Thesis for the Degree of Master of Fisheries Science

Effects of different dietary fishmeal analogue levels in Japanese eel, *Anguilla japonica*

by

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Pukyong National University

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뱀장어 사료 내 어분대체품의 수준별 첨가 효과

Advisor: Sungchul C. Bai

by

Jim Hyacinth Damusaru

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뱀장어 사료 내 어분대체품의 수준별 첨가 효과

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요약

본 실험은 뱀장어 사료 내 어분대체품 (FMA, Fishmeal Analogue) 의 수준별 첨과 효과를 규명하기 위해10주간의 사육 실험이 수행되었다. 평균무게119.61 ± 0.97g (mean ± SD) 인 육성기 뱀장어를 대상으로 한 실험에서 대조구를 포함한 5개의 실험구가 사용되었다. 어분을 대체하지 않은 대조구 (FMA_0), 어분을 FMA로 10% (FMA_{10}), 20% (FMA_{20}), 30% (FMA_{30}) 또는 40% (FMA_{40}) 대체하여 실험사료를 조단백질 및 총 에너지가가 동일하게 제작하였다. 실험종료 후, 증체율과 일간성장률은 FMA_0, FMA_{10}, FMA_{20} 실험구 간에는 유의적인 차이가 없었으며 ($P > 0.05$), 사료효율과 단백질전환효율은 FMA_0 와 FMA_{10}실험구 간에 유의적 차이가 없었다 ($P > 0.05$). 증체율의 Broken line regression 분석을 통한 적정 어분 대체율은15.39%로 나타났다. 혈액분석을

통해서 ALT는 FMA_0, FMA_{10}, FMA_{20}이 FMA_{30}, FMA_{40}보다 유의적으로 낮았으며 ($P < 0.05$), 글루코스 농도는 FMA_0, FMA_{10}, FMA_2간에 유의한 차이가 발생하지 않았다 ($P > 0.05$). 생존율, 간중량지수, 내장중량지수, 비특이적 면역반응 (superoxide dismutase, lysozyme activities 와 myeloperoxidase) 에 있어서 전체실험구간에 유의적인 차이를 보이지 않았다 ($P > 0.05$). 장 조직학적 분석에 있어 FMA_{30}와 FMA_{40}가 FMA_0 와 비교했을 때, 장내 비정상적인 융모배열 (fold breaks, dilation 와 villi dwarfness) 을 나타냈다. 따라서 육성기 뱀장어 사료 내 어분대체품의 최적대체구간은15.39-20%로 사료된다.

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Abstract

A 10–week feeding trial was conducted to evaluate the effects of different dietary fishmeal analogue (FMA) levels as a fishmeal (FM) replacer in Japanese eel, *Anguilla japonica*. Triplicate groups of 13 fish with an average weight of 119.61 ± 0.97g (mean ± SD) were fed one of the five experimental diets replacing FM with FMA at 0, 10, 20, 30 or 40% (FMA_0, FMA_{10}, FMA_{20}, FMA_{30}, and FMA_{40}, respectively). All experimental diets were prepared to be isonitrogenous and isocaloric. At the end of the feeding trial, weight gain and specific growth rate of fish fed FMA_0, FMA_{10} and FMA_{20} were significantly higher than those of fish fed FMA_{30} and FMA_{40} diets ($P < 0.05$). Broken-line regression analysis, however, indicated the optimum FM replacement level of 15.39% for FMA. Intestinal histological analysis of fish fed diets FMA_{30} and FMA_{40} showed abnormal villi structural arrangements (fold breaks, dilation and villi dwarfs) when compared to those of fish fed with FMA_0, FMA_{10} and FMA_{20} diets (with normal and elongated villi). However, survival, hepatosomatic index, viscera somatic index, and non-specific immune parameters (superoxide dismutase, lysozyme activities and

myeloperoxidase) showed no significant differences among fish fed with all the experimental diets ($P > 0.05$). Results indicated that the optimum FM replacement level could be greater than or equal to 15.39% but less than 20% in Japanese eel, *A. japonica*.

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I. Introduction

Japanese eel, *Anguilla japonica*, is one of the most traditionally valued and cultured freshwater fish species in East Asia. It has a high demand and market value as well as great appetite amongst South East Asian population (Lee and Bai, 1997; Ohta et al., 1997; Okorie et al., 2007; Shahkar et al., 2015). In 2015, the total global commercial production of eel was recorded at 266,731 metric tons (MT), valued at US$1,416 million (M) with an average market price of US$25/kg (FAO, 2017). Despite fluctuations in production and price, because of shortage in glass eel supply from the wild, the Korean Republic, still produced 9,009 MT in 2015, with an average market value of US$233 M (KOSTAT, 2017). Amongst other East Asian glass eel producing countries, Korean Republic, in 2014, has been producing 10 MT with a market value of US$110,000 at local price of US$11/kg, with China producing, ahead of Japan and Taiwan with 40, 25 and 3 MT, respectively (FAO, 2016). As a carnivorous fish, Japanese eel prefers to inhabit estuaries, rivers, upper rivers streams and mountain lakes (Okazaki, et al., 2016; Hsu et al., 2016). In the wild, the adults' diets consist mostly of benthic crustacean (crabs), worms, bony fish, insects, clams and frogs while the leptocephali (eel larvae or glass eel) feeds mainly on microscopic plants and animals (Okazaki, et al., 2016; Hsu et al., 2016; Wakiya et al., 2016). As catadromous, Japanese eel begins its life in the Western North Pacific region near West Mariana Ridge, where it spawns and grows into leptocephali before being transported by North Equatorial Current into Kuroshio Current to its coastal recruitment regions in South East Asia (Okazaki et al., 2016; Hsu et al., 2016; Arai, 2016; Aoyama, et al., 2014). The production of eels is based on wild catches of glass eels around the shores of China, Taiwan, the Republic of Korea and Malaysia.

Glass eels are then raised to elvers, ready to be exported to eel farmers in other countries. Grow out of elvers to market size can be achieved in either tank systems, earthen ponds or even in recirculating aquaculture systems (RAS) (Kagawa et al., 2006; Suzuki, 2003). Currently, researchers have been focusing on improving farming systems and nutritional requirements (essential fatty acid, amino acids, vitamins, and minerals) for eels as successes have been made on producing glass eel via artificial fertilisation (Kagawa et al., 2006; Ohta et al., 1997).

The next step is to create hatchery for eels (FRAJ, 2016). Raising hatchery-produced juveniles require cheaper but good quality formulated feed. Vitamin C, E and arachidonic acid (ARA) dietary level of 41.1 to 43.9 mg kg^{-1} diet, 21.2 to 21.6 mg kg^{-1} diet and 0.69–0.71% have been estimated for juvenile eel, respectively. Likewise, vitamin C, E and arachidonic acid dietary level of 410.7 to 911.7 mg kg^{-1} diets, 212.9 to 199.7 mg kg^{-1} diets and 0.71–0.92% have been calculated for male broodstock, respectively (Bee et al., 2013, 2012, and 2010). Broadly, eel feeds are high in carbohydrate (~22%) and fishmeal content (65–70%) and crude protein (CP) level of ~50% (FAO, 2012; NRC, 2011, 1993).

Traditionally, fishmeal (FM) (66% CP) is the prime source of quality protein used in aquaculture diets, however, it is limited and increasingly costly (Tomas et al., 2005; Anderson et al., 2016; FAO, 2016). Conversely, alternative protein source can ease these problems by lowering the cost of aquaculture diets, reducing the amount of wild fish used as protein, and potentially reduces the nutrient levels in effluent waste (Van Weird et al., 1999; Papatryphon and Soares, 2001; Masumoto et al., 2001). However, for most species, there is a limit to the amount of FM that can be replaced by alternative protein sources without causing any negative effect on the health and physiology of fish.

Currently, animal by-products (ABP) are frequently used as aquafeed ingredients to substantiate the short supplies and increasing cost of FM. ABP protein content is higher than 50% and their complement of indispensable amino acids is superior to those of plant and animal origin. ABP are also less expensive than FM. Nevertheless, most single ABP, as well as single plant protein sources, are unable to completely replace FM. However, combination of different ABP and plant protein sources have shown to effectively enrich the diets' nutritional profile, thus, making the diet become complete and balanced than any single protein source in isolation (Glencross, 2016; Nates and Swisher, 2015; Bae et al., 2012; Wang, 2006; Zhang, 2006; Kureshy et al., 2000). Products based on this are referred to as 'fishmeal analogues' (FMAs). FMA used in this study contains ingredients viz; leather meal (LEM), poultry by-product (PBP), feather meal (FEM), soybean meal (SBM), blood meal (BM), fish oil (FO), squid liver powder (SLP) and crystalline amino acids of lysine and methionine (Table 2). Many studies have been done on the beneficial effects of FMAs using blended protein sources with other alternative ingredients that could prevent nutritional deficiencies and ensure a proper supply of essential nutrients in fish. The authors also reported that the combination of plant and animal protein sources, plus the addition of amino acids may help improve potential dietary deficiencies that could adversely affect fish performance and physiology (Richard et al., 2017; Herath et al., 2016; Nates and Swisher, 2015; Lunger et al., 2007; Yigit et al., 2006).

A concentrated mixture of animal and plant protein sources such as SBM, PBP, BM, LEM, and FEM have been extensively used as FM replacers in the diets of many freshwater fish species. The following replacement levels have been done using various animals and plants protein sources; Jo et al., 2016 (12% FMA in shrimp soluble extracts

in rainbow trout, *Oncorhynchus mykiss*); Kirimi et al., 2016 (50% BM in tilapia, *Oreochromis niloticus*); Kasper et al., 2007 (47.6% SBM in yellow perch, *Perca flavescens*); Engin and Carter, 2005 (23% SBM + lupin meal (LUM) + corn gluten meal (CGM) + meat meal (MM) in Australian short-finned eel, *Anguilla australis australis*). Accordingly, Bureau et al. (2000); Cheng et al. (2003); Khan et al. (2003); Van Weird et al., 1999; Masumoto et al. (2001); Papatryphon and Soares (2001) have studied the use of PBP, FEM, BM, SBM, addition of enzymes and amino acids (phytase) that provide good mixture of protein and amino acids enrichment in fish diets and feeding without any significance retardation on health and growth of the fish.

Additionally, several studies have shown using BM, bone meal (BOM), LEM, FEM alone or as FMA for fishmeal replacers in freshwater and saltwater fish, yielding quite similar results; Zhang, 2006 (20% MBM in gibel carp, *Carassius auratus gibel*); Yang et al., 2004 (50% MBM + BM in gibel carp, *C. auratus gibel*); Yanik et al., 2003 (47% BM + BOM + PBP in rainbow trout, *O. mykiss*); Bureau, 2000 (24% MBM in Nile Tilapia, *O. niloticus*); Kikuchi et al. (1994) (12–25% FEM in Japanese Flounder, *Paralichthys olivaceus*).

In recent years, numerous experiments on FM replacement have focused on using dietary FMA or single ingredients as a replacer for fishmeal. Nevertheless, none of the past studies has mentioned specifics of dietary FMA replacement level for fishmeal in the growing Japanese eel, *A. japonica*. Only a few researchers have noted a few studies on fishmeal protein replacement levels using animal by-products and plants protein sources for other related eel fish species viz; European eels, *Anguilla Anguilla* (Gallego et al., 2010), Australian short-finned eel, *A. australis australis* (Richardson) elvers, (Engin and

Carter, 2005), juvenile European eels, *A. anguilla*, (Gallaher et al., 1998) and juvenile Japanese eel, *A. japonica*, (Lee and Bai, 1997). Because of this, there is still a need to conduct an experiment to evaluate the growth performance and the fishmeal protein replacement level using FMA formulated diets for the growing Japanese eel as a freshwater fish.

This study is to further the aquaculture potential of Japanese eel by investigating their growth performance on dietary FMA containing alternative and qualitative protein sources for animals' protein synthesis. Thus, the objective of this study is, therefore, to determine the maximum substitution limit of FM by dietary FMA containing significant compositions of SBM, LEM, FEM, PBM, BM and trace amounts FO, SLP, lysine, and methionine in the growing Japanese eel, *A. japonica*.

It was hypothesized that replacement of FM by dietary FMA levels containing SBM, LEM, FEM, PBM, BM and trace amounts of FO, SLP, lysine, and methionine in Japanese eel reared in an indoor semi-controlled recirculating system, will have no negative effect on the biological performance (growth), feed efficiency and utilization or amino acid composition of the fish.

II. Materials and Methods

This experiment was conducted at the Pukyong National University (PKNU) Aquaculture Facility at the Department of Bio-Materials and Aquaculture in Daeyeon Campus, Busan, South Korea from March 29 to June 24, 2016. Results analysis were done at the Feeds and Foods Nutrition Research Centre (FFNRC) at Yongdang Campus in Busan, South Korea.

2.1 Experimental design and diets

The experiment system consists of fifteen (15) 250-L cubical tanks which are supported by semi-recirculating aquaculture system located in an indoor, climate-controlled laboratory. Each tank receives filtered fresh water at the rate of 3 to 4 L min^{-1}. Water quality was maintained by exchanging water (50%) in the reservoir with filtered fresh water and recirculating it back into the system containing the fifteen tanks. Water replacement was done 2 to 3 times a week. Tanks were subjected to natural photoperiod conditions and ambient light levels from sunlight entering the laboratory windows. This was achieved by regularly covering the tanks with black polyethylene plastic. Water temperature was maintained at 25 ± 1 ºC by heaters in the reservoir tanks during the whole experimental period. Aeration is provided by placing stone bubbles in each tank to maintain oxygen levels near the standard aeration. Tanks are siphoned daily or as needed.

