA). Duncan's multiple range test (Duncan, 1955) was used to compare significant differences among the treatment diets.

Results

Growth performance and survival rate of olive flounder fed different experimental diets are presented in Table 9. Weight gain (WG), specific growth rate (SGR) and feed efficiency (FE) of fish fed $FPPC_0$, $FPPC_{7.5}$, $FPPC_{15}$, $FPPC_{22.5}$ and $FPPC_{30}$ were significantly higher than those of fish fed $FPPC_{40}$ (P<0.05). However, there were no significant differences in WG, SGR and FE among fish fed $FPPC_{40}$ (P>0.05). The survival rate of fish fed six different diets was no significant different (P>0.05). Table 5 also shows results of condition factor (CF), hepatosomatic index (HSI), viscerosomatic index (VSI) of the juvenile fish. CF of fish fed $FPPC_0$ and $FPPC_{7.5}$ was significantly higher than those of fish fed $FPPC_{15}$ and $FPPC_{40}$ (P<0.05), whereas there were no significant differences among the fish fed $FPPC_0$, $FPPC_{7.5}$, $FPPC_{22.5}$ and $FPPC_{30}$ (P>0.05). HSI of fish fed $FPPC_0$ and $FPPC_{7.5}$ was significantly lower than those of fish fed $FPPC_{40}$ (P<0.05). However, significant difference among the fish fed $FPPC_0$, $FPPC_{7.5}$, $FPPC_{15}$, $FPPC_{22.5}$ and $FPPC_{30}$ was not observed (P>0.05). There were no significant differences in VSI of fish fed all the experimental diets (P>0.05).

Whole body proximate composition is provided in Table 10. There are no significant differences among fish fed all the diets (P>0.05). Table 11 shows results of biochemical parameters such as aspartate aminotransferase (AST), alanine aminotransaminase (ALT), total protein (TP) and glucose. AST, ALT, TP and glucose of the fish fed all treatment diets were no significantly different (P>

0.05). AST, ALT, TP and glucose levels in serum were indicated not to be so much affected by the fish meal replacement levels. The results of SOD activity and lysozyme are shown Table 12. Non-specific immune response of juvenile olive flounder shows no significant differences among all the experimental diets.

Discussion

Several studies about fish meal replacement in olive flounder have been investigated (Choi et al., 2004; Kader et al., 2012; Kim et al., 2014). Lim et al., (2010); Pham et al., (2007) attempted to overcome the limitation of dietary availability in plant protein source using mixture of two different plant protein sources in olive flounder diets. The results of the current study indicated that FPPC could be replaced by 30% of fish meal in juvenile olive flounder without growth deterioration. There are several factors could affect growth performance. Essential amino acids requirements are important to make proper feed formulation and to economize fish meal effectively. Alam et al., (2000); Alam et al., (2002); Foster et al., (1998) revealed that essential amino acids requirements. Compare to these researches, arginine, methionie, lysine are sufficient to juvenile olive flounder. It is difficult to conclude that the lack of essential amino acids bring the reason why $FPPC_{40}$ showed low weight gain, feed efficiency and specific growth rate. Another reason of growth retardation is digestibility. Feed utilization and feed efficiency are relative to digestibility. Chou et al., 2004 showed the limitation of growth when 40% substituting of fish meal with soybean meal in diets of juvenile cobia. Marine carnivorous species such as juvenile seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) showed low digestibility when faced with soybean meal as a protein source in aquafeed (Lupatsch et al., 1997; Oliva-Tales, 1998). Otherwise, in this study showed no

significant differences at the 30% replacement level. It probably comes from the improved digestibility compare to non-treated plant protein source. Kim et al.,(2014) showed the similar trend that acid-concentrated soybean meal has high protein digestibility compare to soybean meal despite no significant differences.

Solide-state fermenteation (SSF) is the fermentation process which occurs without liquid or few liquid conditions using microorganism (Cannel et al., 1980). The feature of this process cause some merits compare to submerged fermentation such as low cost, high yield of product, less time consuming (Bhargav et al., 2008). SSF is used for not animal feed but traditional food in Asia. *Bacillus subtilis* is widely used for solid state fermentation by making natto which is a traditional Japanese soybean product and cheonggukjang which is made from cooked soybeans (Cho et al., 2011; Juan et al., 2010). Several studies showed that *B. subtilis* and spores of *B. subtilis* conduct as probiotics for growth improvement and enhanced viability of lactic acid in the intestine of humans and some animals (Hoa et al., 2000). Teng et al., (2012) revealed that *B.subtilis* made trypsin inhibitor contents reduced and small-size protein increased. This study also proved that large-size protein decomposed into small size and increased soluble protein brought about in vitro pepsin digestibility.

Yamamoto et al., (2010) showed the relation between digestibility and weight gain in the different type of fermented soybean meal diets with the results that high nutrient digestibility lead to enhanced weight gain. Kader and Koshio

(2012) indicated that trend of low apparent digestibility reflect low weight gain in red sea bream diet of soybean protein and seafood by-products.

