Thesis for Degree of Master of fisheries science

Effects of the different levels of dietary fish meal analogue (FMA) and two different natural additives on juvenile Japanese eel, *Anguilla japonica*

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A dissertation

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Table of contents

사료 내 어분대체품의 어분대체 수준과 두가지 천연 사료첨가제가 치어기 뱀장어에 미치는 영향

배 진 호

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요약

어분은 양어사료에 있어서 높은 기호성과 단백질 함량, 적절한 아미노산 함량 등의 이유로 인하여 주 단백질원으로 이용되고 있다. 어류 수요량의 증가에 비해, 기후변화와 환경오염 등의 이유로 어분의 생산량이 감소하며, 그 가격이 상승하고 있다. 이에 따라, 양어사료 내 어분을 대체할만한 양질의 단백질원 개발이 필요하다. 어분대체품(FMA)은 축산부산물인 수지박, 가금부산물, 우모분, 혈분과 오징어간분, 참치가공부산물, 라이신, 메치오닌 적정 비율로 배합하여 제작하였다. 또한 두 번째 실험으로는 천연 첨가제인 송강스톤, 유카밀을 첨가하여 어분대체품의 어분대체율 증가 효과를 실험하였다.

9 개 구간의 뱀장어 사료 실험구를 제작하여 대조구에는 어분 100%, 각 실험구에는 어분대비 FMA10% (FMA_{10}), 20% (FMA_{20}), 30% (FMA_{30}), 40% (FMA_{40}),를 첨가하여 사료를 제작하였고 어분대체 상승효과를 실험하기 위해 FMA_0 + 송강스톤 (FMA_0SG), FMA_0 + 유카밀 (FMA_0YM), FMA_{20} + 송강스톤 ($FMA_{20}SG$), FMA_{20} + 유카밀 ($FMA_{20}YM$) 실험구를 추가로 제작 하였다. 사육어는 9g±0.2 의 뱀장어를 40L수조에 15 마리씩 무작위로 3 반복 배치하였고, 27℃ 의 수온을 유지하였다.

8 주의 사육실험 후 성장, 전어체, 비특이적 면역반응, 혈액학적 분석을 실시하였다. 증체율(WG)과 일간성장률(SGR), 사료효율(FE), 단백질효율(PER)에 있어서 FMA_0, FMA_{10} 실험구에서 유의적인 차이를 보이지 않았으며, FMA_{20}, FMA_{30}, FMA_{40}실험구에서는 대조구(FMA_0)에 비해 유의적으로 낮은 값을 나타내었다. 또한 FMA_0 실험구와 FMA_0SG, FMA_0YM, FMA_{20}SG, FMA_{20}YM 실험구에서는 유의적인 차이를 보이지 않았다. 비특이적 면역반응 중 Lysozyme 활성 분석 결과 FMA_{10}, FMA_{20}, FMA_{30}, FMA_{40} 구간이 FMA_0SG, FMA_0YM, FMA_{20}SG, FMA_{20}YM 구간보다 유의적으로 낮은 값을 나타내었으며, 사료 첨가제를 첨가한 실험구에서는 유의적인 차이를 보이지 않았다. *V. Anguillarum*을 이용한 공격실험 결과 균 주입 7 일 후 생존율에 있어서 FMA_{20}SG, FMA_{20}YM 실험구는 FMA_0 실험구와 유의적인 차이를 보이지 않았다. SOD분석과 전어체, 혈액학적 분석 결과 전 실험구간에서 유의적인 차이를 보이지 않았다. 따라서 어분대체품(FMA)의 어분대체율은 10%까지 가능한 것으로 나타났으며, 송강스톤과 유카밀은 어분대체품의 어분대체율을 10%에서 20%까지 증가시키는 효과가 있는 것으로 나타났다.

Effects of the different levels of dietary fish meal analogue (FMA) and two different natural additives on juvenile Japanese eel, *Anguilla Japonica*

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Abstract

Nine experimental diets were formulated to contain fishmeal (FM) and/or fishmeal analogue **(**FMA) as the main animal protein source to determine the optimum FMA level that could replace FM protein and to evaluate antibiotics replacer as song-gang stone and yucca meal. The diets contained 100% FM + 0% FMA in diet (FMA_0), 90% FM + 10% FMA in diet (FMA_{10}), 80% FM + 20% FMA in diet (FMA_{20}), 70% FM + 30% FMA in diet (FMA_{30}), 60% FM + 40% FMA in diet (FMA_{40}), FMA_0 + song-gang stone (FMA_0SG), FMA_0 + yucca meal (FMA_0YM), FMA_{20} + song-gang stone ($FMA_{20}SG$) and FMA_{20} + yucca meal ($FMA_{20}YM$). Nine groups of Japanese eel each with three replicates, with the initial weight of 9 ± 0.2 g were distributed in rectangular tanks receiving flow through water. Each group was fed one of the mentioned diets for 8 weeks. Fish fed FMA_0 and FMA_{10} diets showed no significant differences in weight gain (WG), specific growth rate (SGR), feed efficiency (FE) and protein efficiency ratio (PER). Meanwhile, fish fed FMA_{20}, FMA_{30} and FMA_{40} diets showed significantly lower WG, SGR, FE and PER than the fish fed FMA_0 (control) diet. Fish fed the FMA_0SG, FMA_0YM, $FMA_{20}SG$ and $FMA_{20}YM$ diets showed no significant differences in WG, SGR, FE and PER compared with fish fed the FMA0

diet. In addition, there were no significant differences between diets including SG and YM. However, lysozyme activity of fish fed FMA_{10}, FMA_{20}, FMA_{30} and FMA_{40} was significantly lower than FMA_0SG, FMA_0YM, FMA_{20}SG and FMA_{20}YM diets. At the end of 7 days of challenge test, cumulative survival rate of fish fed FMA20SG, FMA20YM and FMA0 diets showed no significant differences (P<0.05). Body composition, SOD and Hematological indexes showed no significant differences among the fish fed the nine diets. Therefore, our results indicated that FMA could replace up to 10% of FM as a protein source in the diet of Japanese eel and both natural feed additives (SG and YM) could improve replacing rates of FMA from 10% to 20%.

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List of Tables

List of Figures

Ⅰ. Introduction

It has been predicted that world population would increase up to nearly 10 billion by 2050. This means a 33% rise in demands for varieties of healthy and desirable animal protein sources. While aquaculture is increasingly satisfying the growing demand, further progress of other animal protein production sectors does not seem to be feasible due to environmental and economical barriers (FAO 2016; UNSD 2016). But, aquaculture is also facing some major challenges associated with social, financial and ecological sustainability such as the excesses use of fish meal (FM) due to increased production of aquafeeds (FAO 2010; Moutinho 2016). Therefore, lowering the inclusion level of FM in aquafeeds could significantly drop expenses and have meaningful effect on reducing the pressure of wild stock overexploitation (Naylor et al. 2009; Kokou et al 2015).

FM is the most relevant protein source used in commercial diets of carnivorous aquatic species, because of its well-balanced amino acid profile, high palatability and digestibility (Zhou et al. 2004; Glencross et al., 2007; Kokou et al., 2012). A great deal of research has been conducted to replace this valuable commodity in diets of fish and shellfish by less expensive alternatives such as bacteria (Aas et al. 2006), algae (Kiron et al. 2012), plants (Gatlin et al. 2007) invertebrates (Barrows and Frost, 2014) and by-products (Fowler, 1991; Tung and Alfaro 2012). But, plant based protein ingredients contain some anti-nutritional components that negatively influence the digestion or absorption of nutrients (Krogdahl et al. 2010;

Oliva-Teles et al. 2015). Whereas, by-products from terrestrial animals are considered as potent alternatives for FM (Tacon, 1993; Moutinho et al. 2016). Fishmeal analogue (FMA) is a premix of animal by-products and plant ingredients including leather meal, poultry by-product, feather meal, blood meal, squid liver powder, tuna by-product and soybean meal. With high crude protein, low anti-nutritional factors (e.g. proteinase inhibitors, lectins and phytic acid), acceptable palatability (because of tuna by-product and squid liver powder) and rich amino acid profile (by addition of methionine and lysine), FMA could be considered a qualified candidate for substitution of FM (Jo et al. 2016).

Feed additives are known as supplemental and nutritional/non-nutritional ingredients that are added to formulated diets in order to increase physical properties of feed or enhance performance of aquatic species (Bai et al. 2015). A variety of feed additives such as feeding stimulants and palatability enhancers, antimicrobial agents, antioxidants, binders, pigmentation agents, organic acids, enzymes, immunostimulants and hormones are used in aquafeeds (NRC 2011). Song-gang stone (SG) powder is a natural mineral that could be found in Republic of Korea and consists of SiO2, Al2O3, K2O, Na2O Fe2O3 and other trace elements. This compound is considered to be an immunostimulant that could enhance fish health status (Choi et al. 2004; Won, 2016). Yucca meal (YM) is a naturally manufactured powder driven from a flowering plant known as *Yucca schidigera* (Cheeke et al. 2006). YM can act as an antioxidant, anti-stress

and antimicrobial agent that can enhance growth, immunity and appetite of target species (Kelly and Kohler 2003; Piacente et al. 2005; Gaber, 2006; Citarasu, 2010; Güroy et al, 2014; Njagi, 2016). Both of the mentioned additives have the potential to be used in aquafeeds, improve chemical composition of diet and boost the nutritional quality.