At the start of the experiment, each tank was stocked with eels at a density of 6.19 kg m^{-3} (13 eels per tank, 547 g / 250-L tank). The fish were acclimatised to environmental conditions and were fed the basal diets (65% CP) for four weeks, and afterward, their initial weight was recorded before the start of the feeding trial. The fish initial mean

weight was 119.61 ± 0.97 g (mean ± SD) with no significant ($P > 0.05$) differences among each treatment.

Five experimental diets were formulated (Table 1) with 100% FM + 0% FMA (FMA_0, diet 1) as control diet, 90% FM + 10% FMA (FMA_{10}, diet 2), 80% FM + 20% FMA (FMA_{20}, diet 3), 70% FM + 30% FMA (FMA_{30}, diet 4) and 60% FM + 40% FMA (FMA_{40}, diet 5). These substitution levels were determined by consulting published digestibility data on other freshwater finfish by Jo et al. (2016); Bae et al. (2013, 2012, and 2010); Lee and Bae (1997). All diets were formulated to have the same energy (3,800 $kcal.kg^{-1}$), protein percentage (50% CP), lipid level (8%), carbohydrates (26%), fibre (0.40%) and ash (8.20%) (Table 1). All diets were fortified with vitamin and mineral premix. Corn starch was used as a binder as fish oil and soybean oil were used as lipid sources. Additionally, fishmeal, gluten meal were used as protein sources; corn starch as a source of carbohydrates in the experimental diets (Table 1). FMA ingredients and compositions are shown in Table 2.

Diet preparation was done by mixing the dry ingredients in an electric mixer for 5 to 10 min. Filtered water was added slowly until the diet reached the desired moisture and texture level. The amount of water added ranges from 150 to 200 ml. The diets were mixed for another 10 min to texturally incorporate the water. Equal amounts of FMA were substituted along with the proper amount of water in the basal diet. The mixture was then directly doughed and pelleted by passing it through a 1 mm screw-type pelleting machine. The pellets were then air dried for approximately 72 h. After drying, the pellets were placed in labelled Ziploc bags and stored in refrigerators at -20 °C until the actual feeding. This procedure was repeated once (after the fifth week) to create a

second batch of diets, this time with slightly larger pellets (2 mm) used to feed the larger fish. Diets were assigned to each tank at random, with three replicate tanks for each of the five diets. The experiment was conducted for 10 weeks.

2.2 Experimental fish and feeding trial

Japanese eels were purchased and collected from Kyung-gi Aquafarm and were transported directly to Pukyong National University in Busan, both in the Republic of Korea where they were acclimated to experimental conditions for four weeks. During the preconditioning period, fish were raised in semi-recirculating system and fed a basal diet of fishmeal containing 65% protein and 8% lipid until the actual feeding commences.

Fish were fed to apparent satiation twice daily (09:00 and 18:00 h) at the rate of 0.5% of wet body weight per day at the beginning of the first two weeks, 0.7% at third and fourth week and 1.5% of wet body weight per day for 6 weeks until the end of the experiment. Feeding was done during the week, except on Sundays. Fish were considered satiated when the food began to accumulate on the bottom of the tank after approximately 45 min of gradual hand feeding. To monitor the amount of feed administered, each tank had its own labelled containers. The food was weighed before being added to the container. Apparent feed intake was calculated. The total body weight of fish in each tank was determined at the fifth week and the amount of diet fed to the fish was adjusted accordingly as previously stated.

2.3 Sample collection and analysis

At the end of the feeding trial, fish were starved for 24 h, anaesthetized, counted and

weighed for the calculation of weight gain (WG), feed efficiency (FE), specific growth rate (SGR), protein efficiency ratio (PER) and survival rate (SR). Three (3) fish from each tank were pooled by triplicate groups and stored frozen for the analysis of fish whole body composition. The proximate composition categories viz; moisture, ash, crude lipid and protein content of the fish whole body and viscera, plus the experimental diets were analysed using the renowned methods described in AOAC (1995, 2005) as previously stated.

In determining the moisture content, samples of diets and fish whole body composition are dried to a constant weight at 125 ºC for 2 h. Samples are weighed before and after freeze-drying. Moisture content is calculated using the formula below.

$$\text{Moisture} = \left(\frac{\text{Initial mass-Final mass}}{\text{Initial mass}} \right) \times 100$$

The ash content is determined by weighing the samples before and after placing them in a muffle furnace for approximately 4 h or until powdery white. The samples are allowed to cool for 30 min and are then placed directly in a desiccator for additional 30 min, to enhance further cooling to room temperature and thus ensuring constant weight before the final mass is taken. The formula bellow is used to calculate the ash content.

$$\text{Ash} = \left(\frac{\text{Initial mass-Final mass}}{\text{Initial mass}} \right) \times 100$$

To determine the crude lipid content, the Soxtec system 1046 (Tacator AB, Hoganas, Sweden) was used to extract the lipid from the dried samples. Approximately 1 g of the sample was placed in the cellulose thimble and extracted using 150 ml petroleum ether

solvent using the Soxtec unit system by following the standard Soxhlet method as described in AOAC (2005, 1995). The unit system was heated in a water bath for approximately 1.5 h after which time the solvent was evaporated within the system's kiln. The flasks are then placed in a drying oven for 30 min to remove the water. After cooling, the flasks are then placed directly in the desiccator for further cooling for another 30 min. Finally, the flasks are weighed and the lipid content is calculated using the following formula.

$$\text{Lipid} = \left(\frac{\text{Final flask mass-Initial flask mass}}{\text{Initial sample mass}} \right) \times 100$$

The crude protein was determined by using Kjeldahl method (N x 6.25) after acid digestion. Approximately 1 g of the sample was weighed, mixed with 10 g of KSO_4, 0.7 g of HgO and 20 ml H_2SO_4 and are placed in flasks in the boiling unit for 30 min to allow complete acid digestion. The system is cooled by its auto-continuous addition of water. After boiling the flasks were further cooled at room temperature for another 30 min and after were distilled with 50 ml of $NaSO_4$ with an indicator solution. After distillation, the sample solution was then titrated with 50 ml of HCl until the end point is reached (solution color changes from greenish to reddish brown).The crude protein is then calculated by using the following formula.

$$\%\ \text{N in Sample} = 100 \left(\frac{\text{HCl in titration (ml)-normality of standard acid}}{\text{Weight of the sample (g)}} \times 0.014 \right)$$

Crude protein = % nitrogen in sample (N x 6.25)

The amino acid composition analysis of the experimental diets and the fish whole body were conducted by FFNRC Service Laboratory in Yongdang Campus, Busan, using an automatic amino acid analyzer (JLC-500/v; JEOL, Tokyo, Japan). The samples containing the total amino acids were digested at 110 °C for 18 h with 4 M methanosulfonic acid (Sigma–Aldrich, St. Louis, MO, USA). Right after, the digested solution was delivered over a 0.45-μm membrane filter into micro-bottles as it was injected into the analyzer connected to a computer for computation of the acid contents.

For diet evaluations, crude proteins, lipids, ash, and moisture were analysed as described above, except for the moisture content. Moisture content was determined using the oven, instead of the freeze drier. Sample are weighed before and after drying in the oven at 125º C for 2 h and then moisture content are determined using the same formula. Amino acid compositions are determined using the methods described above.

2.4 Growth performance and survival

Mortalities were recorded as they occurred and the fish were lot weighed once in the fifth week. After 10 weeks, the final fish biomass was weighed and five (5) fish were sampled from each tank for biochemical analysis. Three (3) of the fish were used for the whole body proximate analysis and one (1) fish was dissected for its muscle, liver and digestive organs (intestines) for organosmatic measurements. Prior to sampling, fish were starved for 24 h and then immediately anaesthetized with 90% (200 ppm) ethylene glycol phenyl ether, before being weighed and counted. The fish growth performance is calculated as weight gain (WG), specific growth rate (SGR), feed efficiency (FE), protein

efficiency ratio (PER) and survival rate (SR) using the following standard equations (AOAC, 1995, 2005; Tibaldi et al., 2006).

$$\text{WG (\%)} = \left(\frac{\text{Final body weight-Initial body weight}}{\text{Initial body weight (g)}} \right) \times 100$$

$$\text{SGR (\% day}^{-1}\text{)} = \left(\frac{\text{In (Mean final weight)-In (mean initial weight)}}{\text{Number of days (76 d)}} \right) \times 100$$

$$\text{FE (\%)} = \left(\frac{\text{Wet weight gain (g)}}{\text{Dry feed intake}} \right) \times 100$$

$$\text{PER} = \left(\frac{\text{Wet weight gain (g)}}{\text{Total protein intake in dry basic (g)}} \right) \times 100$$

$$\text{SR (\%)} = \left(\frac{\text{Final number of fish}}{\text{Initial number of fish}} \right) \times 100$$

2.5 Biochemical analysis

2.5.1 Organosomatic and hematological parameters

For organosomatic sampling, one (1) fish randomly selected from each tank was independently weighed, has its total length measured and then was directly dissected to isolate the liver and viscera for use in the computation of condition factor (CF), hepatosomatic index (HSI) and vicerosomatic index (VSI). Livers and viscera were then properly persevered in a sealed plastic bottle containing formaldehyde solution in

preparation for later histological analysis.

For hematological analysis, one (1) fish randomly selected from each tank has its blood collected from the caudal vein by using heparinized syringes and directly after, samples of blood plasma were separated and collected by centrifuging them at 3,800 x g for 5 min. The extracted serum was then stored at -70 ºC for the scaling of blood biochemical parameters such as total plasma glucose (GLU), total cholesterol (TCHO), triglycerides (TG), aminotransferase (AST), alanine aminotransferase (ALT), and total protein (TP). This same set of serum was also used for the analysis of non-specific immune responses for the fish lysozyme, superoxide dismutase (SOD) and myeloperoxidase (MPO) activities.

The total glucose, protein, cholesterol, and activities of AST and ALT were measured by using chemical analyser (FujiDRI-CHM 3500i, Fuji Photo Film, Ltd, and Tokyo, Japan).

2.5.2 Histological analysis

Histological analysis of the cross-transversal section of the viscera of the fish was done by the observation of intestinal villi structural arrangements (mucosa) by following the method described by Wang et al. (2016) and Peng et al. (2013). Briefly, the fixed distal guts were prepared, persevered in formaldehyde solution, washed and dehydrated with gradient alcohol; then, the guts were paraffin embedded, sectioned and stained with haematoxylin and eosin. Histological observation was done qualitatively on morphological parameters including microvillus height (HMV), muscular thickness (MT), fold height (HF) and enterocyte height (HE), abnormal submucosa, presence of *lamina propia* (LP), central lacteal (CL), goblet cells (GC), absorption vacuoles (AV), the

appearance of the villi structures and mucosal folds by using light microscope (Olympus, DP72) at a magnification of x100 (a, b & d) and x300 (d & e) at the scale of 100 µm) (Fig 11).

2.5.3 Non-specific immune responses

Serum lysozyme activity was measured using a modified plate technique suggested by Ellis (1999). For this measurement, a standard suspension of 0.72 mg ml^{-1} *Micrococcus lysodeikticus* (Sigma) was prepared in 50 mM phosphate buffer (0.1 M, pH 5.2). A 50 µl of bacterial suspension was placed into each 96-well plate, and 20 µl serum was subsequently added and is directly incubated thereafter at room temperature for 30 min. The decrease in absorbance was then recorded at 450 nm at 0, 30 and 60 min in a spectrophotometer (Shimaszu UV-1601PC). One unit of lysozyme activity was defined as the reduction in absorbance of 0.001 min^{-1}.

The SOD activity was measured in accordance with the percentage reaction inhibition rate of the enzyme with Water Soluble Tetrazolium dye (WST) substrate and xanthine oxidase using a SOD Assay Kit (Sigman-Aldrich, 19160) following the manufacturer's guidelines for users. Each endpoint assay was evaluated by the absorbance at 450 nm (as the absorbance wavelength for the coloured product of WST reaction with superoxidase) after 15 min of the reaction time at 37 ºC in the spectrophotometer (Shimazdu PC-1601 model). A lysozyme activity unit for 10 cm skin was standardly defined as the amount of enzyme producing a decrease in absorbance of 0.000 min^{-1}.

Measurement of myeloperoxidase (MPO) activity was determined as accorded by Quade and Roth (1997) along with the modification made by Park et al. (2016). Broadly,

20 µl of serum was diluted with HBSS (Hanks Balanced Salt Solution) without Ca^{2+} or Mg^{2+} (Sigma Aldrich) in 96-well plates and was consequently added with 35 µl of 3, 3', 5, 5' tetramethylbenzidine hydrochloride (TMB, 20 mM) (Sigma-Aldrich) and H_2O_2 (5mM). The colour change reaction was halted after 2 min by adding 35 µl of 4 M sulphuric acid. Finally, the optical density reading was recorded at 450 nm in a microplate reader.