VSI were not influenced by the dietary fish meal replacement treatments. In (Lim et al., 2011) study, marine carnivorous species Tiger puffer, *Takifugu rubripes* showed increased HSI level as decreasing soybean replacement levels. Mostly HSI is decreasing as elevated soybean replacement levels (Song et al., 2014; Wang et al., 2006). Although there were no significant differences among all treated diets, results of (Gomez-Requeni et al., 2004; Kaushik et al., 2004; Pham et al., 2007) showed the trend in respect of increased HSI levels as elevating mixture of plant protein contents. Also, Ye et al., (2011) indicated that HSI is increasing with increased dietary soybean levels.

In this experiment, whole body proximate compositions were not affected dietary treatment. This observation in accordance with (Kikuch, 1999; Pham et al., 2007; Ye et al., 2011; Zhou et al., 2005).

AST and ALT provide the information on fish metabolism for example detection of liver cell damages. Soltan et al., (2008) presented that increased plant protein in diet affected abnormal liver function due to identified or un-identified anti-nutritional and toxic factors in plant protein source. Nevertheless, in this study showed no significant differences in biochemical results of serum. It seems that anti nutritional facts were reduced in FPPC by bio-processing. Lim and Lee (2011) also presented that plasma chemistry of Nile tilapia (*Oreochromis*

niloticus) was not affected by replacement levels of plant protein sources with fermentation and blend treatments.

Antioxidant enzymes were indicator of animal's physical condition by reflecting external stimulus (Johnson, 2002). Several studies revealed the relation between ingredient or nutritional factors in feed and expression of antioxidant enzymes (Ferna´ndez-Dı´az et al., 2006; Lin et al., 2007; Sagstad et al., 2007; Sitja`-Bobadilla et al., 2005; Tovar-Ramı´rez et al., 2010). Non specific immune responses were not affected by experimental feed and it showed high levels of plane protein with bio-processing could not bring about malfunction of immune system.

In conclusion, this study demonstrated that FPPC could be substituted with fish meal by 30% without any adverse effects on growth performance, proximate composition and non-specific immune responses in juvenile olive flounder diet.

Tables and Figures

Table 5. Proximate composition of the fermented plant-based protein concentrate (FPPC)

Ingredients	%
Moisture (%)	4.90
Crude Protein (%)	67.3
Crude lipid (%)	3.38
Crude ash (%)	6.63

[1] Values are mean of duplicate samples.

Table 6. Amino acid contents of the plant-based fermented protein concentrate (FPPC)

Amino acids	%
Aspartic acid	4.99
Threonine	2.02
Serine	2.67
Glutamic acid	11.64
Proline	5.50
Glycine	1.94
Alanine	4.04
Valine	3.02
Isoleucine	2.82
Leucine	7.20
Tyrosine	2.07
Phenylalanine	3.49
Histidine	1.86
Lysine	2.16
Arginine	2.54
Cystine	2.12
Methionine	1.31

Table 7. Ingredient and proximate composition of the experimental diets (% of DM basis)

Ingredients[1] *(g/100g diet)*	Diets					
	$FPPC_0$	$FPPC_{7.5}$	$FPPC_{15}$	$FPPC_{22.5}$	$FPPC_{30}$	$FPPC_{40}$
Fish meal	70.0	64.75	59.5	54.25	49	42
FPPC[2]		5.45	10.92	16.38	21.84	29.11
Wheat gluten meal	3.0	3.0	3.0	3.0	3.0	3.0
Wheat flour	18.0	17.05	16.09	15.12	14.16	12.88
Fish oil	5.0	5.3	5.59	5.89	6.18	6.58
Vitamin premix[3]	2.0	2.0	2.0	2.0	2.0	2.0
Mineral premix[4]	2.0	2.0	2.0	2.0	2.0	2.0
Lysine		0.39	0.785	1.18	1.57	2.102
Methionine		0.06	0.12	0.18	0.25	0.33
Proximate analyses						
Moisture	8.65	8.33	8.67	9.11	8.74	8.99
Crude protein	52.40	52.27	52.26	52.32	52.24	52.55
Crude lipid	11.51	11.26	11.78	11.43	11.39	11.46
Crude ash	11.78	11.27	10.83	10.42	9.85	9.20

[1] Feed stuffs not mentioned here are the same feed stuffs as the domestic aquaculture feed companies are using currently.

[2] Plant-based fermented protein concentrate (FPPC).

[3] Contains (as mg/kg in diets) : Ascorbic acid, 300; dl-Calcium panthothenate, 150; Choline bitatrate, 3000; Inositol, 150; Menadione, 6; Niacin, 150; Pyridoxine·HCl, 15; Riboflavin, 30; Thiamine mononitrate, 15; dl-α-tocopherol acetate, 201; Retinyl acetate, 6; Biotin, 1.5; Folic acid, 5.4; B_{12}, 0.06

[4] Contains (as mg/kg in diets) : NaCl, 437; $MgSO_4 \cdot 7H_2O$, 1,380; $NaH_2P_4 \cdot 2H_2O$, 878; $Ca(H_2PO_4) \cdot 2H_2O$, 1,367; KH_2PO_4, 2,414; $ZnSO_4 \cdot 7H_2O$, 226; Fe-Citrate, 299; Ca-lactate, 3,004; $MnSO_4$, 0.016; $FeSO_4$, 0.0378; $CuSO_4$, 0.00033; Calcium iodate, 0.0006; MgO, 0.00135; $NaSeO_3$, 0.00025.