Japanese eel, *Anguilla japonica*, is one of the most expensive and important freshwater fish species cultured in East Asia (China, Japan and Republic Korea). According to the international union for conservation of nature and natural resources (IUCN), this species has been listed endangered (Jacoby and Gollock, 2014) and special attention to aquaculture and nutrition of Japanese eel is required. Therefore, according to what was mentioned, the aim of the present study is to evaluate the influence of FM replacement by FMA, with or without YM an SG additives, on growth, whole body proximate composition, hematological indexes and non-specific immune responses of Japanese eel. A challenge test with *Vibrio anguillarum*, a globally distributed pathogen that causes severe hemorrhagic disease in aquaculture farms (Frans 2011), was also designed to examine the effectiveness of diets.

Thus, the objectives of this experiment is to evaluate FMA as a fish meal replacer and to determine the proper inclusion level of FMA, to evaluate the effects of dietary Song-gang stone (SG) and Yucca meal (YM) on growth performance, non-specific immune responses and disease resistance of juvenile Japanese eel,

Anguilla japonica and to identify the one that has more positive responses with a lower cost.

II. Materials and Methods

Experimental Design and Diets

FMA (Fish Meal Analogue) was blended with leather meal (The feed Co., South Korea), poultry by-product (cherry buro Co., South Korea), feather meal (cherry buro Co., South Korea), tuna by-product (Woonam fish Co., South Korea), blood meal, squid liver powder, soybean meal, lysine and methionine. The analysed essential amino acid composition of the FMA is shown in Table 1.

Nine experimental diets were formulated (Table 2) to replace Fishmeal with FMA at 0% (FMA_0), 10% (FMA_{10}), 20% (FMA_{20}), 30% (FMA_{30}), 40% (FMA_{40}), FMA_0 + song-gang stone (FMA_0SG), FMA_0 + yucca meal (FMA_0YM), FMA_{20} + song-gang stone ($FMA_{20}SG$) and FMA_{20} + yucca meal ($FMA_{20}YM$). These experimental diets were formulated to be isonitrogenous and contain 50% crude protein (CP). FM, wheat gluten meal and FMA were used as protein sources. Soybean oil, fish oil and chicken oil were used as lipid sources. Corn starch was used as energy source and cellulose was used as binder. At first, all dry ingredients were mixed using a mechanical mixing machine (HYVM-1214, Hanyoung Food Machinery, Republic of Korea) thoroughly. Then, filtered water (300 ml kg^{-1} diet) was added with fish oil, soybean oil and chicken oil. The experimental diets were pelleted using a screw-type pelleting machine (SFD-GT, Shinsung, Rep. Korea) and stored at -20℃ until use.

Table 1. Amino acid composition of FMA and FM (fishmeal analogue)

Amino acid	% of DM basis
Histidine	2.48
Arginine	2.80
Threonine	1.59
Valine	2.93
Met+Cys	2.63
Phe+Tyr	3.16
Isoleucine	1.40
Leucine	4.29
Tryptophan	0.62
Lysine	3.61

Table 2. Composition of the experimental diets in Japanese eel (% of DM basis)

Ingredients	Diets								
	FMA$_{0}$	FMA$_{10}$	FMA$_{20}$	FMA$_{30}$	FMA$_{40}$	FMA$_{0}$SG	FMA$_{0}$YM	FMA$_{20}$SG	FMA$_{20}$YM
Fishmeal	65.0	58.5	52.0	45.5	39.0	65.0	65.0	52.0	52.0
FMA	0.00	6.50	13.0	19.5	26.0	0.00	0.00	13.0	13.0
Wheat gluten meal	7.90	7.90	7.90	7.90	7.90	7.90	7.90	7.90	7.90
Corn starch	18.6	18.7	18.9	19.1	19.3	18.6	18.6	18.9	18.9
Chicken oil	0.70	0.60	0.40	0.20	0.00	0.70	0.70	0.40	0.40
Soybean oil	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40
Fish oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Cellulose	0.40	0.40	0.40	0.40	0.40	0.00	0.30	0.00	0.30
Song-gang stone[1]	0.00	0.00	0.00	0.00	0.00	0.40	0.00	0.40	0.00
Yucca meal[2]	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.10
Vitamin premix[3]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Mineral premix[4]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Proximate analysis (% of dry matter basis)									
Moisture %	10.2	9.40	9.30	9.40	9.16	8.53	8.99	8.54	9.05
Crude protein %	50.2	50.1	50.5	50.3	50.6	50.6	50.2	50.7	50.3
Crude lipid %	7.70	7.50	8.50	8.10	7.80	8.48	8.40	8.00	7.97
Crude ash %	12.1	12.0	11.7	11.5	12.9	12.9	12.7	12.3	11.5

[1] Davistone Co. Ltd., South Korea

[2] De-Odorase®, Alltech Korea, Seocho-Gu, Seoul, South Korea

[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.

Experimental fish and feeding trial

The experiment was conducted in the department of fisheries biology and Feeds and Foods Nutrition Centre, Pukyong National University, Busan, South Korea. Approximately 500 Juveniles of Japanese eel *Anguilla japonica* were purchased from Nonsan fish farm (Nonsan city, Rep. Korea). The feeding trial was conducted by using an indoor semi-recirculating system with 27 tanks (45L) receiving a continual flow of filtered freshwater at a rate of 2Lmin^{-1} from the central tank. 50% of the water was exchanged daily using pumping filtered fresh water to the central tank. Aeration was used to have enough dissolved oxygen level and water temperature was maintained at 27.0±0.5 ^{o}C using water heaters during the experimental period. 405 fishes averaging 9±0.2g (mean ± standard deviation [SD]) were weighed and randomly distributed into the 27 tanks (15 fish/tank). Each tank was then randomly distributed one of three replicates of the 9 dietary treatments. Fish were fed twice daily (10:00 and 18:00 hrs) from 1.5% to 2% of wet body weight/day until end of feeding trial for 8 weeks. Experimental tanks were siphoned 15:00 pm to clean up the faecal matter and uneaten feed. Mortality of all treatment groups were checked everyday during the experimental period; dead fish were evacuated immediately and weighed. The amounts of feed for each tank was calculated according to the percentage of the remaining fish weight in the tanks. Dead fish were not replaced during the experimental period. Total fish weight in each tank was weighted at the end of the 4th week and the

amount of diet fed to the fish was calculated accordingly and finally at the end of the 8^{th} week. Feeding trial was stopped 24hrs before each measurement to prevent inclusion of ingested feed in the weight measurements as well as to minimize stress.

Sample collection and analysis

After the feeding trial, the number of all fish in each tank, weight gain (WG), specific growth rate (SGR), feed efficiency (FE), protein efficiency ratio (PER) and survival rate (SR) were calculated. Three fishes per tank were randomly selected, weighed, length measured and dissected to obtain liver and visceral mass to calculate Condition Factor (CF), viscerosomatic (VSI) and hepatosomatic (HSI) indices.

The same fish as subjected to CF, HSI and VSI calculations were also used to analyze serological characteristics. Blood samples were obtained from the caudal vein of the fish with 1ml syringes. Serum samples were obtained from blood on clotting by centrifugation at 5,000 × g for 10 minutes and stored at -70^{o}C for the analysis of lysozyme and superoxide dismutase (SOD) activities. A turbidometric assay was used for the determination of serum lysozyme activity by the method described by Hultmark (1980) with slight modifications. Briefly, *Micrococcus lysodeikticus* (0.75mg ml^{-1}) was suspended in sodium phosphate buffer (0.1M, pH 6.4), 200µl of suspension was placed in each well of 96-well plates and 20µl of

test serum was added subsequently. The reduction in absorbance of the samples was recorded at 570 nm after incubation at room temperature for 0 and 60 minutes in a microplate reader (UVM 340, Biochrom, Cambridge, UK). A reduction in absorbance of 0.001 min^{-1} was regarded as one unit of lysozyme activity.

Superoxide dismutase (SOD) activity was measured by the percentage of reaction inhibition rate of the enzyme with WST-1 (Water Soluble Tetrazolium dye) substrate and xanthine oxidase using an SOD Assay Kit (Enzo ADI-900-157, Enzo Life Sciences, Inc.) in accordance with manufacturer's instructions. Each endpoint assay was monitored by absorbance at 450 nm (the absorbance wavelength for the coloured product of WST-1 reaction with superoxide) after 20 minutes of reaction time at 37^{o}C. The inhibition percentage was normalized by mg protein and presented as SOD activity units. Portions of serum were also used for analysis of biochemical parameters including plasma total protein, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and glucose using the commercial clinical kits of Fuji DRI-CHEM 3500i, Fuji Photo Film Ltd, Tokyo, Japan.