2.6 Statistical analysis

After the confirmation of the normality and homogeneity of variance, data were analysed by using the one-way AOVA (SPSS software, version 9.1.3, Chicago, IL, USA) to test for the effects of the different dietary FMA treatments. Given the observance of a significant effect on treatments, a Turkey's honest significant difference test was used to compare means. The treatment's effects were considered at $P < 0.05$ level of significance. Broken-line regression analysis was also used to determine the optimum (economical) FM replacement level by dietary FMA (Fig 7).

III. Results

3.1 Amino acid composition of the diets and fish whole body

Amino acid compositions of the experimental diets and of final (76 d) growing Japanese eel whole body fed the FMA diets are summarised in Table 3 and Table 4 respectively. Among the diets, lysine ranges from 3.39–3.61% of the total amino acids (Table 3). As the dietary FMA substitution level increase, the percentage of lysine composition slightly decreases. Lysine was lowest in FMA_{40} (3.39%) and highest in FMA_0 (3.61%) diet. All diets, including the fish whole body (except fish fed FMA_{20}, 1.20 ± 0.02%, Table 4) contained less lysine than their respective control diets.

Methionine contents of the experimental diets range from 1.15–1.25% of the total amino acids (Table 3). As the dietary FMA substitution level increases, the percentage of methionine composition slightly decreases. A similar trend can also be observed in the fish whole body amino acid compositions (Table 4). There was no large deficit in methionine content observed in all the experimental diet as well as in those of the fish whole body.

Essential amino acid compositions of the experimental diet range from 1.15–3.63% of the total amino acids (Table 3) while the non-essential amino acids range in concentration from 1.05–7.28% of the total amino acids (Table 3). Glutamic acid was the predominant non-essential amino acid, comprising 6.74–7.28% of the total amino acids. Aspartic acid was also found in relatively high concentrations, ranging from 4.18–4.46% of the total amino acids (Table 3). The fish whole body (% of wet matter basis) also reveals a similar trend, however, with slightly lower values (Table 4) when compared to

those amino acid compositions of the experimental diets (% of dry matter basis).

3.2 Survival and growth performance

The survival and growth data of Japanese eel fed the experimental diets with different FMA levels are summarized in Table 5. Survival rate ranges from 90–100% at the conclusion of the 10-week experimentation with no significant ($P < 0.05$) differences among all dietary FMA treatments.

In terms of fish weight, there were no significant differences ($P < 0.05$) found in the fish mean weight among the fish experimental groups at the start of the experiment (wk 0, range, IBW = 119–120 g). However, on wk 10 (FBW), fish mean weights ranges from 146 to 164 g. Mean weight of fish fed the, 0% FMA ($FMA_{0(control)}$), 10 % FMA (FMA_{10}) and 20% FMA (FMA_{20}) diets were significantly ($P > 0.05$) higher than fish fed 30% FMA (FMA_{30}) and 40% FMA (FMA_{40}) diets. (Table 5).

After 10 weeks of feeding trial, WG of the fish fed the FMA_0 (38.11 ± 2.80%), FMA_{10} (37.90 ± 4.37%) and FMA_{20} (35.69 ± 2.05%) diets were significantly higher than those of fish fed the FMA_{30} (28.51 ± 3.99%) and FMA_{40} (25.78 ± 0.63%) diets ($P < 0.05$). However, there were no significant differences in WG of fish fed FMA_0 (the control diet), FMA_{10} and FMA_{20} diets ($P > 0.05$). Likewise, there were no significance differences in WG of fish fed the FMA_{30} and FMA_{40} diets ($P < 0.05$).

SGR displays a similar trend as the WG among the treatment groups with mean growth rate ranging from 0.30–0.42% d^{-1}, and FE of fish fed the FMA_{40} (48.45 ± 1.09%) was significantly lower than those fish fed the other diets, but with no significance difference to those of fish fed dietary FMA_{30}. FE of fish fed the FMA_0 (57.00 ± 2.42%) has the highest percentage composition, nonetheless, there were no significance ($P >$

0.05) differences among fish fed FMA_{10} (55.05 ± 2.12%), FMA_{20} (51.44 ± 3.14%) and FMA_{30} (50.95 ± 2.72%) diets. PER shows a similar trend as FE with a mean value ranging from 0.25 to 0.30 among each dietary FMA treatment (Table 5 and Fig.4). Fish fed FMA_{40} (0.25 ± 0.00) has the lowest PER value and it showed no significance differences ($P < 0.05$) when compared to those of fish fed FMA_{30} (0.26 ± 0.01) and FMA_{40} (0.25 ± 0.00) diets. Fish fed FMA_0 (0.30 ± 0.01) was significantly higher than those of fish fed FMA_{30} and FMA_{40}, however, with no significance differences to those of fish fed diets FMA_{10} (0.29 ± 0.01) and FMA_{20} (0.27 ± 0.01). There were no significance differences in HSI and VSI of fish fed all experimental diets. Fish fed FMA_{40} (0.12 ± 0.01) has a lower CF value than those of fish fed FMA_0 (0.15 ± 0.01) FMA_{10} (0.15 ± 0.01) FMA_{20} (0.13 ± 0.02) and FMA_{30} (0.16 ± 0.03). Additionally, there were no significance differences ($P > 0.05$) among CF value of fish fed diets FMA_0, FMA_{10}, FMA_{20}, and FMA_{30}.

3.3 Proximate composition of the experimental diets

The proximate compositions of the experimental diets with different dietary FMA levels are shown in Table 6. As displayed, there were no significant differences ($P > 0.05$) in the proximate composition of all the experimental diets.

3.4 Whole body proximate composition of the fish

The whole body proximate composition of the fish fed with different dietary FMA levels are shown in Table 7. Fish whole body moisture content ranges from 59.40–63.50% among each treatment, with the lowest moisture value in groups of fish fed diet FMA_{40}

(59.40 ± 2.11%) compared to those of fish fed the other diets. Fish fed FMA_0 (63.50 ± 0.92%) has the moisture value that is significantly higher than those of fish fed dietary FMA_{40}, however, there were no significance differences ($P > 0.05$) when compared to groups of fish fed diets containing FMA_{10} (61.73 ± 1.25%), FMA_{20} (60.80 ± 2.40%) and FMA_{30} (60.33 ± 2.30%). Crude protein contents range from 16.27–16.53% and it showed no significance difference amongst all fish fed the dietary FMA. Crude lipid content ranges from 14.23–14.83% amongst all treatment which also showed insignificant differences. There were no significant differences in crude ash content for all fish fed the experimental diets.

3.5 Hematological parameters

Table 8 shows hematological parameters of the growing Japanese eel fed the experimental diets with different dietary FMA levels. AST of fish fed diet FMA_{30} (90.67 ± 3.06) was significantly higher than those of fish fed diets FMA_0 (67.67 ± 2.52), FMA_{10} (55.00 ± 3.61) and FMA_{20} (59.33 ± 3.61) however, it shows no significance difference ($P > 0.05$) relative to fish fed diet FMA_{40} (86.00 ± 5.29). Fish fed diet FMA_{10} has the lowest AST level. In contrast, there was no significance difference ($P > 0.05$) between fish fed diet FMA_{20} when compared to those fish the control diet (FMA_0). ALT also shows a similar trend as AST. Conversely, for glucose level, fish fed diet FMA_{30} (206.33 ± 7.37) and FMA_{40} (206.33 ± 9.07) were significantly higher than those of fish fed the control diet, FMA_0 (176.67 ± 10.60). However, there were no significance differences ($P > 0.05$) when compared to those of fish fed diets FMA_{10} (195.00 ± 10.00) and FMA_{20} (196.00 ± 7.21). FMA_0 has the lowest glucose level, however, it shows no significance

differences ($P < 0.05$) when compared to those of fish fed FMA_{10} and FMA_{20} diets. Total protein was at max in fish fed diet FMA_{10} (5.53 ± 0.55) among other diets. However, there were no significance differences ($P > 0.05$) with those of fish fed the experimental diets viz; FMA_0 (4.60 ± 0.85), FMA_{20} (4.83 ± 0.47) FMA_{30} (4.80 ± 0.10) and FMA_{40} (4.80 ± 0.44). Fish fed diet FMA_0 (4.60 ± 0.85) has the lowest total protein level, however, there were no significant differences ($P < 0.05$) when compared to those of fish fed FMA_{20} (4.83 ± 0.47), FMA_{30} (4.80 ± 0.10) and FMA_{40} (4.80 ± 0.44) diets. Additionally, there were no significance differences in total cholesterol contents of fish fed the experimental diets ($P > 0.05$). Triglyceride level was lowest in fish fed diet FMA_0 (427.33 ± 6.43) amongst group of fish fed other diets, however, it shows no significance difference ($P < 0.05$) relative to those of fish fed diet FMA_{20} (455 ± 7.00). Triglyceride level of fish fed diet FMA_{40} (480.33 ± 10.79) was significantly higher than to those of fish fed other diets, however, there was no significance difference ($P > 0.05$) when compared to those of fish fed diet FMA_{30} (470.67 ± 2.52). There were also no significance differences in the level of triglycerides for fish fed FMA_{10} and FMA_{20} diets.

3.6 Non-specific immune responses

The non-specific immune responses of the growing Japanese eel fed the experimental diets with different dietary FMA levels are shown in Table 9. The highest SOD value was recorded for fish fed diet FMA_{40} (102.52 ± 0.09), among fish fed other diets. However, they were no significance differences ($P > 0.05$) in comparison to those of fish fed the other experimental diets. Fish fed diet FMA_{10} (97.94 ± 0.03) recorded the lowest SOD level among fish fed other diets. However, they were no significance differences ($P <$

0.05) when compared to those of fish fed the other experimental diets. Likewise, the value of lysozyme activity shows virtually a similar trend as SOD. The value of lysozyme activity of fish fed diet FMA_{40} (0.69 ± 0.01) records the max value, among those fish fed other diets, however, there were no significant differences ($P > 0.05$) on the lysozyme content levels of fish fed the experimental diets, inclusive of those fish fed the control diet (FMA_0). MPO is higher in value in fish fed diet FMA_{10} (2.28 ± 0.19) and low in fish fed diet FMA_{40} (1.99 ± 0.06) relative to those fish fed the control diet, however, there were no significance differences ($P > 0.05$) on MPO levels in fish fed all the experimental diets.

3.7 Histological analysis

The histological analysis of the growing Japanese eel fed the experimental diets for 10 weeks with different dietary FMA levels are shown in Fig. 11. The transversal section of eel's intestinal villi was qualitatively observed in the microscope at a magnification of x100 (Fig 11 a, b, & c) and x300 (Fig 11 d & e) at the scale of 100 μm, showing varying structural arrangements of villi and microvilli of fish fed with different level of FMA diets. Fish fed dietary FMA_0, FMA_{10} and FMA_{20} had spacious (intestinal glands) villus which appears to be elongated and showing wide surface area of villi arrangements, packed with dense goblet cells (GC), normal microvilli and absorption vacuoles (AV) compared to those fish fed dietary FMA_{30} and FMA_{40} which appear to be round and dwarf in shape and with low number of GC and AV, respectively. Also present in fish fed diets FMA_{30} and FMA_{40} are broken folds, severe enlargement of enteritis parameters and the presence of abnormal submucosa (Fig 11.c & d).

IV. Tables and Figures

Table 1. Diet formula and proximate analysis for basal diets with different protein sources (FM, FMA, corn starch, wheat gluten meal (WGM), and soybean oil (SO), and fish oil (FO). All values are calculated as a percentage (%) of the diet's protein unless otherwise noted.

FMA protein replacement levels	0% FMA_0	10% FMA_{10}	20% FMA_{20}	30% FMA_{30}	40% FMA_{40}
Fish meal [Menhaden][1]	65.00	58.50	52.00	45.50	39.00
FMA[2]	0.00	6.70	13.50	20.40	27.20
Corn starch[3]	20.20	20.40	20.50	20.50	20.60
Wheat gluten meal[4]	7.90	7.90	7.90	7.90	7.90
Soybean oil[4]	0.90	0.90	0.90	0.90	0.90
Fish oil[5]	2.20	1.60	1.20	0.80	0.40
Vitamin premix [6]	2.00	2.00	2.00	2.00	2.00
Mineral premix[7]	2.00	2.00	2.00	2.00	2.00
	100.00	100.00	100.00	100.00	100.00
Protein (%)*	50.00	50.00	50.00	50.00	50.00
Fat (%)*	8.40	8.40	8.40	8.40	8.40
Carbohydrate*	26.40	26.40	26.40	26.40	26.40
Fibre (%)*	0.40	0.40	0.40	0.40	0.40
Ash (%)*	8.20	8.20	8.20	8.20	8.20
Gross Energy ($kcal.kg^{-1}$, dry diet)[8]	3800	3800	3800	3800	3800
Price (Won/kg)**	2,026.46	1,960.25	1,895.49	1,829.28	1,763.07
Proximate analysis (% of DM basis)					
Moisture	8.15	7.59	7.70	8.59	8.47
Crude protein	51.85	51.92	51.82	51.40	51.86
Crude lipid	7.03	7.02	7.08	7.22	7.11
Crude ash	11.36	11.14	11.00	11.03	11.08

[1] Norse LT-94®, low-temperature dried fish meal, Norsildmel, Bergen, Norway. Purchased from Su-hyup Feed Co.Ltd., Uiryeong, and Republic of Korea.