Table 8. Amino acid compositions of the experimental diets

Amino acids	Diets					
	$FPPC_0$	$FPPC_{7.5}$	$FPPC_{15}$	$FPPC_{22.5}$	$FPPC_{30}$	$FPPC_{40}$
Aspartic acid	4.39	4.35	4.19	4.11	4.07	4.03
Threonine	2.06	2.06	1.93	1.89	1.82	1.83
Serine	1.93	1.96	1.89	1.91	1.99	1.93
Glutamic acid	6.31	6.32	6.47	6.58	6.65	6.73
Proline	2.58	2.61	2.73	2.93	2.96	3.03
Glycine	2.83	2.70	2.51	2.37	2.23	2.19
Alanine	2.92	2.95	2.93	2.90	2.88	2.86
Valine	2.72	2.68	2.60	2.58	2.56	2.53
Isoleucine	2.33	2.34	2.36	2.30	2.26	2.26
Leucine	3.51	3.80	3.93	2.05	4.26	4.43
Tyrosine	1.38	1.34	1.36	1.30	1.37	1.32
Phenylalanine	1.94	2.06	2.06	2.10	2.20	2.33
Histidine	1.82	1.79	1.71	1.72	1.74	1.62
Lysine	4.85	4.86	4.87	4.85	4.88	4.84
Arginine	2.68	2.67	2.56	2.44	2.41	2.41
Cystine	0.82	0.90	0.99	1.11	1.07	1.20
Methionine	1.68	1.66	1.65	1.69	1.67	1.67

Table 9. Growth performance of juvenile olive flounder *Paralichthys olivaceus* fed experimental diets for 8 weeks[1].

	Diets					
	$FPPC_0$	$FPPC_{7.5}$	$FPPC_{15}$	$FPPC_{22.5}$	$FPPC_{30}$	$FPPC_{40}$
WG (%)[2]	244 [a] ± 18.1	232 [a] ± 5.63	232 [a] ± 11.4	229 [a] ± 10.5	224 [a] ± 7.58	204 [b] ± 2.44
FE (%)[3]	146 [a] ± 4.42	144 [a] ± 3.29	143 [a] ± 7.11	141 [a] ± 6.49	138 [a] ± 4.68	127 [b] ± 2.32
SGR (%/day)[4]	2.91 [a] ± 0.09	2.86 [a] ± 0.04	2.85 [a] ± 0.08	2.83 [a] ± 0.08	2.80 [a] ± 0.06	2.65 [b] ± 0.02
HSI (%)[5]	1.71 [c] ± 0.10	1.75 [bc] ± 0.09	1.77 [bc] ± 0.08	1.78 [bc] ± 0.04	1.89 [ab] ± 0.10	1.98 [a] ± 0.04
VSI (%)[6]	2.64 [ns] ± 0.11	2.67 ± 0.07	2.74 ± 0.23	2.65 ± 0.18	2.95 ± 0.14	2.95 ± 0.16
CF[7]	0.81 [a] ± 0.01	0.81 [a] ± 0.01	0.77 [b] ± 0.03	0.78 [ab] ± 0.02	0.78 [ab] ± 0.02	0.77 [b] ± 0.01
Survival (%)[8]	100 [ns] ± 0.00	96.7 ± 2.89	96.7 ± 2.89	98.3 ± 2.89	98.3 ± 2.89	96.7 ± 2.89

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

[2]Weight gain (WG, %) = (final wt. - initial wt.) × 100 / initial wt

[3]Feed efficiency ratio (FE, %) = (wet weight gain / dry feed intake) × 100

[4]Specific growth rate (SGR, %) = ($\log_e$ final wt. - $\log_e$ initial wt.) × 100 / days

[5] Hematosonatic index (HSI, %) = liver wt. × 100 / body wt.

[6]Visceralsomatic index (VSI, %) = viscera wt. x 100 / body wt.