Four fish per tank were collected and pooled together according to the type of diet fed; homogenised and freeze-dried at -80^{o}C for analysis. Proximate compositions of basal diet and fish were determined according to standard procedures of AOAC (2000) in duplicates. Samples were dried to a constant weight at 105^{o}C to

determine moisture content. Ash content was determined by incineration in a muffle furnace at 550^{o}C for 3.5h, crude lipid by the Soxhlet extraction using Soxtec system 1046 (Tecator AB, Foss, Hoganas, Sweden) and crude protein by the Kjedahl method (N×6.25) after acid digestion.

Challenge test

At the end of the 8 weeks of experiment, fish were redistributed based on their earlier dietary treatments in 45-L tank to evaluate the effects of diets on disease resistance. The water temperature was maintained at 27±0.5 ℃ (mean±SD).

The pathogenic bacterium, *V. anguillarum* KCCM 40293 was obtained from the Department of Biotechnology, Pukyong National University, Busan, Korea. The bacterial strain originally sourced from diseased Japanese eel. *V. anguillarum* was cultured in tryptic soy broth (TSB; Sigma-Aldrich) at 26°C for 24 h and stored with 20% glycerol at -80°C for further use. Bacterial growth was measured at OD_{600} by spectrophotometer followed by plate counting by colony counter on tryptic soy agar (TSA; Sigma-Aldrich). The bacterial colony was identified by API 20 E pathogenic bacteria identification kit (BioMerieux, Durham, NC, USA). Five fishes per aquarium were injected intraperitoneally (i.p.) with 0.1 mL per fish at a suitable concentration of 5×10^{7} CFU/mL ($2\times LD_{50}$). The mortality of fish in each aquarium was recorded daily during 10 days.

Statistical Analysis

All data were subjected to one-way analysis of variance (ANOVA) using SAS Program Version 9.4(SAS Institute, Cary, NC, USA) to test the effects of the different dietary treatments. When a significant effect was observed, a Least Significant Difference (LSD) test was used to compare the means. Treatment effects were considered significant at confidence level of $P<0.05$. The mean and SD of the mean was calculated for each treatment.

III. Results

Growth Performance

Growth performance of juvenile Japanese eel, *Anguilla japonica*, fed the experimental diets for 8 weeks is summarized in Table 3 and graphically presented in Figures 1 (a-d). After the feeding trial, weight gain (WG), specific growth rate (SGR), feed efficiency (FE) and protein efficiency ratio (PER) of fish fed FMA_0 was significantly higher than fish fed FMA_{20} diet. However, there were no significant differences between fish fed FMA_0, FMA_{10}, FMA_0SG, FMA_0YM, $FMA_{20}SG$ and $FMA_{20}YM$. Hepatosomatic index (HSI) of fish fed FMA_0 was significantly higher than fish fed FMA_{20} and FMA_{40} diets. However, there were no significant differences between fish fed FMA_0, FMA_{10}, FMA_{30}, FMA_0SG, FMA_0YM, $FMA_{20}SG$ and $FMA_{20}YM$. Viscerosomatic index (VSI) of fish fed FMA_0 was significantly higher than fish fed FMA_{40} diet. However, there were no significant differences between fish fed FMA_0, FMA_{10}, FMA_{20}, FMA_{30}, FMA_0SG, FMA_0YM, $FMA_{20}SG$ and $FMA_{20}YM$. There were no significant differences in condition factor (CF) and survival among fish fed all the experimental diets.

Whole Body Proximate Analysis

Whole body proximate compositions of juvenile Japanese eel fed the experimental diets are summarized in Table 4. There were no significant differences (P<0.05) in whole body crude protein, crude lipid, moisture and ash contents among fish fed all the experimental diets after the feeding trial.

Non-specific Immune Responses

Non-specific immune responses of juvenile Japanese eel fed the experimental diets are summarized in Table 5 and graphically presented in Figures 2 (a-b). Lysozyme activity of fish fed FMA_0, FMA_{10}, FMA_{20}, FMA_{30} and FMA_{40} was significantly lower than FMA_0SG, FMA_0YM, $FMA_{20}SG$ and $FMA_{20}YM$. However, there were no significant differences among the fish fed FMA_0SG, FMA_0YM, $FMA_{20}SG$ and $FMA_{20}YM$. There were no significant differences (P<0.05) in Superoxide dismutase (SOD) inhibition among fish fed all the experimental diets

Hematological indexes

Serum parameters of juvenile Japanese eel fed the experimental diets are summarized in Table 6 and graphically presented in Figures 3 (a-d). There were no significant differences (P<0.05) in serum glucose, total protein (TP), glutamic

oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) among fish fed all the experimental diets after the feeding trial.

Challenge test

Cumulative survival rate of juvenile Japanese eel challenged with *V. Anguillarum* for 7 days is shown in Fig 4. During the challenge test, the first mortalities occurred on the second day and it was pronounced after the third day of injection. At the end of 7 days of challenge test, cumulative survival rates of fish fed FMA0SG and FMA0YM diets were significantly higher than FMA0 diet ($P<0.05$). However, cumulative survival rate of fish fed FMA20SG, FMA20YM and FMA0 diets showed no significant differences ($P<0.05$).

Table 3. Growth performance of juvenile Japanese eel fed the experimental diets for 8 weeks[1]

	Diets									Pooled SEM[10]
	FMA $_{0}$	FMA $_{10}$	FMA $_{20}$	FMA $_{30}$	FMA $_{40}$	FMA $_{0}$SG	FMA $_{0}$YM	FMA $_{20}$SG	FMA $_{20}$YM	
WG[2]	120^{a}	113^{ab}	107^{b}	94.4^{c}	78.1^{d}	124^{a}	121^{a}	118^{ab}	118^{ab}	2.89
SGR[3]	1.88^{a}	1.80^{ab}	1.73^{b}	1.58^{c}	1.37^{d}	1.92^{a}	1.89^{ab}	1.85^{ab}	1.86^{ab}	0.03
FE[4]	90.0^{a}	87.5^{ab}	81.0^{b}	72.4^{c}	53.7^{d}	93.3^{a}	93.5^{a}	90.1^{a}	89.1^{a}	2.53
PER[4]	1.79^{a}	1.75^{ab}	1.60^{b}	1.44^{c}	1.06^{d}	1.84^{a}	1.86^{a}	1.78^{a}	1.77^{a}	0.05
HSI[6]	1.31^{a}	1.18^{ab}	1.01^{b}	1.08^{ab}	0.95^{b}	1.04^{ab}	1.13^{ab}	1.19^{ab}	1.08^{ab}	0.03
VSI[7]	1.13^{a}	1.03^{ab}	1.06^{ab}	0.97^{ab}	0.82^{b}	1.06^{ab}	1.05^{ab}	1.10^{a}	1.04^{ab}	0.03
CF[8]	0.09	0.08	0.10	0.10	0.09	0.10	0.10	0.09	0.09	0.01
Sur[9]	75.6	84.4	84.4	82.2	73.3	84.4	75.6	86.7	77.8	1.55

[1]Values are means from triplicate groups of fish (n = 3) where values in each row with different superscripts are significantly different ($p < 0.05$)
[2]Weight gain (WG) = (final weight -initial weight) ×100/initial weight
[3]Specific growth rate (SGR; %) = (ln final weight - ln initial weight) × 100/d
[4]Feed efficiency (FE; %) = wet WG (g) × 100/dry feed intake (g)
[5]Protein efficiency ratio (PER) =wet weight gain/protein intake
[6]Hepatosomatic index (HSI; %) = (liver weight/body weight) × 100
[7]Viscerosomatic index (VSI; %) = (visceral weight/body weight) × 100
[8]Condition factor (VSI) = {fish weight (g)/fish length (cm)^{3}} ×100
[9]Survival (%) = (final number of fish/initial number of fish) ×100
[10]Pooled standard error of mean = SD/√n

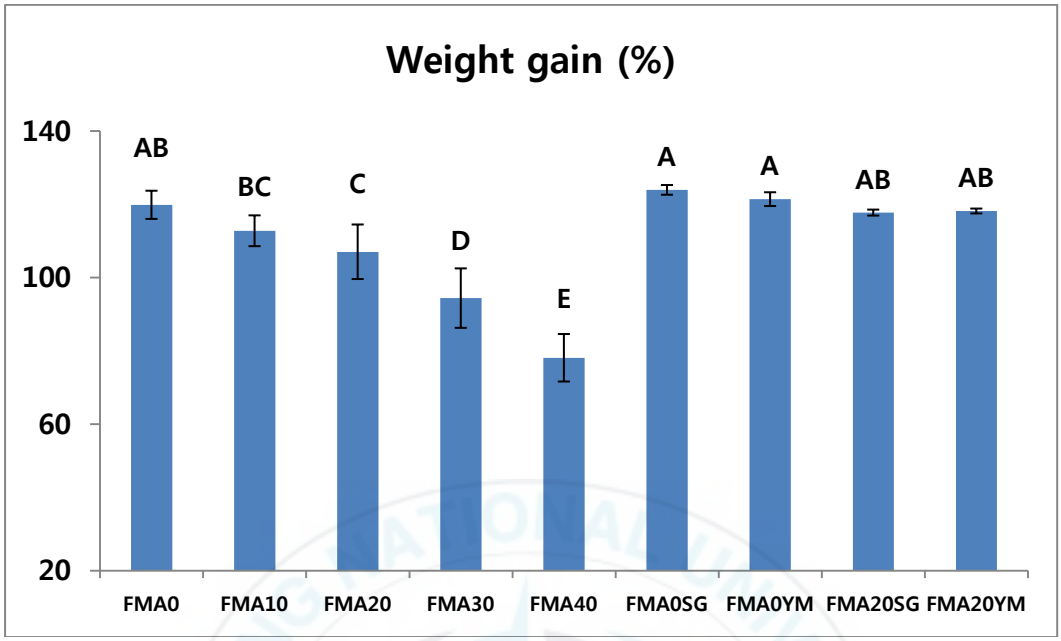

Fig.1-a. Weight gain of juvenile Japanese eel fed the experimental diets for 8 weeks[1].