[2] Poultry by-product meal (17.00%), leather meal (20.00%), feather meal (16.00%), Soybean

meal (17.60%), Blood meal (19.90%), Fish oil (1.50%), Squid liver powder (5.00%), lysine (2.00%) and methionine (1.00%). See Table 2.

[3] Purchased from Su-hyup Feed Co.Ltd. Uiryeong, Republic of Korea

[4] Purchased from Pung-Chung Co.Ltd., Republic of Korea

[5] Purchased from Dong Suh Oil & Fats, Changwon, Republic of Korea

[6] Contains (as mg kg^{-1} in diets): Ascorbic acid, 300; DL-Calcium pantothenate,150; Choline bitatrate, 3000; Inositol,150; Menadione, 6; Niacin, 150; Pyridoxine HCl,15; Riboflavin,30; Thiamine mononitrate,15; dl-∝-Tocopheral acetate, 201; Rectinyl acetate,6; Biotin,1.5; Folic acid, 5.4; B12,0.06.

[7] Contains (as mg kg^{-1} in diets): NaCl, 437.4; $MgSO_4.7H_2O$, 1379.8; $NaH_2P_4.2H_20$, 877.8;$Ca(H_2PO_4)_2.2H_2O$, 1366.7; KH_2PO_4, 2414; $ZnSO_4.7H_2O$, 226.4 Fe-Citrate, 299; Ca-lactate, 3004; $MnSO_4$, 0.016; $FeSO_4$, 0.0378; $CuSO_4$, 0.00033; Calcium iodate, 0.0006; MgO, 0.00135; $NaSeO_3$,0.00025.

[8] Calculated on the basis of sum of energy in each ingredients in the diets for protein, carbohydrates and fat (Jauncey, 1982 in Khan and Maqbool, 2017; Gümüş et al., 2017)

* Estimated value, NRC, 2011.

**Calculated price in the year 2017.

Table 2. Fishmeal analogue with different protein sources; feather meal (FEM), blood meal (BM), leather meal (LEM), soybean meal (SBM), poultry by-product (PBP), squid liver powder (SLP) and fish oil (FO). All values are in percentage (rate used in the FMA diet) unless otherwise noted.

FMA Ingredients	Rate (%)
Feather meal[3]	16.00
Blood meal[3]	19.90
Leather meal[4]	20.00
Poultry by-product[3]	17.00
Soybean meal[4]	17.60
Squid liver powder[3]	5.00
Fish oil[3]	1.50
Lysine	2.00
Methionine	1.00
	100.00
Crude protein (%)*	64.00
Crude lipid (%)*	13.50
Ash*	12.30
Moisture*	5.24
Price (Won/kg)**	1006.55

[3] Purchased from Su-hyup Feed Co.Ltd., Uiryeong, and Republic of Korea.

[4] Purchased from Pung-Chung Co.Ltd, Republic of Koreas

*Estimated value, NRC, 2011.

**Calculated price in the year 2017.

Table 3. Amino acid compositions of the experimental diets (g kg^{-1}, % of wet matter basis) used for feeding the growing Japanese eel, *Anguilla japonica* (n = 3) for 10 wks.

Amino Acids	Experimental Diets[2]				
	$FMA_{0(control)}$	FMA_{10}	FMA_{20}	FMA_{30}	FMA_{40}
Essential amino acids					
Arginine	2.65	2.66	2.65	2.67	2.69
Histidine	1.75	1.73	1.74	1.72	1.69
Isoleucine	2.26	2.18	2.11	2.02	1.98
Valine	2.53	2.55	2.54	2.56	2.57
Leucine	3.54	3.55	3.58	3.61	3.63
Lysine	3.61	3.55	3.48	3.43	3.39
Threonine	2.04	1.97	1.94	1.88	1.82
Methionine	1.25	1.23	1.22	1.17	1.15
Phenylalanine	1.96	1.94	1.97	1.98	1.97
Non-essential amino acids					
Alanine	2.88	2.83	2.79	2.76	2.70
Aspartic acid	4.46	4.39	4.35	4.21	4.18
Cystein[1]	1.05	1.06	1.08	1.12	1.13
Proline	2.65	2.72	2.83	3.88	2.96
Serine	1.85	1.88	1.90	1.91	1.94
Glutamic acid	7.28	7.17	7.05	6.85	6.74
Glycine	2.74	2.76	2.85	2.93	2.98
Tyrosine	1.31	1.30	1.27	1.20	1.15

[1]Sulphur-containing amino acids

[2] Five experimental diets were formulated to contain graded level of fishmeal analogue: 0 (FMA_0), 10 (FMA_{10}), 20 (FMA_{20}), 30 (FMA_{30}) and 40 (FMA_{40}) % of fishmeal analogue.

Table 4. Amino acid compositions of the fish whole body (g kg^{-1}, % of wet matter basis), of the growing Japanese eel, *Anguilla japonica* (n=3) fed the experimental diets for 10 wks.

Amino Acids	Experimental Diets[2]				
	$FMA_{0(control)}$	FMA_{10}	FMA_{20}	FMA_{30}	FMA_{40}
Essential amino acids					
Arginine	0.87 ± 0.02	0.89 ± 0.03	0.90 ± 0.01	0.91 ± 0.04	0.91 ± 0.04
Histidine	0.59 ± 0.04	0.60 ± 0.02	0.59 ± 0.02	0.57 ± 0.02	0.56 ± 0.03
Isoleucine	0.75 ± 0.01	0.74 ± 0.06	0.73 ± 0.02	0.71 ± 0.04	0.71 ± 0.02
Valine	0.83 ± 0.03	0.84 ± 0.04	0.83 ± 0.04	0.84 ± 0.03	0.87 ± 0.04
Leucine	1.16 ± 0.03	1.22 ± 0.07	1.20 ± 0.04	1.22 ± 0.03	1.23 ± 0.06
Lysine	1.19 ± 0.04	1.20 ± 0.02	1.19 ± 0.04	1.17 ± 0.01	1.19 ± 0.03
Threonine	0.67 ± 0.03	0.66 ± 0.04	0.61 ± 0.04	0.63 ± 0.06	0.62 ± 0.02
Methionine[1]	0.42 ± 0.02	0.41 ± 0.01	0.41 ± 0.03	0.39 ± 0.02	0.39 ± 0.03
Phenylalanine	0.65 ± 0.04	0.67 ± 0.01	0.65 ± 0.05	0.65 ± 0.04	0.68 ± 0.04
Non-essential amino acids					
Alanine	0.96 ± 0.04	0.94 ± 0.01	0.92 ± 0.02	0.93 ± 0.01	0.92 ± 0.03
Aspartic acid	1.46 ± 0.05	1.47 ± 0.04	1.46 ± 0.05	1.44 ± 0.03	1.44 ± 0.03
Cystein[1]	0.34 ± 0.01	0.35 ± 0.02	0.35 ± 0.03	0.38 ± 0.01	0.37 ± 0.03
Proline	0.88 ± 0.03	0.91 ± 0.03	0.91 ± 0.04	0.94 ± 0.04	0.95 ± 0.05
Serine	0.62 ± 0.01	0.63 ± 0.01	0.63 ± 0.03	0.63 ± 0.01	0.65 ± 0.03
Glutamic acid	2.36 ± 0.08	2.39 ± 0.10	2.35 ± 0.08	2.33 ± 0.06	2.34 ± 0.04
Glycine	0.90 ± 0.02	0.92 ± 0.03	0.92 ± 0.04	0.94 ± 0.04	0.95 ± 0.05
Tyrosine	0.43 ± 0.03	0.41 ± 0.02	0.43 ± 0.03	0.41 ± 0.04	0.42 ± 0.01

[1]Sulphur-containing amino acids

[2] Five experimental diets were formulated to contain graded level of fishmeal analogue: 0 (FMA_0), 10 (FMA_{10}), 20 (FMA_{20}), 30 (FMA_{30}) and 40 (FMA_{40}) % of fishmeal analogue.

Table 5. Growth performance, feed efficiency, and organosomatic indices of growing Japanese eel fed the experimental diets for 10 wks[1].

	Experimental Diets[2]				
Parameters	$FMA_{0(control)}$	FMA_{10}	FMA_{20}	FMA_{30}	FMA_{40}
IBW[3]	119.64 ± 0.71	119.04 ± 0.44	120.78 ± 1.24	119.70 ± 0.75	119.22 ± 1.56
FBW[4]	164.99 ± 16.16^{a}	164.17 ± 5.76^{a}	163.91 ± 5.70^{a}	153.83 ± 5.21^{b}	146.14 ± 7.99^{b}
WG (%)[5]	38.11 ± 2.80^{a}	37.90 ± 4.37^{a}	35.69 ± 2.05^{a}	28.51 ± 3.99^{b}	25.78 ± 0.63^{b}
SGR (%.day^{-1})[6]	0.42 ± 0.03^{a}	0.42 ± 0.04^{a}	0.40 ± 0.02^{a}	0.33 ± 0.04^{b}	0.30 ± 0.01^{b}
FE (%)[7]	57.00 ± 2.42^{a}	55.05 ± 2.12^{ab}	51.44 ± 3.14^{bc}	50.95 ± 2.72^{bc}	48.45 ± 1.09^{c}
PER[8]	0.30 ± 0.013^{a}	0.29 ± 0.011^{ab}	0.27 ± 0.013^{bc}	0.26 ± 0.014^{c}	0.25 ± 0.006^{c}
SR (%)[9]	89.74 ± 15.49	97.22 ± 4.81	100.00 ± 0.00	100.00 ± 0.00	97.44 ± 4.81
HSI (%)[10]	1.00 ± 0.01^{a}	0.99 ± 0.03^{a}	1.16 ± 0.25^{a}	1.09 ± 0.17^{a}	1.13 ± 0.15^{a}
VSI (%)[11]	1.45 ± 0.91^{a}	1.47 ± 0.05^{a}	1.46 ± 0.24^{a}	1.34 ± 0.21^{a}	1.47 ± 0.43^{a}
CF[12]	0.15 ± 0.01^{ab}	0.15 ± 0.01^{ab}	0.13 ± 0.02^{ab}	0.16 ± 0.03^{a}	0.12 ± 0.01^{b}

FMA = fishmeal analogue

[1] Value are mean from triplicate groups of fish, where the means in each row with different superscripts are significantly different at ($P < 0.05$).

[2] Five experimental diets were formulated to contain graded level of fishmeal analogue: 0 (FMA_0), 10 (FMA_{10}), 20 (FMA_{20}), 30 (FMA_{30}) and 40 (FMA_{40}) % of fishmeal analogue.

[3] Initial body weight (g)

[4] Final body weight (g)

[5-9] WG: Weight gain; SGR: Specific growth rate; FE: Feed efficiency; PER: Protein efficiency ratio, SR: Survival rate.[10] HSI: Hepatosomatic index = Liver weight x 100/body weight.

[11] VSI: Viscerosomatic index (Total intestine ratio) = viscera weight x 100/body weight.

[12] CF: Condition factor = 100 x (Live weight, g)/ (body length, cm)^{3}.

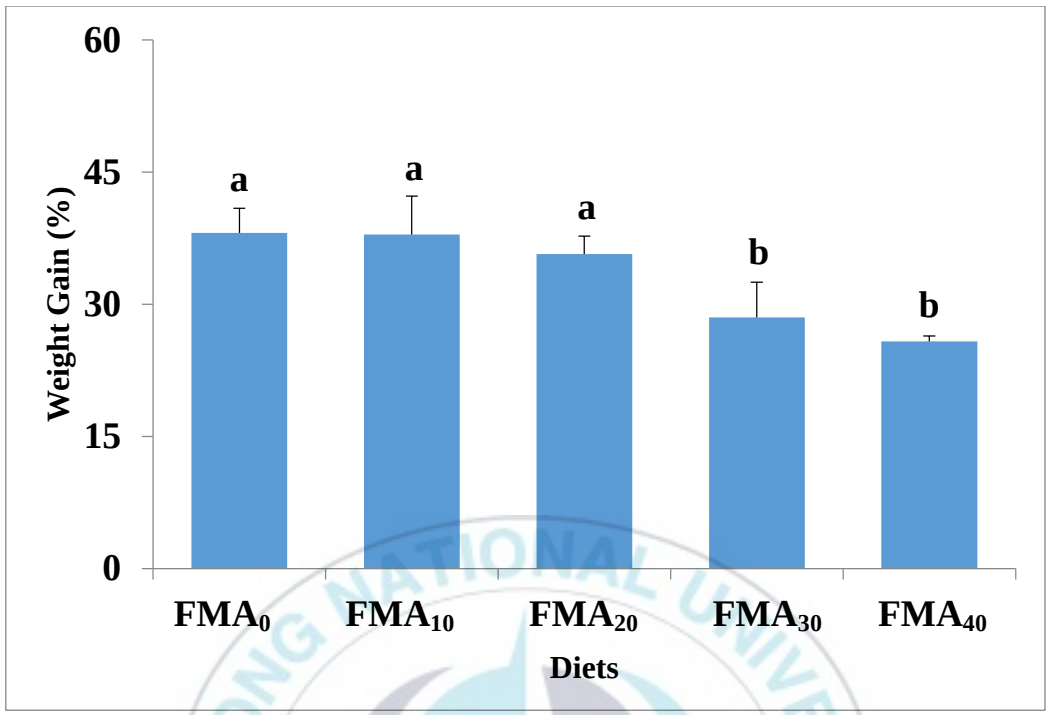


Figure 1. Weight gain of the growing Japanese eel fed the experimental diets for 10 wks.