[7]Condition factor = (wet weight / total length^3) × 100

[8]Survival rate = (total fish - dead fish) × 100 / total fish

[ns] No Significant difference

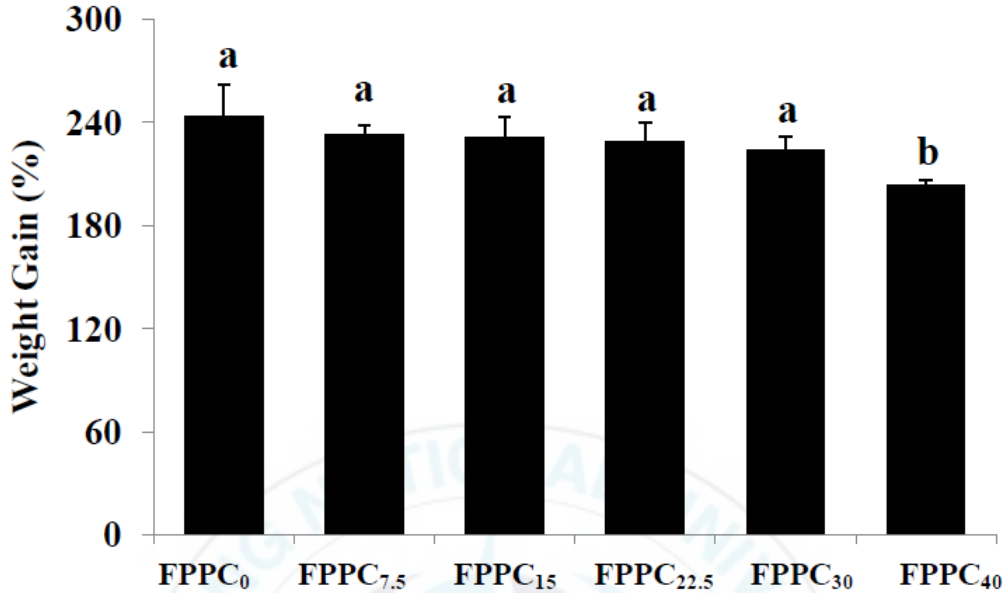

Fig 2. Weight gain of juvenile olive flounder fed the experimental diets for 8 weeks.

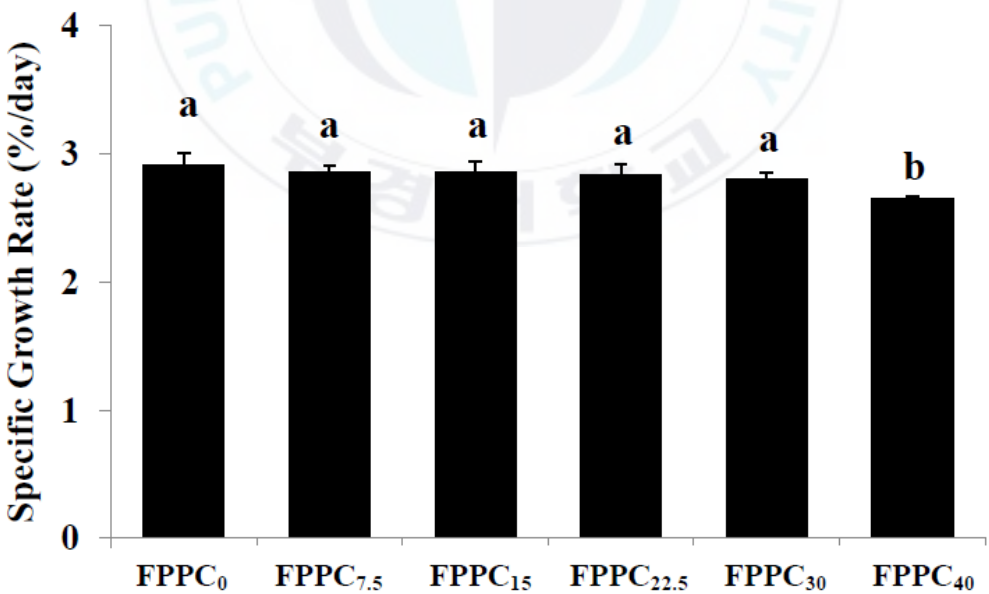

Fig 3. Specific growth rate of juvenile olive flounder fed the experimental diets for 8 weeks.