FMA_0 (control) - Basal diet (FMA0 % of fishmeal)

FMA_{10} – FMA 10% of fishmeal

FMA_{20} – FMA 20% of fishmeal

FMA_{30} – FMA 30% of fishmeal

FMA_{40} – FMA 40% of fishmeal

FMA_0SG – FMA0 + SG

FMA_0YM – FMA0 + YM

FMA_{20}SG – FMA20 + SG

FMA_{20}YM – FMA20 + YM

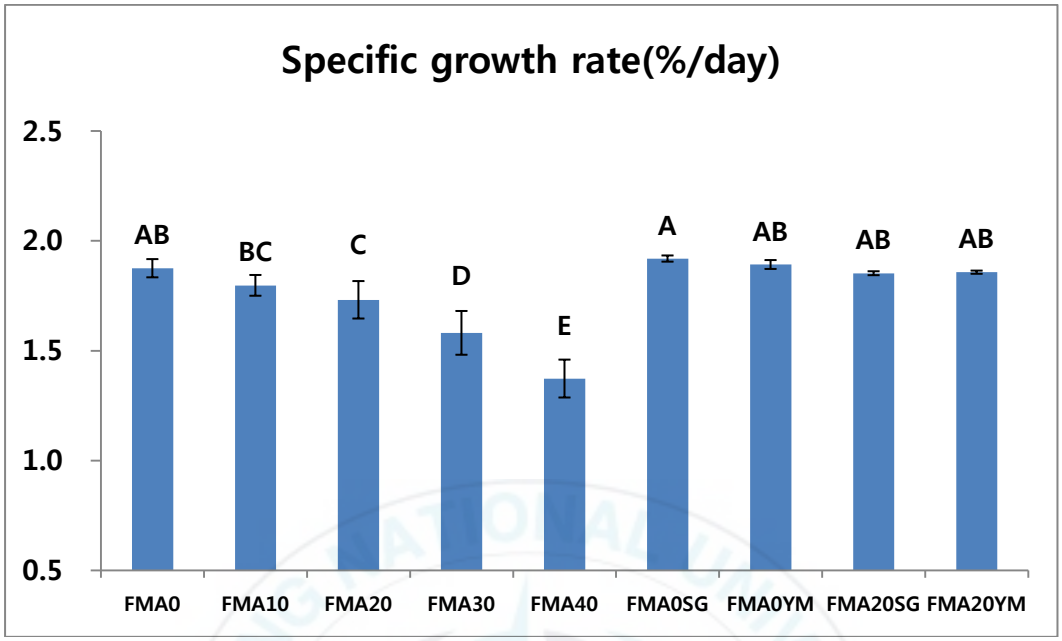

Fig.1-b. Specific growth rate of juvenile Japanese eel fed the experimental diets for 8 Weeks[1]

FMA_0 (control) - Basal diet (FMA0 % of fishmeal)

FMA_{10} – FMA 10% of fishmeal

FMA_{20} – FMA 20% of fishmeal

FMA_{30} – FMA 30% of fishmeal

FMA_{40} – FMA 40% of fishmeal

FMA_0SG – FMA0 + SG

FMA_0YM – FMA0 + YM

FMA_{20}SG – FMA20 + SG

FMA_{20}YM – FMA20 + YM

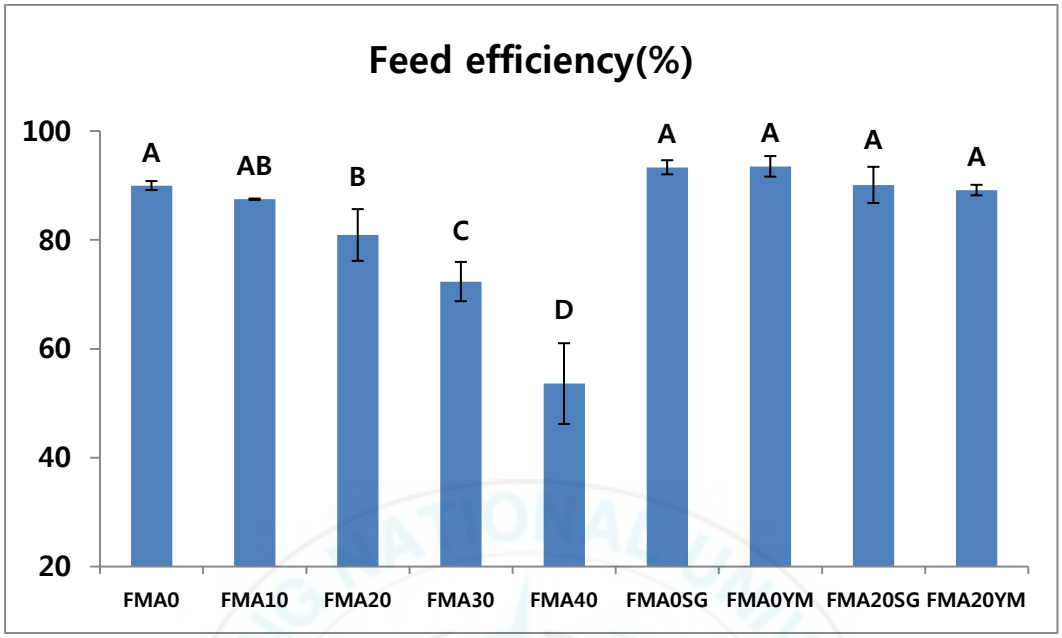

Fig. 1-c. Feed efficiency of juvenile Japanese eel fed the experimental diets for 8 weeks[1].

FMA_0 (control) - Basal diet (FMA0 % of fishmeal)

FMA_{10} – FMA 10% of fishmeal

FMA_{20} – FMA 20% of fishmeal

FMA_{30} – FMA 30% of fishmeal

FMA_{40} – FMA 40% of fishmeal

FMA_0SG – FMA0 + SG

FMA_0YM – FMA0 + YM

FMA_{20}SG – FMA20 + SG

FMA_{20}YM – FMA20 + YM

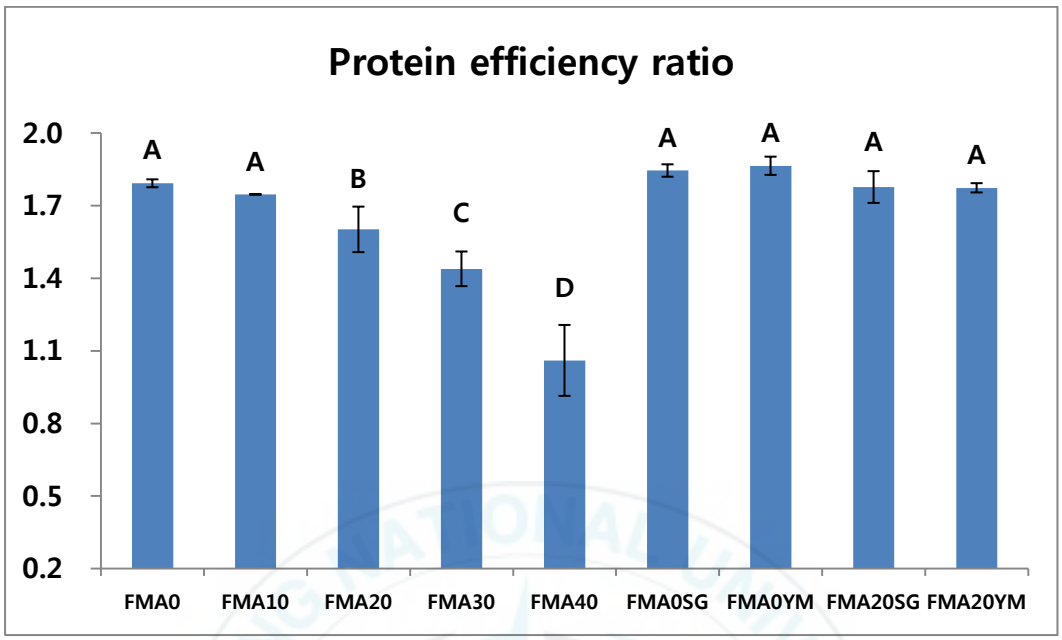

Fig. 1-d. Protein efficiency ratio of juvenile Japanese eel fed the experimental diets for 8 Weeks[1].