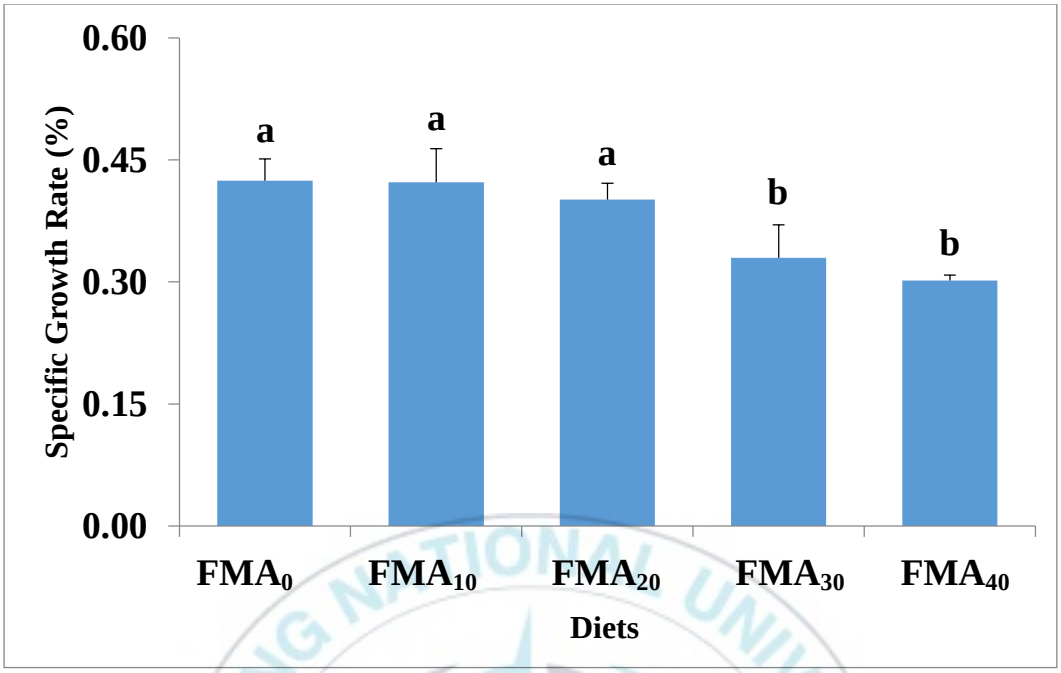


Figure 2. Specific growth rate of the growing Japanese eel fed the experimental diets for 10 wks.

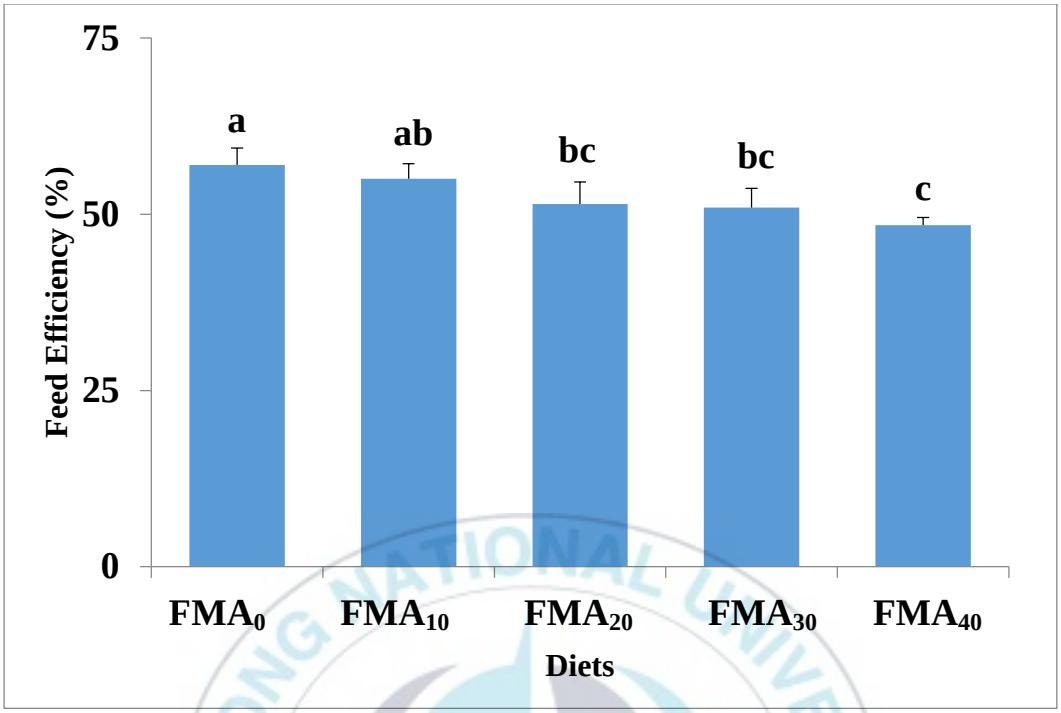


Figure 3. Feed efficiency of the growing Japanese eel fed the experimental diets for 10 wks.

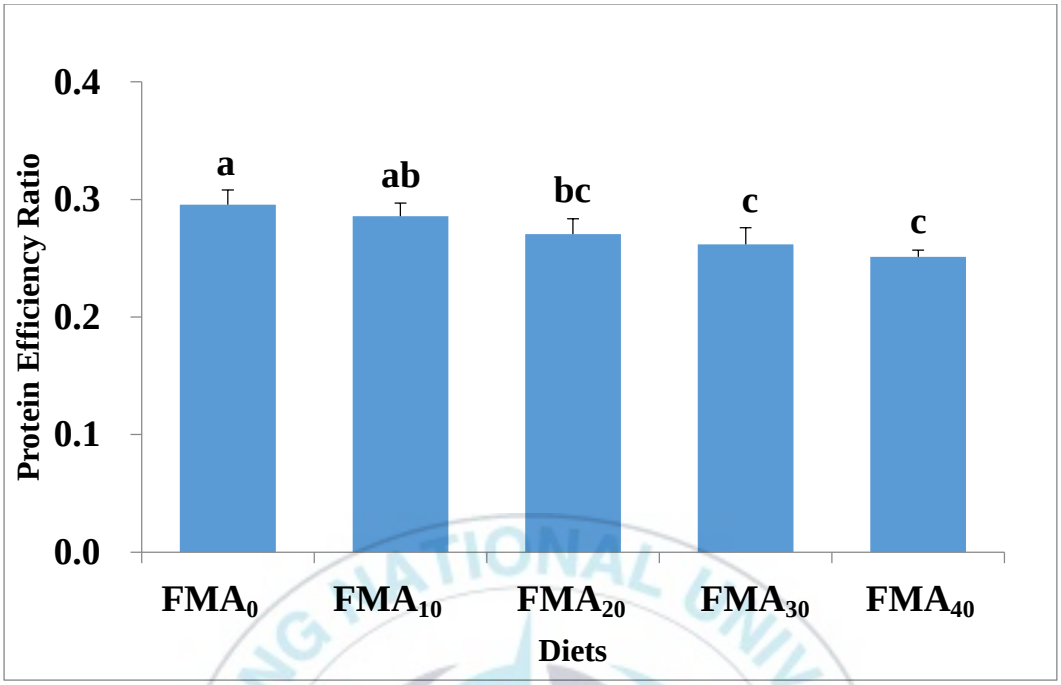


Figure 4. Protein efficiency ratio of the growing Japanese eel fed the experimental diets for 10 wks.

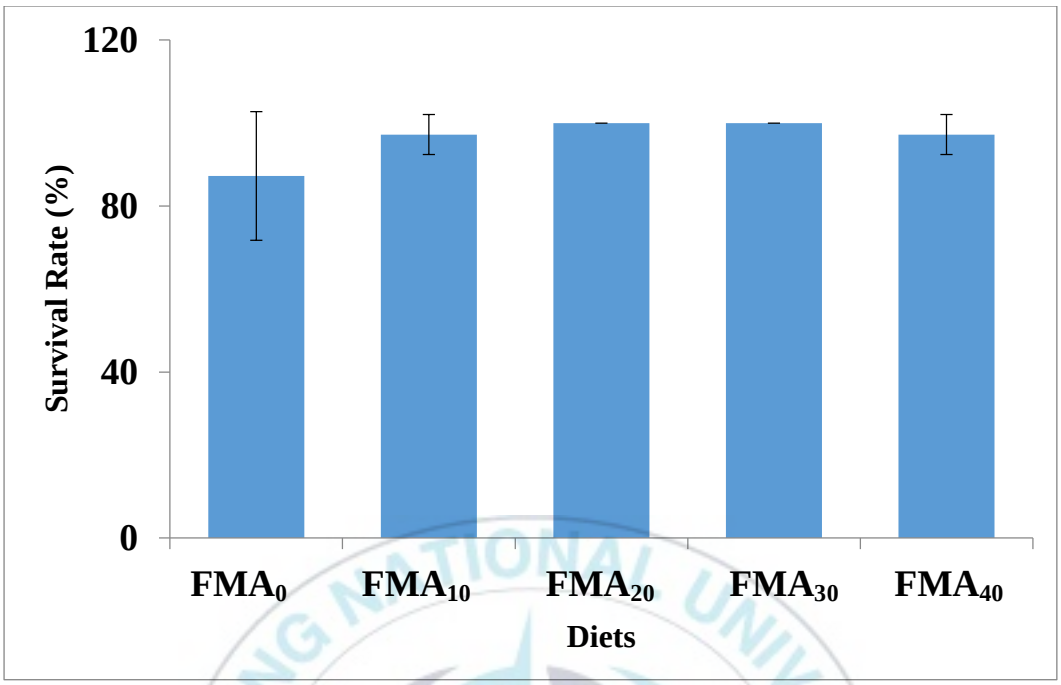


Figure 5. The survival rate of the growing Japanese eel fed the experimental diets of 10 wks.

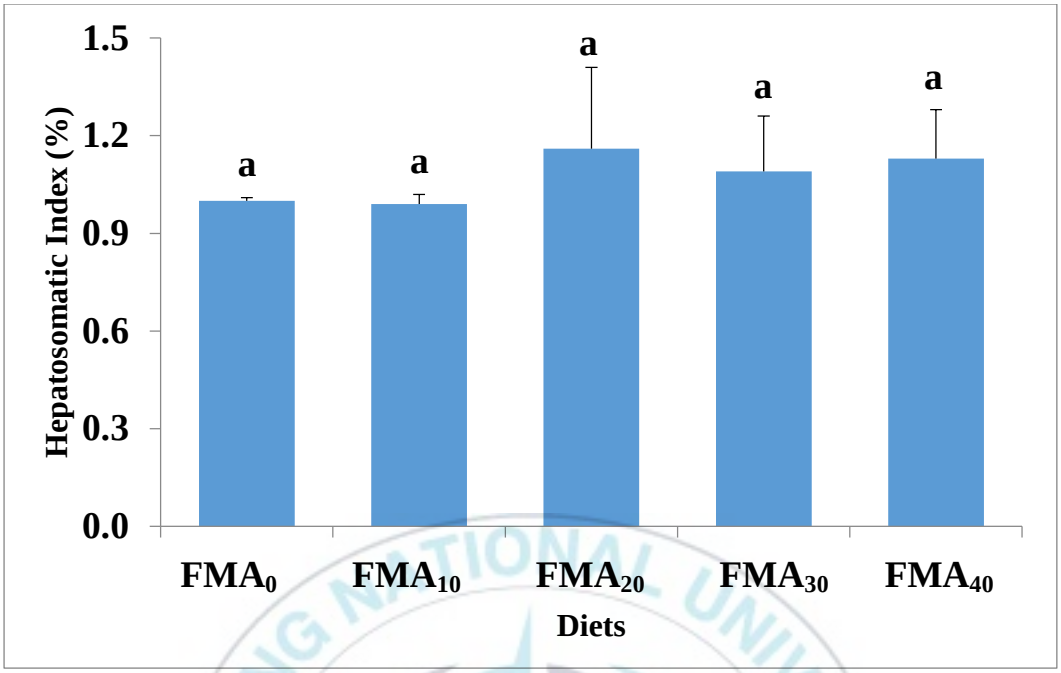


Figure 6. Hepatosomatic index of the growing Japanese eel fed the experimental diets for10 wks.