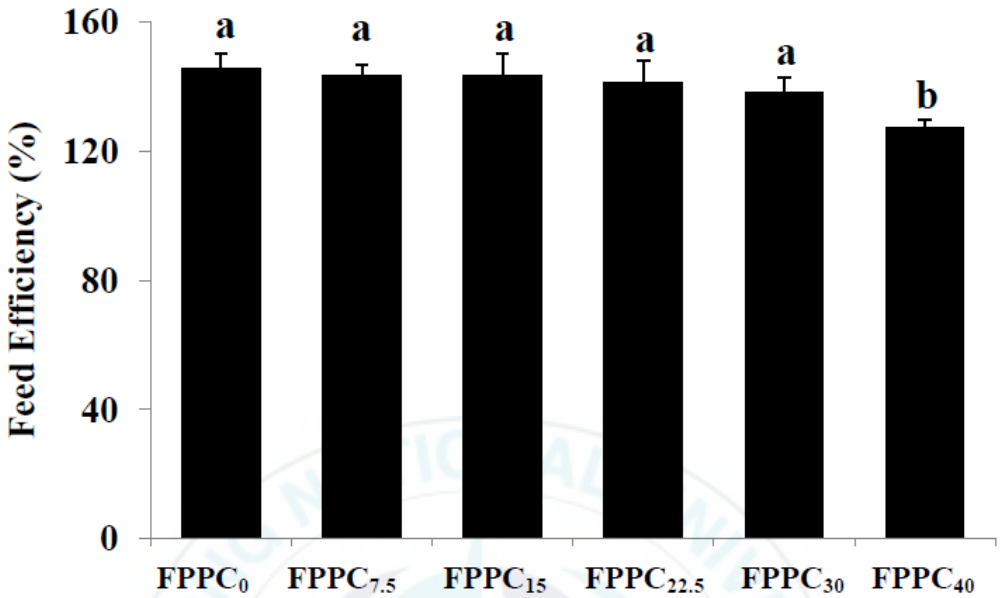

Fig 4. Feed efficiency of juvenile olive flounder fed the experimental diets for 8 weeks.

Table 10. Whole-body proximate compositions (%, DM) of juvenile olive flounder *Paralichthys olivaceus* fed the experimental diets for 8 weeks[1].

	Diets					
	$FPPC_0$	$FPPC_{7.5}$	$FPPC_{15}$	$FPPC_{22.5}$	$FPPC_{30}$	$FPPC_{40}$
Moisture(%)	76.5 [ns] ± 1.31	75.7 ± 1.58	76.9 ± 2.00	76.0 ± 1.02	76.8 ± 1.61	76.2 ± 1.34
Crude Protein(%)	17.7 [ns] ± 0.48	17.2 ± 0.28	16.9 ± 0.43	17.1 ± 1.18	17.2 ± 0.55	16.2 ± 0.35
Crude Lipid(%)	2.47 [ns] ± 0.20	2.43 ± 0.26	2.17 ± 0.31	2.18 ± 0.14	2.35 ± 0.21	2.36 ± 0.25
Crude Ash(%)	3.76 [ns] ± 0.19	3.62 ± 0.32	3.37 ± 0.25	3.61 ± 0.06	3.35 ± 0.10	3.62 ± 0.26

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

[ns] No Significant difference

Table 11. Hematological parameters of juvenile olive flounder fed the experimental diets for 8 weeks[1]

	Diets					
	$FPPC_0$	$FPPC_{7.5}$	$FPPC_{15}$	$FPPC_{22.5}$	$FPPC_{30}$	$FPPC_{40}$
AST (U/L)[2]	10.0 [ns] ± 1.73	10.7 ± 2.08	9.67 ± 3.06	11.3 ± 2.89	10.0 ± 2.65	10.7 ± 1.15
ALT (U/L)[3]	5.00 [ns] ± 2.00	5.00 ± 1.73	5.33 ± 1.53	4.67 ± 1.53	3.67 ± 1.15	5.33 ± 2.31
Glucose (mg/dl)	21.7 [ns] ± 4.04	28.7 ± 9.24	27.0 ± 5.57	30.0 ± 4.58	29.7 ± 8.39	31.3 ± 4.16
T-Protein (g/dl)[4]	2.03 [ns] ± 0.06	2.03 ± 0.38	2.13 ± 0.12	2.13 ± 0.15	2.27 ± 0.06	2.17 ± 0.21

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

[2] AST (U/L): Aspartate transaminase

[3] ALT (U/L): Alanine transferase

[4] T-Protein (g/dl): Total protein

[ns] No Significant difference

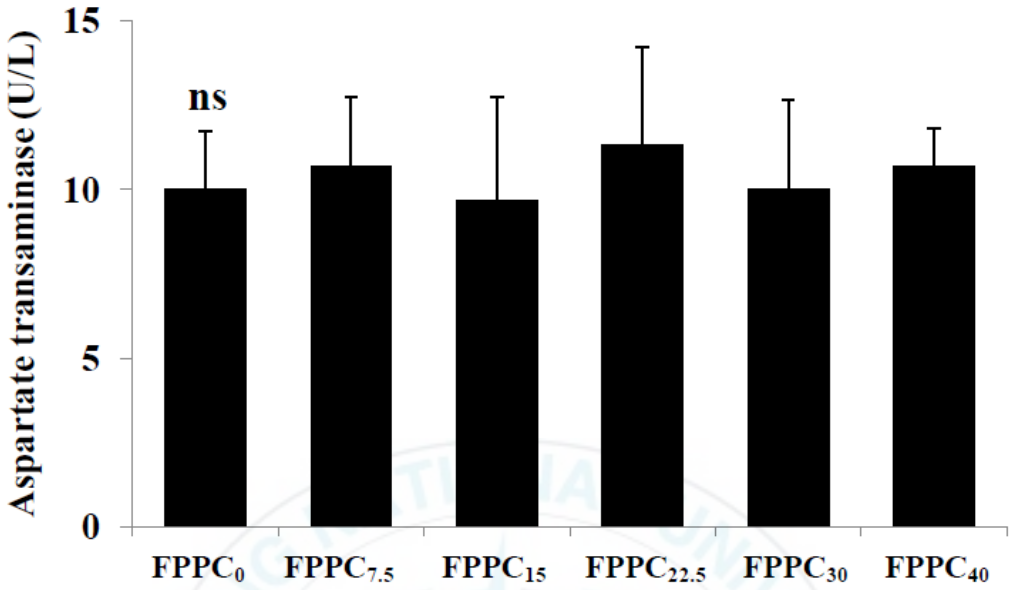

Fig 5. AST (Aspartate transaminase) of juvenile olive flounder fed the experimental diets for 8 weeks.