FMA_0 (control) - Basal diet (FMA0 % of fishmeal)

FMA_{10} – FMA 10% of fishmeal

FMA_{20} – FMA 20% of fishmeal

FMA_{30} – FMA 30% of fishmeal

FMA_{40} – FMA 40% of fishmeal

FMA_0SG – FMA0 + SG

FMA_0YM – FMA0 + YM

FMA_{20}SG – FMA20 + SG

FMA_{20}YM – FMA20 + YM

Table 4. Whole body proximate composition of juvenile Japanese eel fed the experimental diets for 8 weeks (% dry matter basis) [1]

Content (%)	Diets[2]									Pooled SEM[3]
	FMA_0	FMA_{10}	FMA_{20}	FMA_{30}	FMA_{40}	FMA_0SG	FMA_0YM	$FMA_{20}SG$	$FMA_{20}YM$	
Moisture	69.8	71.2	69.0	69.4	69.1	69.5	69.3	69.5	69.7	0.25
Crude protein	19.0	18.2	18.8	19.2	19.5	19.1	18.9	19.7	18.7	0.12
Crude lipid	8.45	8.00	9.51	8.74	8.56	8.60	9.56	7.64	8.15	0.32
Ash	2.59	2.71	2.45	2.55	2.78	2.48	2.69	2.79	2.68	0.05

[1]Values are means from triplicate groups of fish (n = 3) where values in each row with different superscripts are significantly different ($p < 0.05$)

[2]Diets

FMA_0 (control) - Basal diet (FMA0 % of fishmeal)

FMA_{10} – FMA 10% of fishmeal

FMA_{20} – FMA 20% of fishmeal

FMA_{30} – FMA 30% of fishmeal

FMA_{40} – FMA 40% of fishmeal

FMA_0SG – FMA0 + SG

FMA_0YM – FMA0 + YM

$FMA_{20}SG$ – FMA20 + SG

$FMA_{20}YM$ – FMA20 + YM

[3]Pooled standard error of mean = SD/√n

Table 5. Non-specific immune responses in juvenile Japanese eel fed the experimental diets for 8 weeks[1]

Activity	Diets[2]									Pooled SEM[4]
	FMA_0	FMA_{10}	FMA_{20}	FMA_{30}	FMA_{40}	FMA_0SG	FMA_0YM	$FMA_{20}SG$	$FMA_{20}YM$	
SOD[3] (% of inhibition)	67.0	68.1	67.5	66.2	65.0	66.5	66.5	67.5	67.8	0.35
Lysozyme (U/ml)	0.62^{bc}	0.60^{c}	0.77^{c}	0.66^{c}	0.64^{c}	0.94^{a}	1.05^{a}	0.90^{ab}	0.91^{a}	0.33

[1]Values are means from triplicate groups of fish (n = 3) where values in each row with different superscripts are significantly different ($p < 0.05$)

[2]Diets

FMA_0 (control) - Basal diet (FMA0 % of fishmeal)

FMA_{10} – FMA 10% of fishmeal

FMA_{20} – FMA 20% of fishmeal

FMA_{30} – FMA 30% of fishmeal

FMA_{40} – FMA 40% of fishmeal

FMA_0SG – FMA0 + SG

FMA_0YM – FMA0 + YM

$FMA_{20}SG$ – FMA20 + SG

$FMA_{20}YM$ – FMA20 + YM

[3]SOD: Superoxide dismutase

[4]Pooled standard error of mean = $SD/\sqrt{n}$

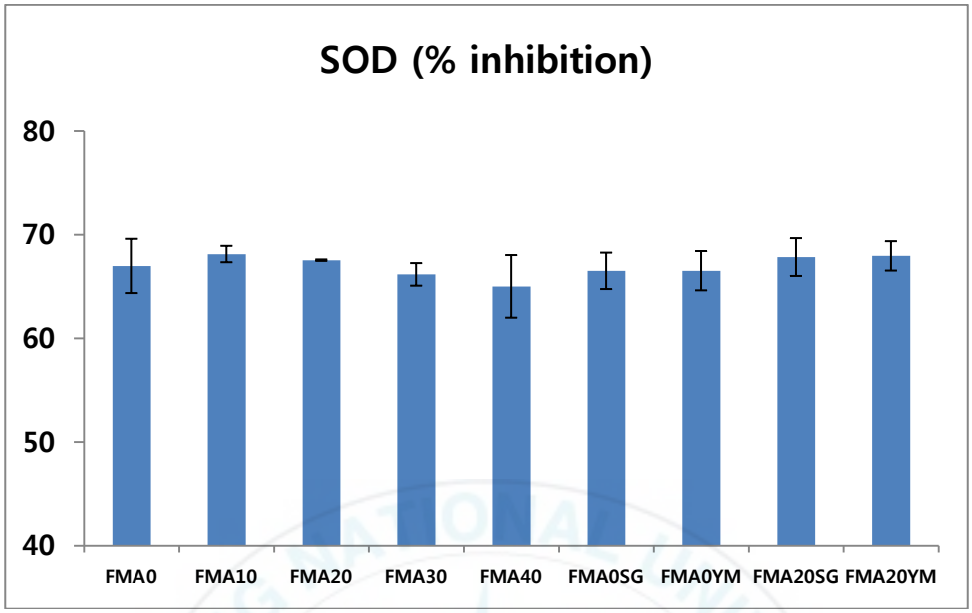

Fig. 2-a. Superoxide dismutase inhibition in juvenile Japanese eel fed the experimental diets for 8 weeks1.

FMA_0 (control) - Basal diet (FMA0 % of fishmeal)

FMA_{10} – FMA 10% of fishmeal

FMA_{20} – FMA 20% of fishmeal

FMA_{30} – FMA 30% of fishmeal

FMA_{40} – FMA 40% of fishmeal

FMA_0SG – FMA0 + SG

FMA_0YM – FMA0 + YM

FMA_{20}SG – FMA20 + SG

FMA_{20}YM – FMA20 + YM

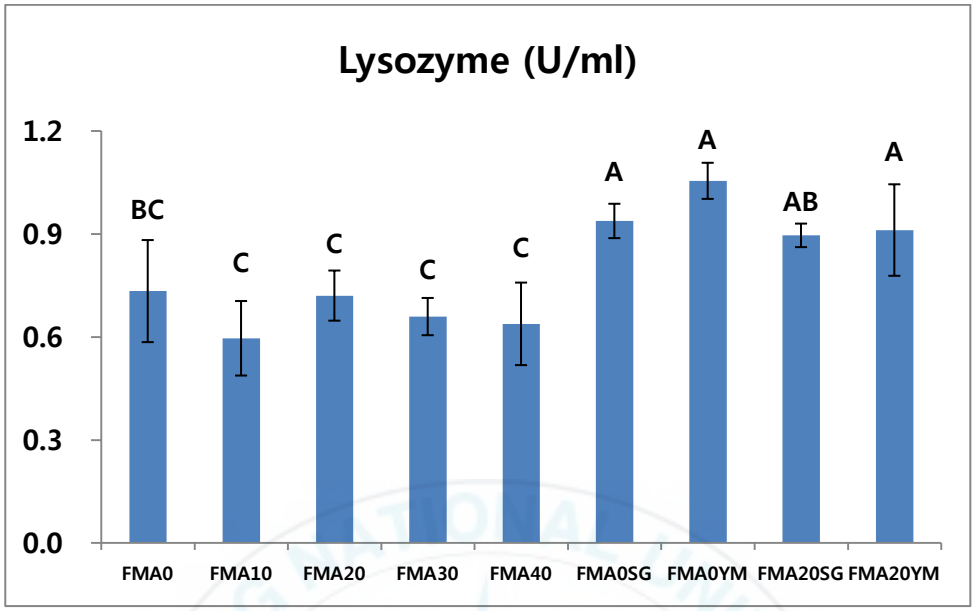

Fig. 2-b. Lysozyme activity in juvenile Japanese eel fed the experimental diets for 8 weeks[1].

FMA_0 (control) - Basal diet (FMA0 % of fishmeal)

FMA_{10} – FMA 10% of fishmeal

FMA_{20} – FMA 20% of fishmeal

FMA_{30} – FMA 30% of fishmeal

FMA_{40} – FMA 40% of fishmeal

FMA_0SG – FMA0 + SG

FMA_0YM – FMA0 + YM

FMA_{20}SG – FMA20 + SG

FMA_{20}YM – FMA20 + YM

Table 6. Serum parameters in juvenile Japanese eel the experimental diets for 8 weeks[1]

Content (%)	Diets[2]									Pooled SEM[5]
	FMA_0	FMA_{10}	FMA_{20}	FMA_{30}	FMA_{40}	FMA_0SG	FMA_0YM	$FMA_{20}SG$	$FMA_{20}YM$	
Glucose (mg/dl)	98.7	97.7	90.3	86.0	91.0	101	90.7	91.7	92.7	2.16
Total protein (g/dl)	4.33	4.20	4.27	4.70	4.77	4.43	4.53	4.40	4.10	0.11
GOT (U/l)[3]	113	117	116	119	121	115	115	115	114	1.69
GPT (U/l)[4]	7.67	9.33	7.33	8.00	7.67	7.67	7.67	8.00	6.67	0.21

[1]Values are means from triplicate groups of fish (n = 3) where values in each row with different superscripts are significantly different ($p < 0.05$)

[2]Diets

FMA_0 (control) - Basal diet (FMA0 % of fishmeal)

FMA_{10} – FMA 10% of fishmeal

FMA_{20} – FMA 20% of fishmeal

FMA_{30} – FMA 30% of fishmeal

FMA_{40} – FMA 40% of fishmeal

FMA_0SG – FMA0 + SG

FMA_0YM – FMA0 + YM

$FMA_{20}SG$ – FMA20 + SG

$FMA_{20}YM$ – FMA20 + YM

[3]GOT - Glutamic oxaloacetic transaminase - 1U is defined as the amount of enzyme causing the transamination of 1.0 μmol of L-aspartate/min at 25 C and pH 7.4.