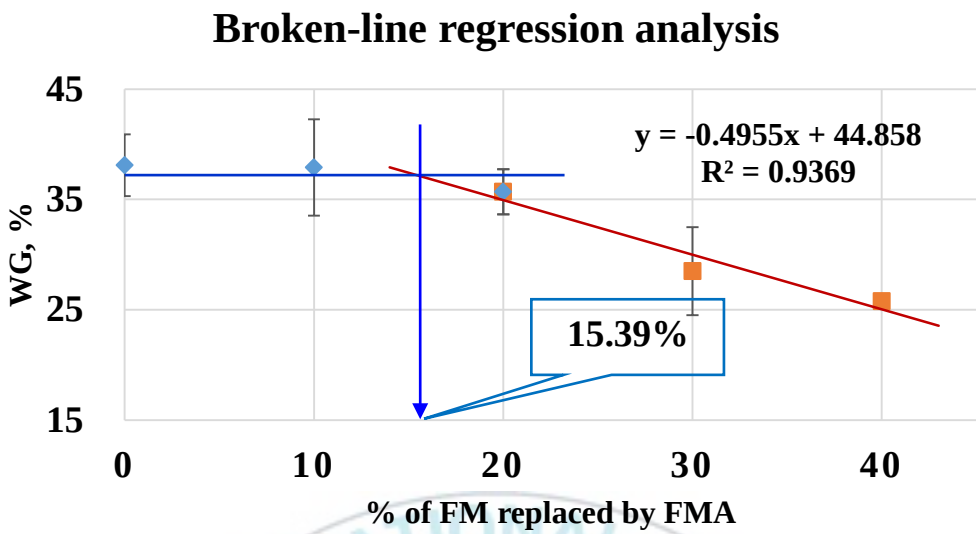


Figure 7. Broken-line regression analysis of weight gain (WG, %) of the growing Japanese eel fed the experimental diets for 10 wks.

Table 6. Proximate composition of experimental diets with different FMA levels (% of dry matter basis)[1].

	Experimental Diets[2]				
Parameters	$FMA_{0(control)}$	FMA_{10}	FMA_{20}	FMA_{30}	FMA_{40}
Moisture (%)	8.15 ± 0.24	7.59 ± 0.10	7.70 ± 0.02	8.59 ± 0.02	8.47 ± 0.07
Crude protein (%)	51.85 ± 0.60	51.92 ± 0.23	51.82 ± 0.49	51.40 ± 0.03	51.86 ± 0.64
Crude lipid (%)	7.03 ± 0.00	7.02 ± 0.14	7.08 ± 0.18	7.22 ± 0.56	7.11 ± 0.13
Crude ash (%)	11.36 ± 0.13	11.14 ± 0.12	11.00 ± 0.08	11.03 ± 0.05	11.08 ± 0.10

FMA = fishmeal analogue

[1] Value are mean from triplicate groups of fish, where the means in each row with different superscripts are significantly different at ($P < 0.05$).

[2] Five experimental diets were formulated to contain a graded level of fishmeal analogue: 0 (FMA_0), 10 (FMA_{10}), 20 (FMA_{20}), 30 (FMA_{30}) and 40 (FMA_{40}) % of fishmeal analogue.

Table 7. Whole body proximate composition of the growing Japanese eel fed the experimental diets for 10 wks (% of wet matter basis)[1].

Parameters	Experimental diets[2]				
	$FMA_{0(control)}$	FMA_{10}	FMA_{20}	FMA_{30}	FMA_{40}
Moisture (%)	63.50 ± 0.92^{a}	61.73 ± 1.25^{ab}	60.80 ± 2.40^{ab}	60.33 ± 2.30^{ab}	59.40 ± 2.11^{b}
Crude protein (%)	16.43 ± 1.10^{a}	16.30 ± 0.80^{a}	16.27 ± 0.60^{a}	16.40 ± 1.55^{a}	16.53 ± 1.40^{a}
Crude lipid (%)	14.40 ± 0.30^{a}	14.83 ± 0.32^{a}	14.40 ± 1.40^{a}	14.23 ± 0.67^{a}	14.73 ± 1.50^{a}
Crude ash (%)	6.60 ± 1.01^{a}	6.17 ± 1.30^{a}	6.87 ± 0.60^{a}	7.03 ± 0.81^{a}	7.47 ± 0.85^{a}

FMA = fishmeal analogue

[1] Value are mean from triplicate groups of fish, where the means in each row with different superscripts are significantly different at ($P < 0.05$).

[2] Five experimental diets were formulated to contain a graded level of fishmeal analogue: 0 (FMA_0), 10 (FMA_{10}), 20 (FMA_{20}), 30 (FMA_{30}) and 40 (FMA_{40}) % of fishmeal analogue.

Table 8. Hematological parameters of the growing Japanese eel fed the experimental diets for 10 wks (% of dry matter basis)[1].

Parameters	Experimental Diets[2]				
	$FMA_{0\ (control)}$	FMA_{10}	FMA_{20}	FMA_{30}	FMA_{40}
AST(U/L)[3]	67.67 ± 2.52^{b}	55.00 ± 3.61^{c}	59.33 ± 3.61^{bc}	90.67 ± 3.06^{a}	86.00 ± 5.29^{a}
ALT(U/L)[4]	16.67 ± 5.51^{b}	15.00 ± 2.65^{b}	16.33 ± 1.53^{b}	26.33 ± 11.06^{a}	28.00 ± 7.37^{a}
Glucose (mg/dL)	176.67 ± 10.60^{b}	195.00 ± 10.00^{ab}	196.00 ± 7.21^{ab}	206.33 ± 7.37^{a}	206.33 ± 9.07^{a}
T-Protein (g/dL)	4.60 ± 0.85^{ab}	5.53 ± 0.55^{a}	4.83 ± 0.47^{ab}	4.80 ± 0.10^{ab}	4.80 ± 0.44^{ab}
T-Cholesterol (mg/dL)	442.33 ± 13.28^{a}	450.00 ± 0.00^{a}	435.00 ± 18.73^{a}	450.00 ± 0.00^{a}	442.00 ± 13.86^{a}
Triglycerides (mg/dL)	427.33 ± 6.43^{d}	455.00 ± 11.68^{bc}	455.00 ± 7.00^{cd}	470.67 ± 2.52^{ab}	480.33 ± 10.79^{a}

FMA = fishmeal analogue

[1] Value are mean from triplicate groups of fish, where the means in each row with different superscripts are significantly different at ($P < 0.05$).

[2] Five experimental diets were formulated to contain a graded level of fishmeal analogue: 0 (FMA_0), 10 (FMA_{10}), 20 (FMA_{20}), 30 (FMA_{30}) and 40 (FMA_{40}) % of fishmeal analogue.

[3] AST (U/L): Aspartate aminotransferase. [4] ALT (U/L): Alanine aminotransferase.

Table 9. Nonspecific immune responses of the growing Japanese eel fed the experimental diets for 10 wks[1].

Parameters	Experimental Diets [2]				
	$FMA_{0(control)}$	FMA_{10}	FMA_{20}	FMA_{30}	FMA_{40}
SOD[3]	99.63 ± 1.63[a]	97.94 ± 0.03[a]	98.99 ± 0.44[a]	98.38 ± 2.37[a]	102.52 ± 0.09[a]
Lysozyme (U/ml)[4]	0.67 ± 0.01[a]	0.68 ± 0.00[a]	0.70 ± 0.01[a]	0.68 ± 0.01[a]	0.69 ± 0.01[a]
MPO[5]	2.42 ± 0.07[a]	2.28 ± 0.19[a]	2.20 ± 0.23[a]	2.06 ± 0.08[a]	1.99 ± 0.06[a]

FMA = fishmeal analogue; SOD: Superoxide dismutase

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

[2] Five experimental diets were formulated to contain a graded level of fishmeal analogue: 0 (FMA_0), 10 (FMA_{10}), 20 (FMA_{20}), 30 (FMA_{30}) and 40 (FMA_{40}) % of fishmeal analogue.

[3] SOD (% inhibition): Superoxide dismutase.

[4] Lysozyme activity.

[5] MPO: Myeloperoxidase

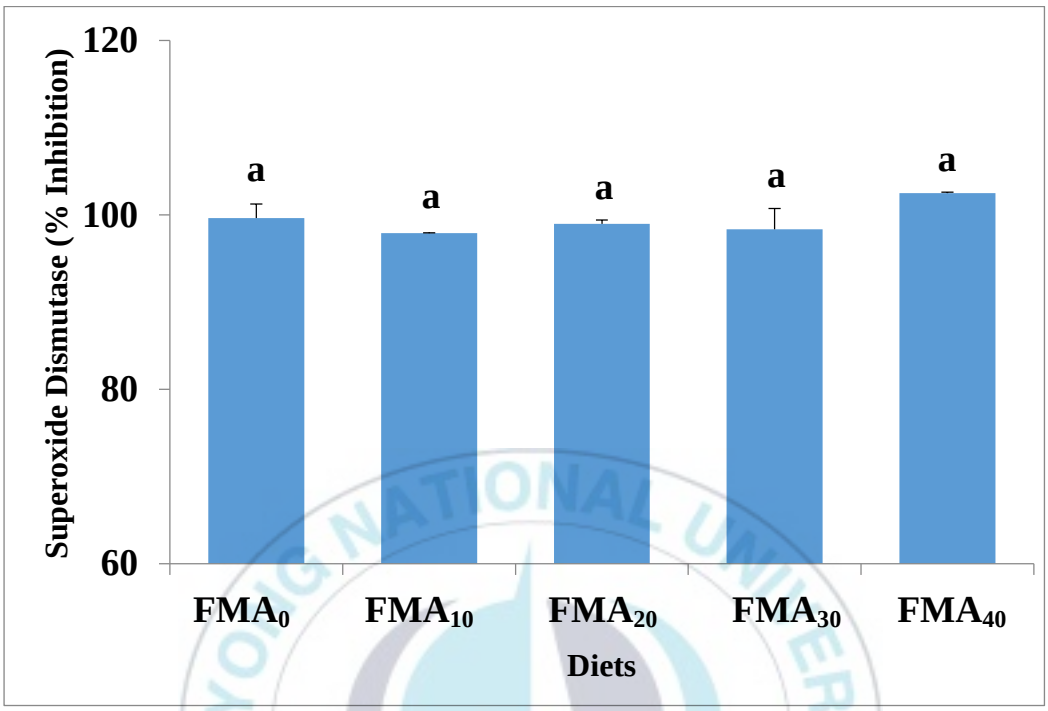


Figure 8. Superoxide dismutase activity of the growing Japanese eel fed the experimental diets for 10 wks.

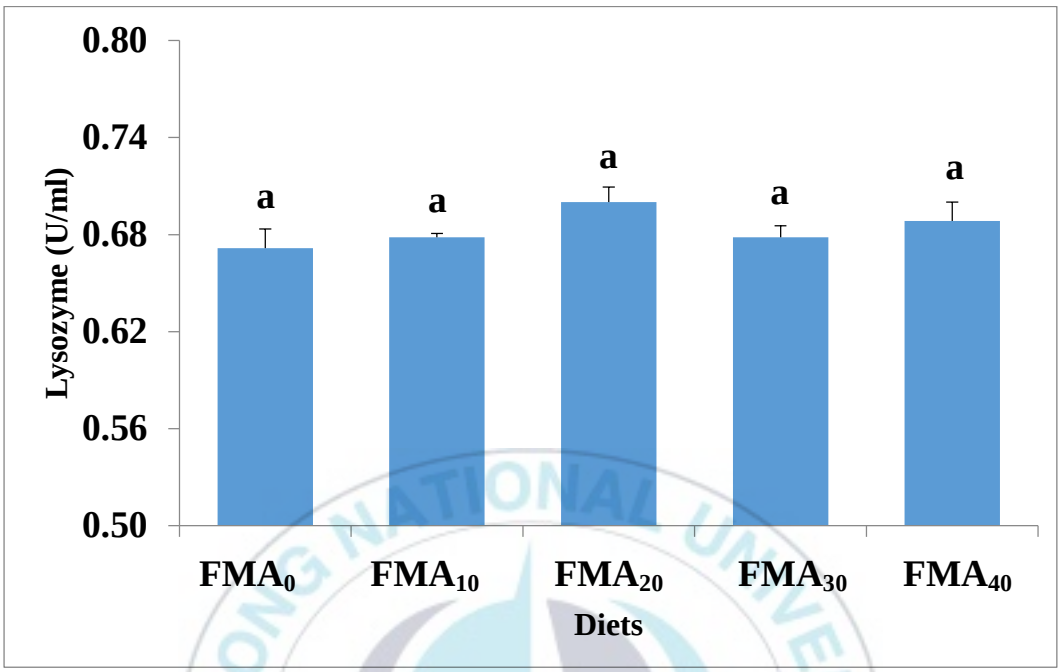


Figure 9. Lysozyme activity of the growing Japanese eel fed the experiment diets for 10 wks.