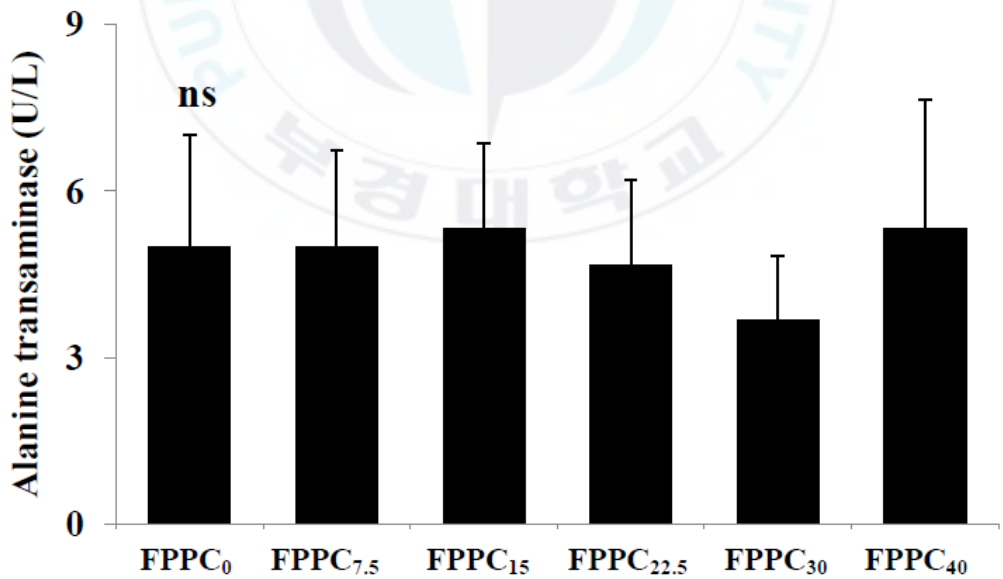

Fig 6. ALT (Alanine transferase) of juvenile olive flounder fed the experimental diets for 8 weeks.

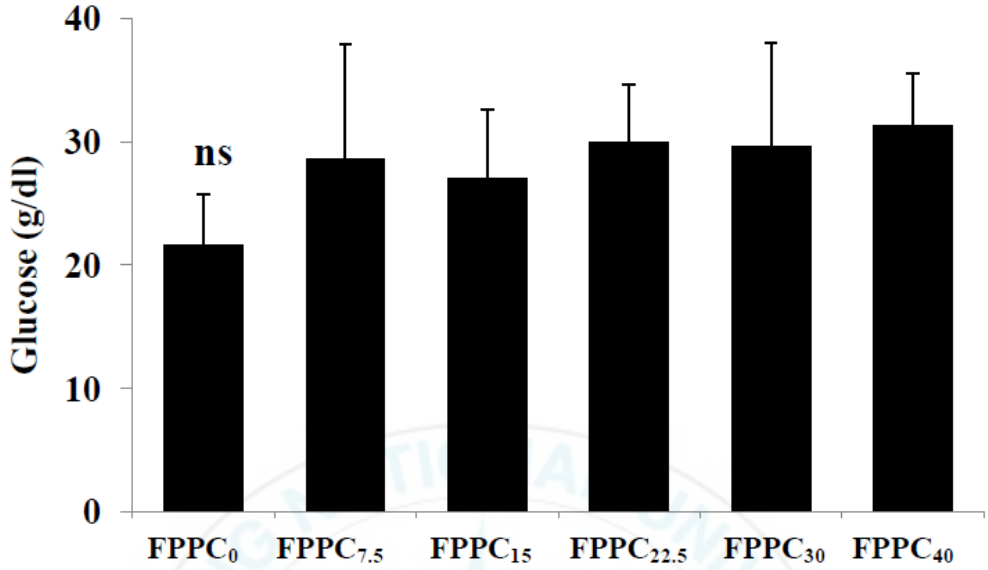

Fig 7. Glucose levels of juvenile olive flounder fed the experimental diets for 8 weeks.