[4]GPT - Glutamic pyruvic transaminase - 1U is defined as the amount of enzyme causing the transamination of 1.0 μmol of L-alanine/min at 25 C and pH 7.4.

[5]Pooled standard error of mean = SD/√n

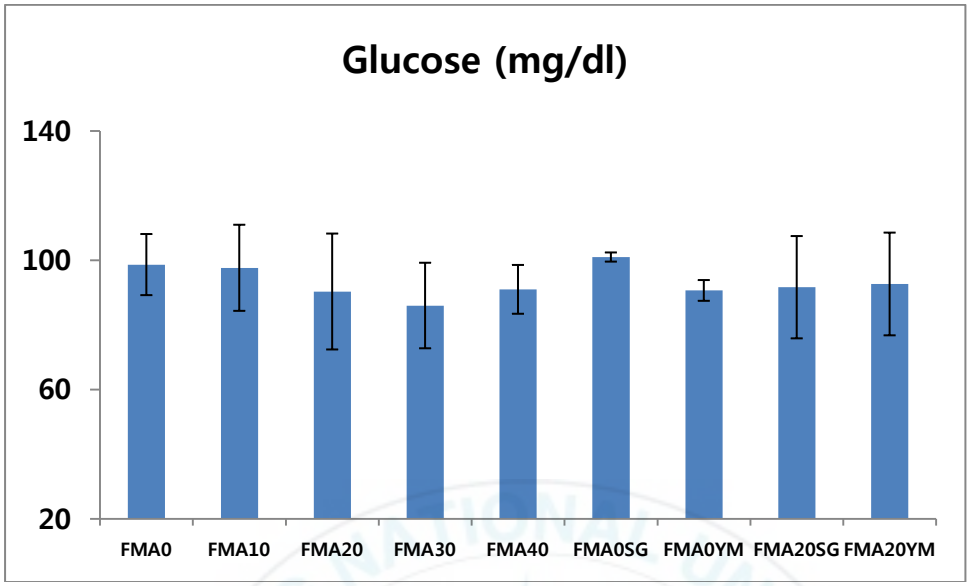

Fig.3-a. Glucose in juvenile Japanese eel fed the experimental diets for 8 weeks.

FMA_0 (control) - Basal diet (FMA0 % of fishmeal)

FMA_{10} – FMA 10% of fishmeal

FMA_{20} – FMA 20% of fishmeal

FMA_{30} – FMA 30% of fishmeal

FMA_{40} – FMA 40% of fishmeal

FMA_0SG – FMA0 + SG

FMA_0YM – FMA0 + YM

FMA_{20}SG – FMA20 + SG

FMA_{20}YM – FMA20 + YM

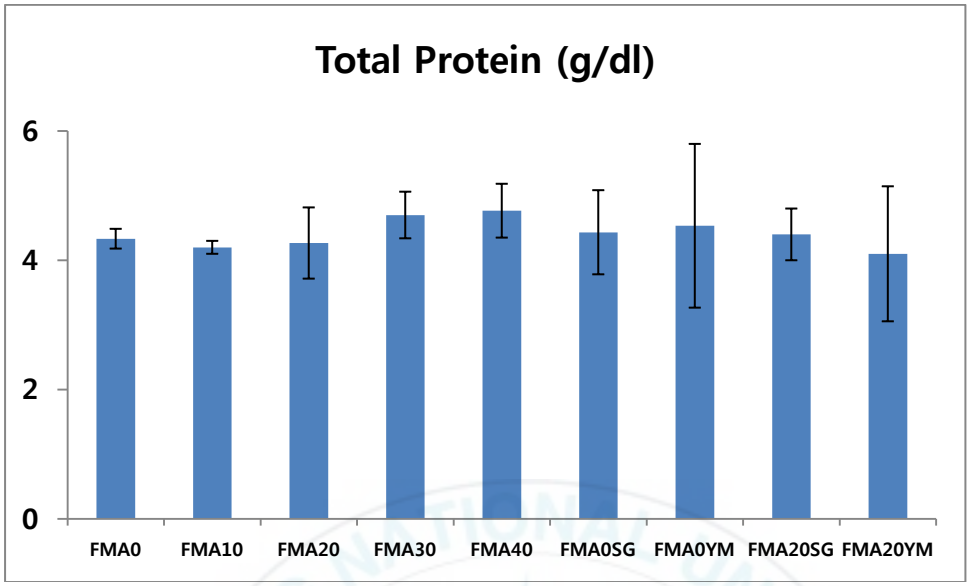

Fig.3-b. Total protein in juvenile Japanese eel fed the experimental diets for 8 weeks.

FMA_0 (control) - Basal diet (FMA0 % of fishmeal)

FMA_{10} – FMA 10% of fishmeal

FMA_{20} – FMA 20% of fishmeal

FMA_{30} – FMA 30% of fishmeal

FMA_{40} – FMA 40% of fishmeal

FMA_0SG – FMA0 + SG

FMA_0YM – FMA0 + YM

FMA_{20}SG – FMA20 + SG

FMA_{20}YM – FMA20 + YM

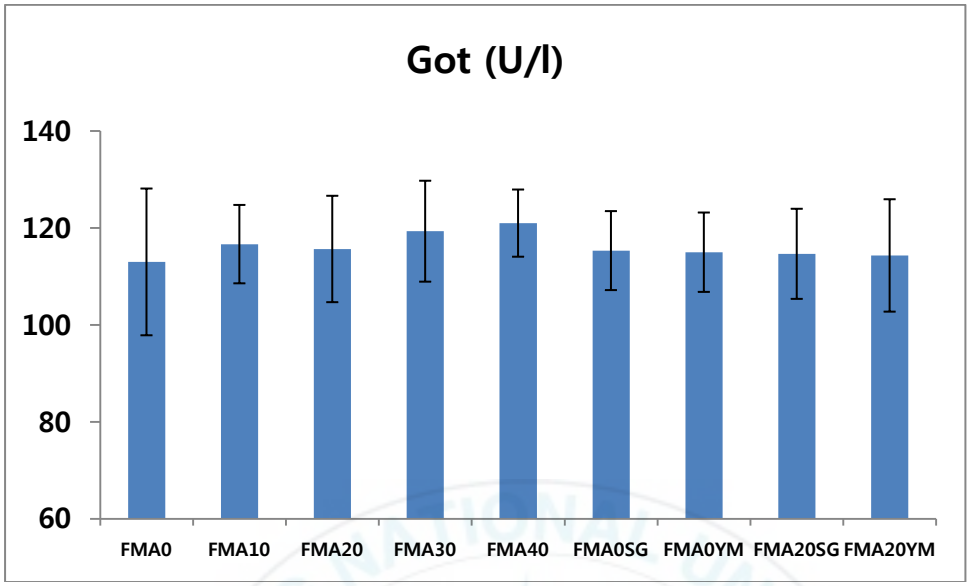

Fig.3-c. Glutamic oxaloacetic transaminase (GOT) in juvenile Japanese eel fed the experimental diets for 8 weeks.

FMA_0 (control) - Basal diet (FMA0 % of fishmeal)

FMA_{10} – FMA 10% of fishmeal

FMA_{20} – FMA 20% of fishmeal

FMA_{30} – FMA 30% of fishmeal

FMA_{40} – FMA 40% of fishmeal

FMA_0SG – FMA0 + SG

FMA_0YM – FMA0 + YM

FMA_{20}SG – FMA20 + SG

FMA_{20}YM – FMA20 + YM

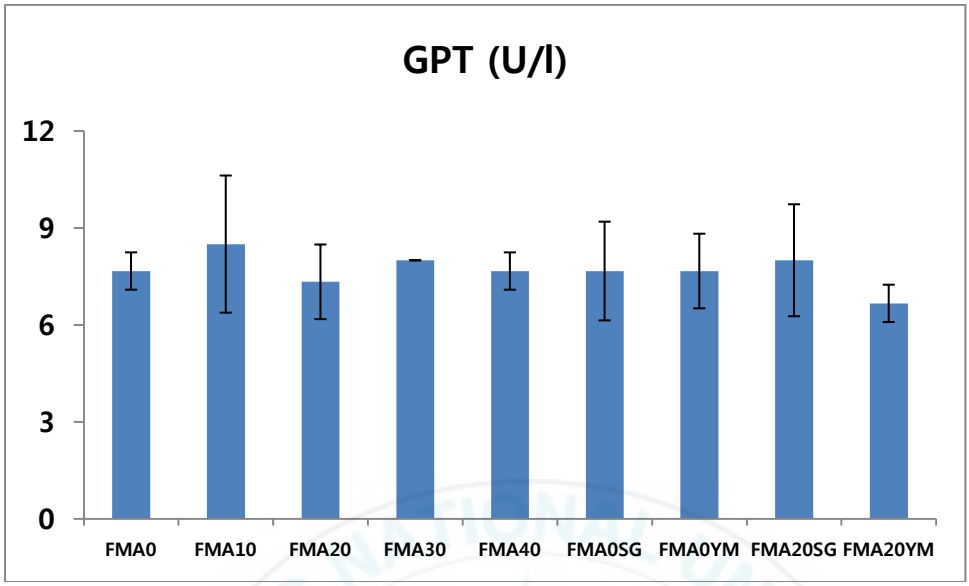

Fig.3-d. Glutamic pyruvic transaminase (GPT) in juvenile Japanese eel fed the experimental diets for 8 weeks.