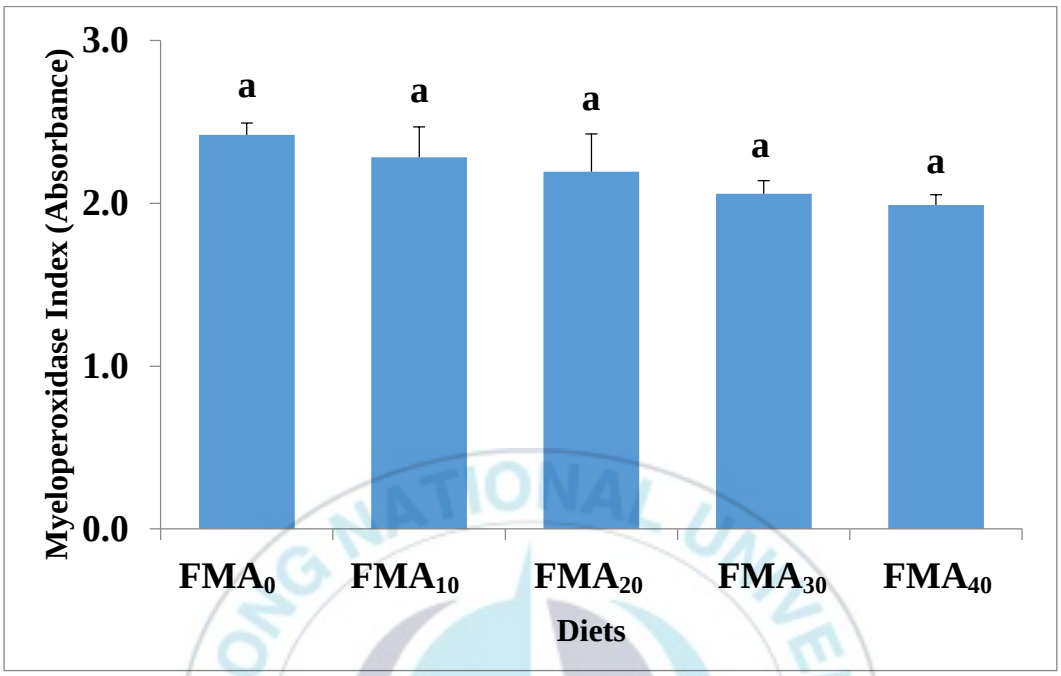
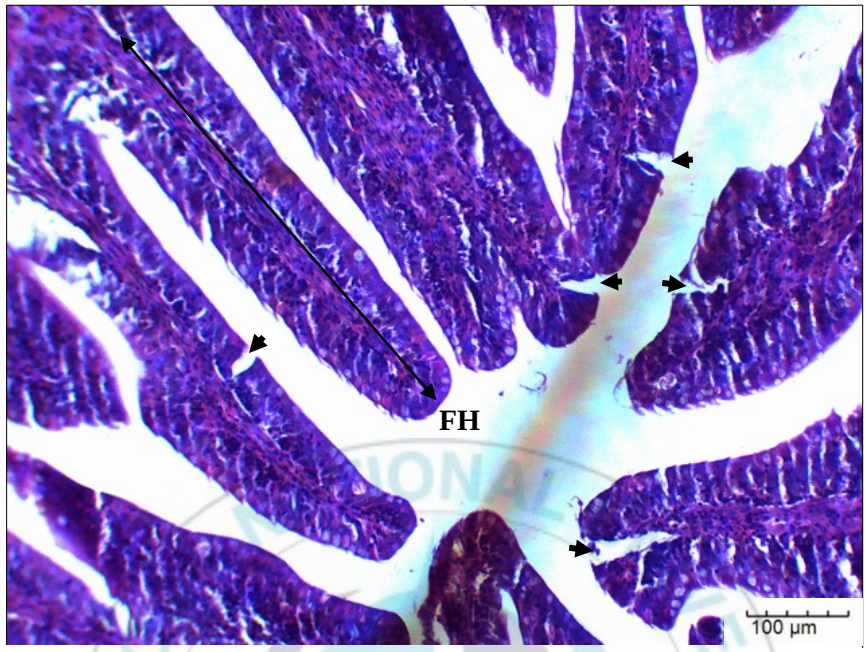
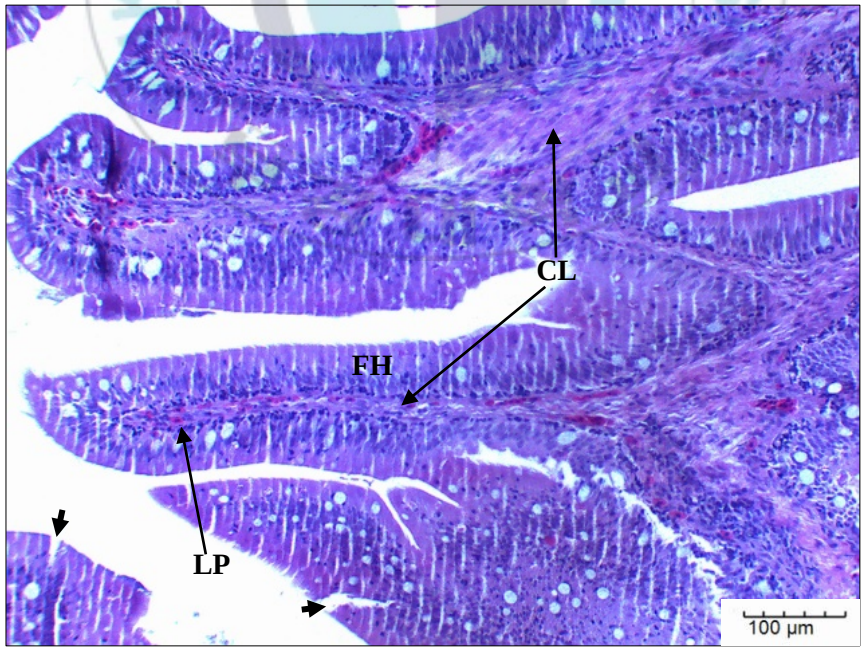


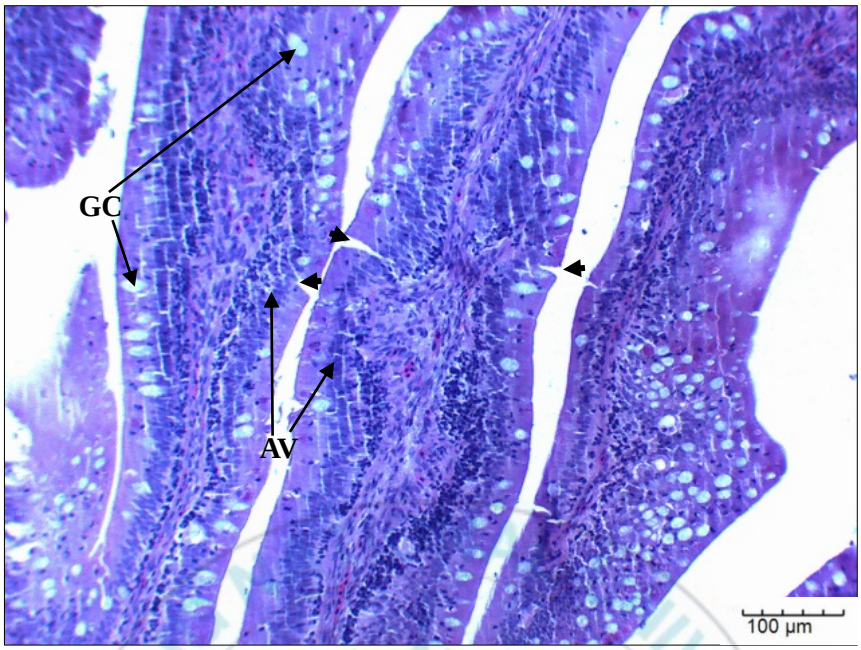
Figure 10. Myeloperoxidase index of the growing Japanese eel fed the experimental diets for 10 wks.



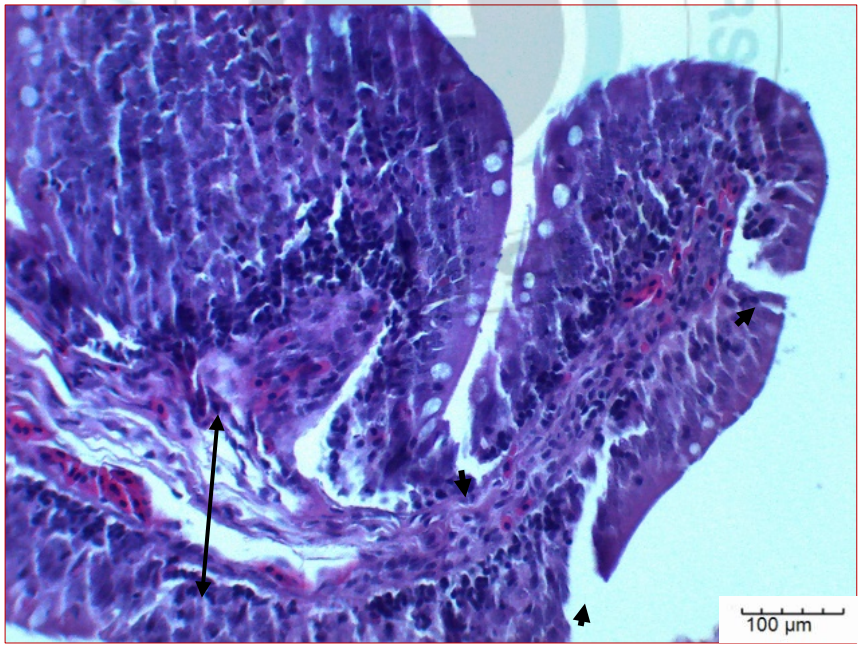
(a) FMA_0



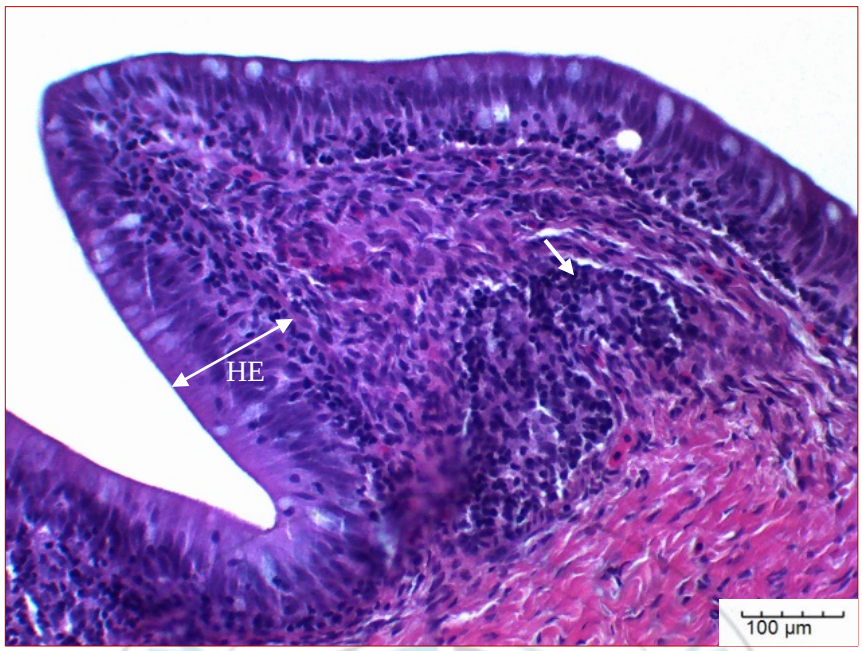
(b) FMA_{10}



(c) FMA_{20}



(d) FMA_{30}



(e) FMA_{40}

Figure 11. Displays the photomicrographic transversal section of the intestinal villi of the growing Japanese eel fed the experimental diets with different FMA levels for 10 weeks as observed at a magnification of x100 for (a), (b), (c) and x300 for (d) and (e) at the scale of 100µm. Fish fed diets FMA_0 (a), FMA_{10} (b) and FMA_{20} (c) were observed to be embraced with normal and elongated villi with wide surface area being enhanced by the presence of densely packed goblet cells (GC), *Lamina propria* (LP), central lacteal (CL), fold heights (FH) and absorptive vacuoles (AV) as shown in arrows in a, b & c. Severe enlargement of enteritis parameters (double-edged arrow) in fish is also shown in (c). Fish fed diet FMA_{30} (d) showed irregular villi shapes with cuts, dilations, and engrossment of LP (shown in double-end arrow). Those fish fed diet FMA_{40} (e)

were fused with short (dwarf) villus and reduced surface area for absorption as well as the heightening of the enterocyte (HE). Unlabeled arrows (short arrows) showed broken folds and the presence of the abnormal submucosa of the fish (white short arrow (d)).

V. Discussion and Conclusion

The amino acid compositions of the experimental diets and that of the fish whole body are shown in Table 3 and Table 4, respectively. Aspartic acid was the leading non-essential acid of the total amino acids as leucine and lysine were predominant essential acids. The sum of amino acid composition of the fish whole body was relatively similar in all fish fed the FMA diets, however with slightly lower value when compared to those of fish fed the experimental diets. This similar trend of acid compositions in dietary FMA in the fish whole body was also reported by Sullivan (2009) when replacing 60% of FM by PBP and 30% of FM by MBM. Slight variation in values of each individual amino acids in each fish groups may be caused by the feeding activities of the fish and the changes in experimental conditions (temperature and water quality) that may affect the biological performance of the fish (Sullivan, 2009).