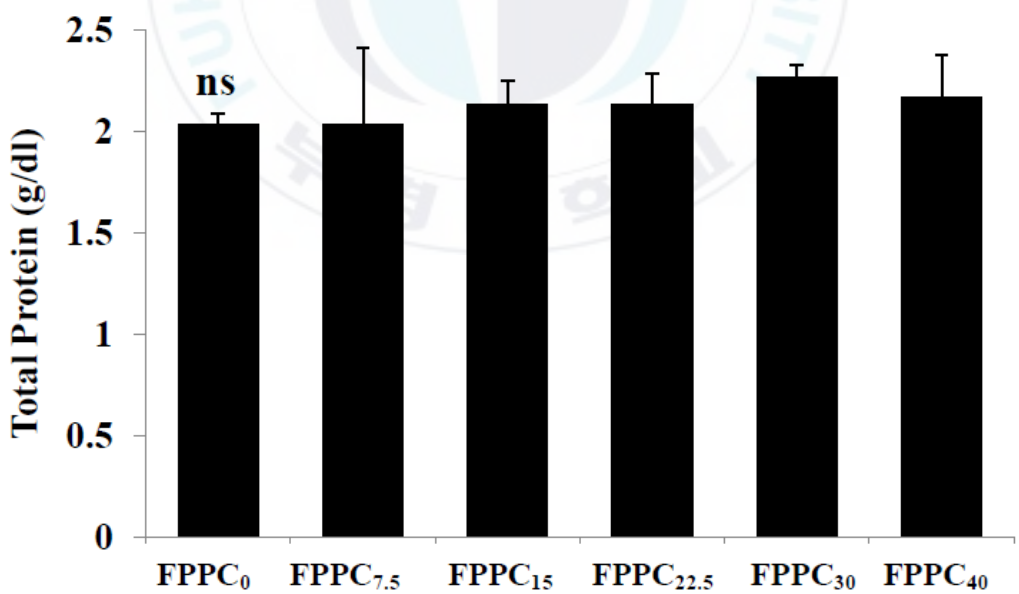

Fig 8. Total protein of juvenile olive flounder fed the experimental diets for 8 weeks.

Table 12. Non-specific immune responses of juvenile olive flounder fed experimental diets for 8weeks[1]

	Diets					
	$FPPC_0$	$FPPC_{7.5}$	$FPPC_{15}$	$FPPC_{22.5}$	$FPPC_{30}$	$FPPC_{40}$
SOD[2]	72.6 [ns] ± 4.89	75.5 ± 4.27	70.3 ± 3.07	72.8 ± 2.81	73.4 ± 2.01	73.4 ± 3.12
Lysozyme (U/ml)	2.57 [ns] ± 0.08	2.41 ± 0.21	2.40 ± 0.23	2.52 ± 0.03	2.62 ± 0.02	2.46 ± 0.04

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

[2] SOD (% inhibition): Superoxide dismutase

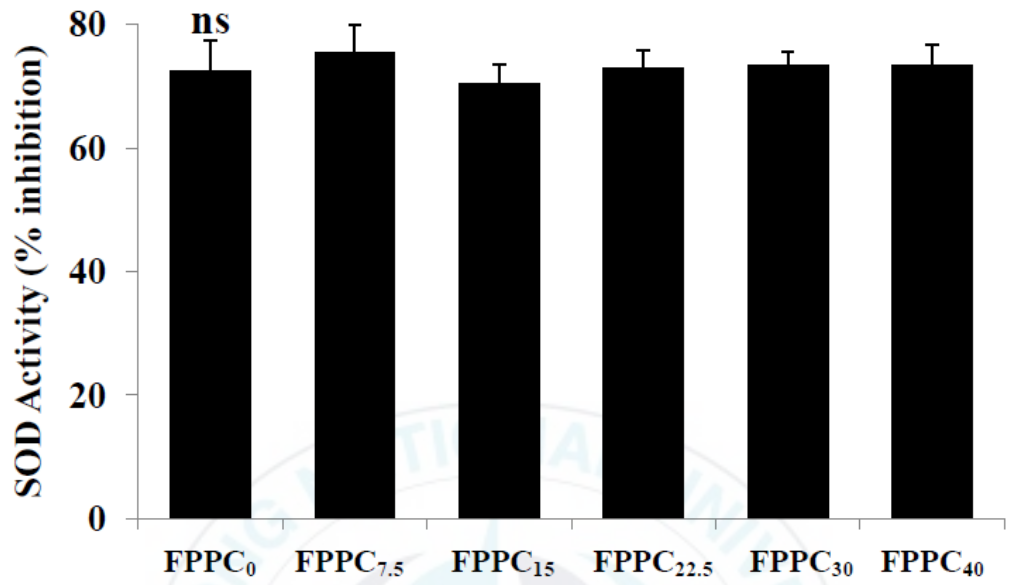

Fig 9. Superoxide dismutase activity of juvenile olive flounder fed the experimental diets for 8 weeks.