FMA_0 (control) - Basal diet (FMA0 % of fishmeal)

FMA_{10} – FMA 10% of fishmeal

FMA_{20} – FMA 20% of fishmeal

FMA_{30} – FMA 30% of fishmeal

FMA_{40} – FMA 40% of fishmeal

FMA_0SG – FMA0 + SG

FMA_0YM – FMA0 + YM

FMA_{20}SG – FMA20 + SG

FMA_{20}YM – FMA20 + YM

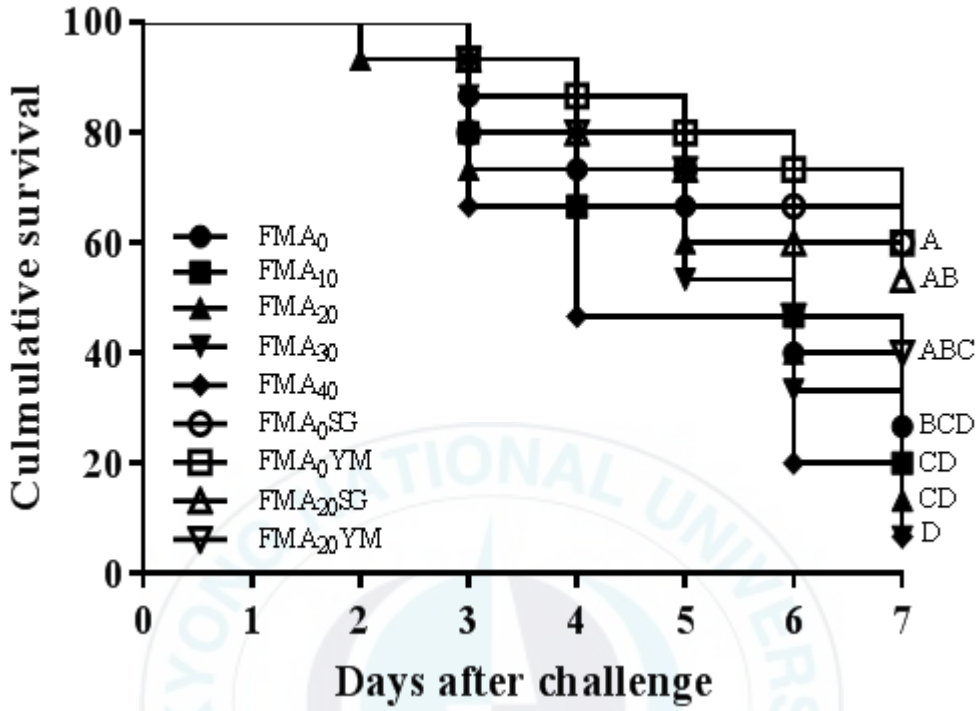

Fig 4. Cumulative survival rate after challenge with *V. anguillarum* for 7 days in Japanese eel fed the nine experimental diets for 8 weeks.

FMA_0 (control) - Basal diet (FMA0 % of fishmeal)

FMA_{10} – FMA 10% of fishmeal

FMA_{20} – FMA 20% of fishmeal

FMA_{30} – FMA 30% of fishmeal

FMA_{40} – FMA 40% of fishmeal

FMA_0SG – FMA_0 + SG

FMA_0YM – FMA_0 + YM

$FMA_{20}SG$ – FMA_{20} + SG

$FMA_{20}YM$ – FMA_{20} + YM

IV. Discussion and Conclusion

The high survival rate recorded during this study could be attributed to high acceptability of the experimental diets by the juvenile Japanese eel. In the present study, WG, SGR and FE of fish fed 20, 30 and 40% of FMA were significantly lower than those of control (FMA0) group. Same results were observed by Wang et al. (2013), while replacing FM by dietary poultry by-product meal for Japanese sea bass, *Lateolabrax japonicas.* Although it should be mentioned that there is scarce information on the replacement of FM by FMA in diet of any type of fish. Previously it was demonstrated that inclusion of 25% of feather meal in the diet of juvenile tench, *Tinca tinca*, showed significantly lower growth performance (total length, total weight, SGR and FCR) than the control group with no replacement (González-Rodríguez et al. 2014). One of the main ingredients of FMA is feather meal, thus, our results could be in consistence with findings of the mentioned research. Most individual animal protein sources, such as meat and bone meal, feather meal, blood meal, and poultry by-product meal, have been able to replace less than 50% of FM in diets of salmonids. Fowler (1991) found that poultry by-product meal could consist of 20% of a practical diet for chinook salmon *Oncorhynchus tshawytscha*, with concurrent reduction of the FM by 50%, without impairing growth and feed efficiency. In another study, MAB (mixture of animal by-product ; mixture of leather meal, meat & bone meal, feather meal,

squid liver powder, poultry by-product meal and blood meal) was used as fishmeal replacer in juvenile rainbow trout, and the result indicated lower WG, SGR, FE and PER for 20% of replacement level (Lee et al. 2001). These results are significant because it is the first time, to our knowledge, that six animal protein sources, leather meal, poultry by-product, feather meal, tuna by-product, blood meal and squid liver powder, have been shown to be effective FMA components in replacing FM in fish. Feather meal was reported as a minor replacer for FM in diets for coho salmon *O. kisutch* (Higgs et al. 1979), chinook salmon (Fowler, 1982), and Nile tilapia (Bishop et al. 1995). Blood meal was also studied in rainbow trout (Luzier et al. 1995), eel (Lee and Bai, 1997), and tilapia (Lee and Bai 1998). Ma et al (2014), indicate that more than 21% fish meal must be retained in diets for golden pompano, *Trachinotus ovatus*, when poultry by-product meal is used alone as a fish meal substitute. Also, the present study demonstrated that the dietary additives produced different response levels in terms of growth, feed utilization and immunity in juvenile Japanese eel. The results demonstrated a more positive response of juvenile Japanese eel to Song-gang® stone (SG) and Yucca Meal (YM) compared to control diet. Fish fed FMA_0SG, FMA_0YM, $FMA_{20}SG$ and $FMA_{20}YM$ diets exhibited significantly higher WG, SGR, FE and PER compared to fish fed FMA_{20} diets but they were not significantly different between FMA_0, FMA_0SG, FMA_0YM, $FMA_{20}SG$ and $FMA_{20}YM$ diets. Growth enhancement of several fish species by Yucca extract

additives have been reported previously (Kelly & Kohler, 2003; Gaber, 2006; Güroy et al. 2016). For Nile tilapia, growth was promoted by a diet containing Quillaja saponin with 150 mg kg^{-1} inclusion level (Francis et al. 2001). Another study showed that Striped catfish (*Pangasianodon hypophthalmus*) fed on a diet containing 0.15% *Yucca Schidigera* extract had improved growth performance as compared to the control diet (Güroy et al. 2014). Consistent with these results are the findings that showed 150 mg kg^{-1} *Quillaja saponaria* inclusion level in the diet increased 18% of average weight for common carp, *Cyprinus carpio* (Francis et al. 2002). In addition, Yucca inclusion enhanced feed utilization as revealed by Gaber (2006) in which plant-protein-based diets supplemented with Yucca had a higher apparent protein digestibility coefficient and increased whole body protein content of Nile tilapia. The observations are comparable to findings by Lee et al. (2015), Won et al. (2016) and Shakhar et al. (2015), with yellow loess and Song-gang® stone in rainbow trout *Onchorynchus mykiss* and dietary Macsumsuk® in Nile tilapia *Oreochromis niloticus*, respectively. It has been suggested that several plant extracts and minerals boost lipid metabolism that breakdown body fatty acids as the main energy source and this results in an efficient protein accretion and more growth (Ji et al. 2007). According to our results more than 10% replacement of FM resulted in reduction in growth, the lower growth performance is probably related to the inferior quality of the animal protein sources, while leather meal and squid liver powder seem to have potential as animal protein

sources in fresh water fish feed (Lee et al. 2001). Sugiura et al. (1998) reported that the levels of Ca and ash in salmonid diets formulated with animal by-product sources were inversely correlated to the percentage of net absorption of Ca, Fe, and Mn, indicating possible antagonistic interactions of Ca with these minerals. When FM is replaced with combined ingredients, the interpretation of results is difficult because many interactions between nutrients may be involved in nutrient metabolism. Consequently, the findings of the present study are in agreement with previous reports and suggest that up to 10% of FM replacement by FMA could be possible and YM and SG could be used as a natural feed additive with FMA in juvenile Japanese eel diets to improve growth performance.

Measurement and analysis of condition factor (CF), hepatosomatic and viscerosomatic indices (HSI and VSI) are important in assessing food value in an animal, its health status and general well-being and the degree of pollutants in the environment where the animal lives (Ighwela et al. 2014). In the present study, there were significant differences in HIS between the control and FMA40 group. Less differences in fish fed the control diet and other experimental diets, is an indication of good conditions and health status of fish during the feeding trial. Abdel-Warith et al. (2001) found that liver tissue showed alterations in hepatic structure when African catfish were fed diets containing high levels of poultry by-product meal. Thereby, future studies that focus on evaluating the rendered animal protein ingredients to replace FM could also include histological

examination of live tissue of fish fed the experimental diets. The high survival rate recorded during this study could be attributed to high acceptability of the experimental diets by the juvenile Japanese eel.

Whole body proximate composition in fish fed all diets were not significantly affected after whole body proximate analysis. Gaber (2006), reported higher body protein and low body lipid levels in Nile tilapia fed Yucca supplemented diets compared to those of the control diet. In a related study, Yilmaz et al., 2012 also reported lower crude lipid levels in sea bass, *Dicentrarchus labrax,* fed herbal extracts compared to the control diet. According to these studies, plant extracts could reduce the crude lipid contents in cultured fish. But these observations were in contrary to our results and the reason could be related to different diet compositions and culture conditions.

Non-specific immune response is a fundamental defence mechanism in fish and also plays a key role in the acquired immune response and homeostasis through a system of receptor proteins (Halver and Hardy, 2002; Uribe et al. 2011; Reverter et al. 2014; Srivastava and Pandey, 2015). Lysozyme is produced mainly by macrophages in response to microbial components and many other immune stimulants (Siwicki and Anderson, 1993; Ringø et al. 2012) and it is a preferred marker of the immune response due to its close association with leucocytes (Kiron, 2012). SOD on the other hand, is a metallo-enzyme that catalyses the dismutation of the superoxide radical (O_2) into hydrogen peroxide (H_2O_2) and

molecular oxygen (O_2) (Shao et al. 2010), providing an important defence against oxidative damage. In the present study, no significant differences in the amount of SOD was observed. But lysozyme activity of fish fed diets consisting of SG and YM were significantly higher than other diets. Improved immunity have been recorded in cultured fish with several feed additives such as herbal extracts (Chelladurai et al. 2014), yellow loess (Lee et al. 2015), Song-gang® stone (Lee et al. 2015) and Yucca meal (Njagi, 2016). Ji et al. (2013), demonstrated that 70% replacement of FM by silkworm pupae meal in diet of juvenile Jian carp (*Cyprinus carpio*) significantly decreased non-specific immune responses. These results are partly comparable with our results showing the beneficial effects of SG and YM for improving non-specific immune responses.

Haematological and serum parameters are good indicators of the health status of an organism and they can vary with season, temperature and nutritional status (Blaxhall, 1972). Lee et al. 2016 suggested that any unhealthy condition caused by poor nutrition could affect the haematological characteristics of fish. Glucose is one of the stress indicators in fish. Stress hormones in conjunction with cortisol, mobilize and elevate glucose production in fish to cope with the energy demand of a stressor (Wallace et. al. 1994). Total plasma protein is considered a strong innate response in fish (Reverter et al. 2014) and measurements can reflect nutritional status (Acharya and Mohanty, 2014). It is the protein component of the blood and it increases with starvation or any other stress. Plasma GOT and GPT

on the other hand, are proteins or enzymes found mainly in liver cells and elevated levels in the blood indicate an inflammation or damage of liver cells (Giannini et al. 2005). In the present study, no significant differences were recorded in all serum parameters in juvenile Japanese eel fed the experimental diets, indicating good health status of the fish. Challenge test, support the results of the immune responses such as lysozyme.

In summary, the results indicated that WG, SGR, FE and PER were significantly different in fish fed FMA_0 (control diet) and fish fed diets FMA_{20}, FMA_{30} and FMA_{40}. This is while no significant differences were observed between fish fed FMA_0, FMA_{10}, FMA_0SG, FMA_0YM, $FMA_{20}SG$ and $FMA_{20}YM$ diets. Whole body proximate composition of fish did not show significant differences among groups. Fish fed diets consisting of SG and YM showed a higher lysozyme activity than all other diets that replaced FM. Also, there were no significant differences in the haematological indexes among fish fed all the experimental diets. The cost of each diet per kilogram based on different additives and the supplementation level was: ₩2,027 ₩1,958 ₩1,888 ₩1,818 ₩1,748 ₩2,031 ₩2,044 ₩1,892 ₩1,905 for FMA_0, FMA_{10}, FMA_{20}, FMA_{30}, FMA_{40}, FMA_0SG, FMA_0YM, $FMA_{20}SG$ and $FMA_{20}YM$ diets, respectively. Therefore, based on results for WG, SGR, FE, PER, Lysozyme, SOD, and haematological indexes, FMA could replace up to 10% of FM in diet. But with inclusion of dietary song-gang® stone and combination of song-gang stone and yucca meal, FMA could

replace up to 20% of FM in diet of juvenile Japanese eel. However, considering the local availability and cost/kg of diet, song-gang stone is proposed to be a more practical feed additive for juvenile Japanese eel culture.

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VII. Appendix

Initial data

	Tank No	Total Weight	Number	Individual weight	Total feeding	Individual feeding
FMA_0	29	132.4	15	8.8	2.65	0.09
	19	133.1	15	8.9	2.66	0.09
	14	134.8	15	9.0	2.70	0.09
FMA_{10}	27	136.2	15	9.1	2.72	0.09
	10	133.8	15	8.9	2.68	0.09
	3	134.3	15	9.0	2.69	0.09
FMA_{20}	11	134.4	15	9.0	2.69	0.09
	4	137.3	15	9.2	2.75	0.09
	20	136.4	15	9.1	2.73	0.09
FMA_{30}	28	132.2	15	8.8	2.64	0.09
	17	133.9	15	8.9	2.68	0.09
	15	134.8	15	9.0	2.70	0.09
FMA_{40}	9	135.1	15	9.0	2.70	0.09
	23	137.6	15	9.2	2.75	0.09
	1	132.3	15	8.8	2.65	0.09
FMA_0SG	5	135.4	15	9.0	2.71	0.09
	26	133.5	15	8.9	2.67	0.09
	25	134.8	15	9.0	2.70	0.09
FMA_0YM	6	133.9	15	8.9	2.68	0.09
	18	134.2	15	8.9	2.68	0.09
	7	134.7	15	9.0	2.69	0.09

$FMA_{20}SG$	16	135.1	15	9.0	2.70	0.09
	24	133.7	15	8.9	2.67	0.09
	22	136.9	15	9.1	2.74	0.09
$FMA_{20}YM$	13	134.9	15	9.0	2.70	0.09
	8	138.4	15	9.2	2.77	0.09
	2	132.2	15	8.8	2.64	0.09

8th weeks data

	Tank No	Total Weight	Number	Individual weight	Total feeding	Individual feeding
FMA_0	29	229.7	12	19.1	141.7	11.51
	19	258.6	13	19.9	142.4	12.12
	14	176.7	9	19.6	144.2	11.91
FMA_{10}	27	233.1	12	19.4	145.73	11.84
	10	241.3	13	18.6	143.17	11.01
	3	251.7	13	19.4	143.70	11.90
FMA_{20}	11	231.2	12	19.3	143.81	12.31
	4	256.7	14	18.3	146.91	10.99
	20	224.5	12	18.7	145.95	12.75
FMA_{30}	28	213.9	12	17.8	141.45	11.79
	17	215.9	13	16.6	143.27	11.02
	15	210.1	12	17.5	144.24	12.02
FMA_{40}	9	191.1	12	15.9	144.56	12.05
	23	158.1	10	15.8	147.23	14.72
	1	179.6	11	16.3	141.56	12.87
FMA_0SG	5	282.3	14	20.2	144.88	10.35
	26	238.1	12	19.8	142.85	11.84
	25	243.1	12	20.3	144.24	11.96
FMA_0YM	6	198	10	19.8	143.27	11.74
	18	255.1	13	19.6	143.59	11.16
	7	220.3	11	20.0	144.13	11.98

$FMA_{20}SG$	16	255.7	13	19.7	144.56	11.96
	24	231.9	12	19.3	143.06	11.92
	22	278.5	14	19.9	146.48	11.48
$FMA_{20}YM$	13	236.1	12	19.7	144.34	12.03
	8	220.7	11	20.1	148.09	12.01
	2	230.8	12	19.2	141.45	11.79