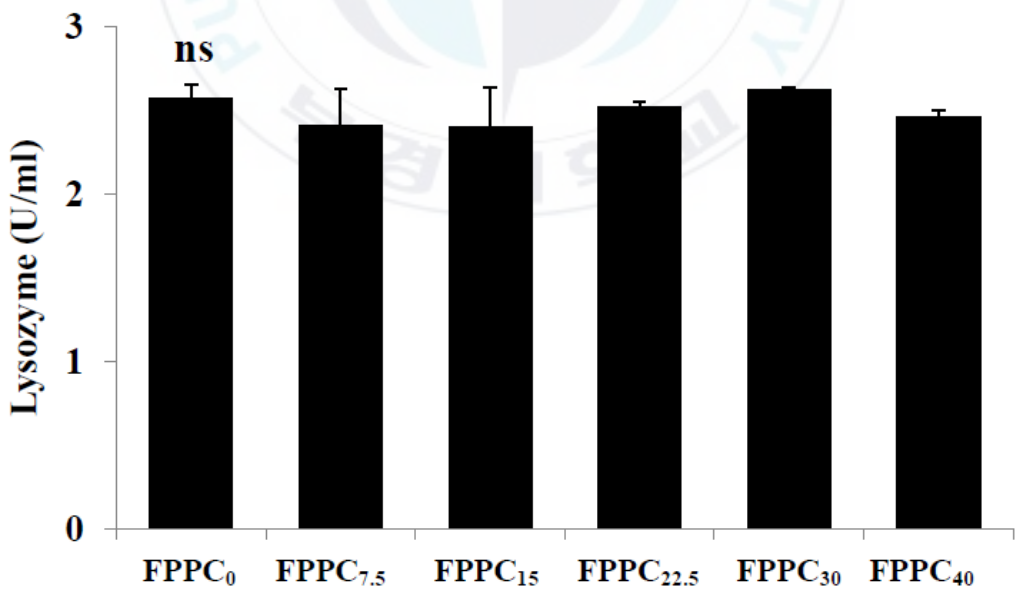

Fig 10. Lysozyme activity of juvenile olive flounder fed the experimental diets for 8 weeks.

IV. Acknowledgment

마지막 감사의 말을 영어로 쓸까 우리말로 쓸까 한참을 고민한 끝에 제 마음을 표현하기에는 부족한 영어보다는 우리말이 더 나을 것 같아 이렇게 한글로 씁니다. 감사의 말을 가장 마지막에 남겨두었는데 이제 이 페이지를 작성하면서 논문이 정말로 끝이 났다는 것을 실감하게 됩니다.

아무것도 모르고 해양바이오신소재학과에 입학 한 게 엊그제 같은데, 학부를 졸업하고 어느새 수산생물학과 석사과정을 끝낸다고 하니 시간은 빠르게 흘러간다는 어른들의 말씀이 틀리지 않았다는 것을 깨닫게 됩니다. 그동안 많은 분들께 도움을 받았으며, 이 자리를 빌어 감사 인사를 올립니다.

무사히 석사를 졸업할 수 있도록 석사 과정 동안 아낌없는 가르침을 주신 배승철 교수님께 감사의 인사를 드립니다. 또한 매번 맛있는 음식을 손수 만들어주시며 댁에 초대해주신 사모님께도 감사하다는 말을 전하고 싶습니다. 졸업 논문의 심사를 해주신 김종명교수님과 공승표교수님께도 진심으로 감사 드립니다. 학부 때부터 지금까지 많은 가르침을 주신 허성범 교수님, 장영진 교수님, 김창훈 교수님, 김동수 교수님, 남윤권 교수님, 박정환 교수님, 최윤희 교수님께도 깊은 감사를 드립니다.

실험을 진행하는 동안 많은 분들의 도움이 있었습니다. 여기서 다 언급을 하지 못하였지만 여러 사람들의 도움이 없었다면 실험이 무사히 진행되지도 않았을 것이고, 논문이 나오지도 못했을 것입니다. 주영이오빠, 진혁이오빠, 현호오빠, 정휘오빠, 승한이오빠, 영진이오빠와 성훈이오빠한테 많은 것을 배웠으며 도움을 받았습니다. 또한 하윤, 도영, 유진, 하함, 형우, 민혜,

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모두들 진심으로 고맙습니다. 사랑합니다. 다들 행복하시길 빌게요.

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Appendix

Exp.2 Raw data

	Rep	WG(%)	SGR(%/day)	FE(%)	Survival (%)
$FPPC_0$	1	223.00	2.83	140.74	100
	2	254.00	2.90	147.07	100
	3	254.51	3.01	149.25	100
$FPPC_{7.5}$	1	232.10	2.86	142.76	95.0
	2	227.07	2.82	140.66	95.0
	3	238.32	2.90	147.11	100
$FPPC_{15}$	1	218.52	2.76	135.00	95.0
	2	239.02	2.91	147.54	100
	3	237.33	2.89	147.08	95.0
$FPPC_{22.5}$	1	234.50	2.87	144.76	100
	2	235.70	2.88	145.46	95.0
	3	216.90	2.75	133.89	100
$FPPC_{30}$	1	225.18	2.81	139.00	100
	2	215.73	2.74	133.17	100
	3	230.72	2.85	142.42	95.0
$FPPC_{40}$	1	201.21	2.63	124.78	95.0
	2	204.88	2.65	129.27	95.0
	3	205.83	2.66	128.03	100