Findings of this study showed that fish fed FMA_{10} and FMA_{20} diets were attributed to significantly higher growth and biological performance in relation to WG, SGR, FE, and PER with no significance differences compared to fish fed dietary FMA_0 (control diet). The consistency of these observations were also reported in studies done on other related freshwater fish species that used combinations of various protein sources in the diets of rainbow trout, *Oncorhynchus mykiss* (Jo et al., 2016; Bureau, 2000); European eel, *Anguilla anguilla* (Gallego et al., 2010; Gallaher et al., 1998); Australian short-finned eel, *Anguilla australis australis* (Engin and Carter, 2005); Nile tilapia, *Oreochromis niloticus* (Kimiri et al., 2016) and kuruma shrimp, *Marsupenaeus japonicus* (Bulbul et al., 2016). Most of these studies have clearly demonstrated limitations of replacing FM with a higher level of different combinations of protein sources and

adverse effects on fish performance in terms of deficiencies of certain amino acids, vitamins, and minerals in the formulated diets. Due to these limitations, most fish nutritionists have supplemented the fish diets with amino acids, including the first limiting amino acids such as methionine and lysine, phytase, taurine, and attractants along with fish solubles that could enhance growth, feed utilization, body composition and the physiological health of the fish (Hardy, 2010; Jo et al., 2016; Bulbul et al., 2016). However, this study focuses on demonstrating the blended use of alternative sources of protein as dietary FMA, including the addition of methionine and lysine in the formulation of the diet so as to meet the nutrient requirement levels that were reported for Japanese eel and other freshwater fish species in NRC (2011). The key ingredients used in the FMA are mainly composed of FEM, BM, SBM, LEM, PBP and minor compositions of FO and SLP. In accordance with this study, NRC (2011); Jo et al. (2016); Herath et al. (2016); Nates and Swisher (2015); Hernandez and Roman (2016) have reported that dietary FMA that has the mixture of SBM, PBP, BM, and FEM is able to balance the amino acid requirements in fish diets, along with the enhancement of animals' protein synthesis, increasing feed palatability and sustainability, reducing production cost and piping of less affluent of waste to the environment. In agreement with the present study, some other studies postulated the utilization of various protein sources as dietary FMA that combined ingredients such as, BM, PBP, FEM, SBM and poultry meal (PM) that had a positive impact on growth performance in rainbow trout, *O mykiss* (Jo et al., 2016; Yanik et al., 2003); Nile tilapia, *O. niloticus* (Kirimi et al., 2016); kuruma shrimp, *M japonicas* (Bulbul et al., 2016)*;* Rohu, *Labeo rohita* (Khan et al., 2003)*;* European eel, *A anguilla* (Gallego et al., 2010; Gallagher and Degani,1998)*;* Australian short-finned

eel, *A. australis australis* (Engin and Carter, 2005) and Japanese eel, *Anguilla japonica* (Bae et al., 2016, unpublished paper).

In this study, contrary to the proximate composition of the experimental diets, the fish whole body showed a slight variation in terms of its significance differences ($P < 0.05$) with fish fed diet FMA_{40} having a lower moisture content value (% of diet on wet basis) relative to those of fish fed the control diets. This study also showed the minimal higher value of crude protein and lipids for fish fed dietary FMA_{40} compared to those fish fed the control diets, respectively. Some other studies have reported similar trend in lower moisture value with increments in dietary FMA and other protein sources (ingredients) that slightly affects the growth performance and feed efficiency in rainbow trout, *O. mykiss* (Jo et al., 2016); kuruma shrimp, *M. japonicas* (Bulbul et al., 2016) and Australian short-finned eel, *A. australis australis* (Engin and Carter, 2005).

Hemato-biochemical indices are suitable indicators for evaluating the health, stress level and physiological welfare of fish (Simide et al., 2016; Raina, 2016). Conversely, this study showed an increment of AST and ALT level in fish fed FMA_{30} with no obvious difference to those of fish fed FMA_{40} in relation to fish fed the control diets. An increase in AST and ALT with FMA addition in the fish diets can be attributed to various dynamics such as reduction in FM content, tolerance of anti-nutritional factors, digestibility and palatability problems that arbitrate with high inclusion of FMA in the mixture of PBP, BM, and wheat gluten meal (WGM) contents. Other factors may also associate with experimental conditions and handling methods that can intensely affect the physiological condition of the fish (Jo et al., 2016; NRC, 2011). Similarly, glucose and triglycerides level also showed an increase in value on fish fed dietary FMA_{10}, FMA_{20},

FMA_{30}, and FMA_{40} in relation to those fish fed the control diets. This can also be attributed to influences of higher inclusion of corn starch (CS), soybean oil (SO) and SBM in the diets containing FMA (Nates, 2016; Nates and Swisher, 2015, 2016; NRC, 2011). While studies have shown successes in replacing FM using other proteins sources to certain levels in Mediterranean yellowtail, *Seriolla dumerilli*, using SBM (20–30%) (Tomas, 2005); rainbow trout, *O.mykiss*, with FMA (12%) (Jo et al., 2016); red snapper *Lutjanus argentimaculatatus* with SBM (12%) (Catacutan and Pagador, 2004), and in Japanese eel, *A. japonica* with similar FMA (10%) (Bae et al., 2016, unpublished paper), however, Catcutan and Pagador (2004) argued that fish fed with high replacement diets (36% and 48% of SBM) had lipid deposits in the liver and low hematocrit levels, which ultimately affected the fish health. Zhou et al. (2005) also tested that fish fed diets containing 0–60% SBM in increments of 10% in juvenile cobia, *Rachycentron caladium*, showed that 40% SBM was successful for maximising growth rate, whereas lipid levels in the liver, blood cell composition, and feed conversion ratio was negatively affected as SBM content increases. Zhang (2006) also reported that using BM, BOM, LEM, and FEM as FMA replacers for FM in the diets of gibel carp, *Carassius gibelio* can only replace 20%, however, higher replacement levels have resulted in reduced growth, weight gain and feed efficiency of the fish without compromising the economical utilization of the diet.

In this study, total protein in blood serum showed a little variation with a high significant value in fish fed diet FMA_{10} and the lowest value in fish fed FMA_{20} diet. This variation can be caused by experimental condition and feeding activities of the fish, otherwise, the values fall within the standard range as reported in Australian short-finned

eel, *A. australis australis* (Engin and Carter, 2005), Japanese eel, *A. japonica* (Bae et al., 2016, unpublished paper) and in related freshwater fish such as rainbow trout, *O. mykiss* (Jo et al., 2016; Yanik et al., 2003) and Nile tilapia, *O.niloticus*, (Kirimi et al., 2016). Total cholesterol, in contrary, albeit with very high value, seems not to be affected by the experimental diets, and the values are within the range that is common for many freshwater eel species (NRC, 2011; Nates, 2016).

A non-specific defense mechanism is an efficient clearance and degradation system needed during microbial invasion which otherwise would lead to severe inflammation and eventually death of an animal. It includes neutrophil activation, the production of peroxidase, oxidative radicals, proteases, lysine, agglutins, and the initiation of other inflammatory factors such as mucosal secretion as the first line of defense in fish (Dalmo et.al., 1997). These mechanisms play an important role in all stages of infection by controlling the balance of the release and clearing reactive oxygen species in immune cells (Dalmo et al., 1997; Xu et al., 2010). Jo et al. (2016) and Bae et al. (2010) also postulated that an FM diet added with hydrolysates and use of quality protein sources as FMA can enhance the innate immunity of fish. Inconsistent with Jo et al. (2016); Bae et al. (2016, unpublished paper); Lee and Bai (1997), the activity of SOD in this study showed an increase in value in fish fed diets FMA_{40} with an increment level of dietary FMA. Along with SOD, lysozyme is also one of the important enzymes of defensive mechanism that powered lysis of bacteria and activation of the phagocytes complementary systems as opsonin (Magnadòttir, 2006, in Jo et al., 2016). Bae et al. (2012) showed that inclusion of 0.5% of propolis in the hemoglobin meal in Japanese eel, *A. japonica* had improved the serum lysozyme activities of the fish. A similar analogy

was also observed in this result, with no significant differences amongst each fish groups, indicating a steady response of the fish immune system by the inclusion of dietary FMA that contains 24% and 25% of BM and FEM, respectively. The effects of FMA diets are surely attributed to their bioactive peptide contents that have antioxidative, antimicrobial, and immunomodulatory activities (Korhonen and Pihlanto, 2006; Korhonen et al., 1998). Like lysozyme and SOD activity, MPO is also an important enzyme which has the ability to kill pathogens. During the oxidative respiratory burst, the MPO was mostly released by the azurophilic granules of neutrophils (Park et al., 2016). This study showed that even though there were no significant differences of MPO values in each fish groups, MPO absorbance slightly decreases with dietary FMA increments. This means that fish fed dietary FMA_{10} and FMA_{20} have good MPO performance (immune system health status) relative to those of fish fed dietary FMA_{30} and FMA_{40}. In correlation to this study, Park et al. (2016) also showed that rainbow trout, *O.mykiss*, fed probiotics-supplemented diets showed a decrease in plasma MPO level when fed with multi-probiotics (*Bacillus subtilis* + *B. subtilis* + *B. licheniformis*; MP, 0.5%) compared to fish fed single probiotic 1 (*B. subtilies*. SP_1, 0.5%) and single probiotic 2 (*B. licheniformis*; SP_2, 0.5%). Likewise, similar trends of MPO levels were also reported by Salinas et al. (2008) when gilhead seabream, *Sparus aurata*, were fed *B. subtilis* supplemented diets. The nitroblue tetrazolium reduction after reaction with superoxides is considered as an indicator of the health status or the immune system in fish (Park et al., 2016).

Histological technique is an important research tool used for assessing the gastrointestinal environment in fish, particularly in detecting pathological processes (parasites, bacteria, and fungi), abnormalities and for overall fish health supervision

(Wang et al., 2016; Peng et al., 2013). The gastrointestinal tract serves as a diverse range of function in fish from nutrient absorption to ionic and osmotic regulation and even air breathing (Ajala et al., 2015; Wilson, 2010; Gonçalves et al., 2007). In the present study, qualitative observations made on the cross-transversal section of the growing Japanese eel gastrointestinal villi showed that fish fed dietary FMA_{10} and FMA_{20} have developed normal, elongated and intact intestinal mucosal epithelium with well-organized villus and presence of a few broken folds relative to those of fish fed the control diet (FMA_0). Additionally, the villi, central lacteal (CL), the density of the goblet cells (GC) and absorption vacuoles (AV) appeared normal and were not affected by the treatment when compared to those fish fed dietary FMA_{30} and FMA_{40}. Results also showed that dietary FMA increments over FM have severely affected those fish fed dietary FMA_{30} and FMA_{40} with engrossment of LP and presence of broken folds. Studies related to intestinal morphological evaluation in increment of vegetable oil for fishmeal replacement in European seabass, *Decentrarchus labrax*, showed the engrossment of LP in fish fed 95% of vegetable meals and oils as a result of the accumulation of lipoprotein in the fish diet (Torrecillas et al., 2017). Wang et al. (2016) also confirmed that there has been the presence of broken folds, abnormal submucosa and enlargement of enteritis parameters in Japanese seabass, *Lateolabrax japonicas*, fed 30% SBM.

In argument, based on the quality of FMA diets, the experimental conditions, the results of the parameters shown by the fish weight gain, feed utilization and amino acid compositions, there is a potential that the dietary FMA could replace up to 20% of FM protein in growing Japanese eel, *A. japonica*, as per say to the basis on the fish body weight gain by analysis of variance (ANOVA). Nevertheless, results based on the

broken-line regression analysis indicated that the optimum level of FM replacement by dietary FMA in the growing Japanese eel, *A. japonica*, was 15.39%.

In conclusion, the results of the present study demonstrated that the optimum fishmeal replacement level with dietary FMA could be greater than or equal to 15.39% but less than 20% for growing Japanese eel based on broken-line analysis model and ANOVA. This study could possibly provide additional information regarding the potential application of dietary FMA as a valuable alternative protein source for the growing freshwater fish such as the Japanese eel, *A. japonica*.

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Jim Hyacinth Damusaru

VII. Dedications

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VIII. Appendix

Growth Performance

(a) Weight Gain, Feed Intake & Survival

Initial Data: Weight & Feeding (g) Feeding Rate: 0.50% BW								
Diets	**Tank No**	**No. of Fish**	**Total Weight**	**Individual Weight**	**DM**	**Total Feeding**	**Individual Feeding**	**Total Feed Intake**
D1 FMA_0	1	13	1557	120	0.82	9.55	0.73	9.55
	9	13	1564	120	0.82	9.59	0.74	9.59
	15	13	1545	119	0.82	9.48	0.73	9.48
D2 FMA_{10}	2	13	1547	119	0.76	10.19	0.78	10.19
	6	13	1554	120	0.76	10.23	0.79	10.23
	12	13	1542	119	0.76	10.15	0.78	10.15
D3 FMA_{20}	3	13	1559	120	0.77	10.12	0.78	10.12
	7	13	1558	120	0.77	10.12	0.78	10.12
	11	13	1582	122	0.77	10.27	0.79	10.27
D4 FMA_{30}	4	13	1561	120	0.86	9.09	0.70	9.09
	10	13	1562	120	0.86	9.09	0.70	9.09
	13	13	1545	119	0.86	8.99	0.69	8.99
D5 FMA_{40}	5	13	1527	117	0.85	9.01	0.69	9.01
	8	13	1559	120	0.85	9.20	0.71	9.20
	14	13	1564	120	0.85	9.23	0.71	9.23
D = Diets, **FMA** = Fish Meal Analogue, **DM** = Dry Matter, **BW** = Body Weight								

Data at the 5th Week Weight & Feeding (g) Feeding Rate: 0.70–1.00%								
Diets	**Tank No**	**No. of Fish**	**Total Weight**	**Individual Weight**	**DM**	**Total Feeding**	**Individual Feeding**	**Total Feed Intake**
D1 FMA_0	1	13	1758	135	0.82	21.57	1.66	9.93
	9	10	1390	107	0.82	17.05	1.71	8.40
	15	13	1819	140	0.82	22.31	1.72	14.02
D2 FMA_{10}	2	13	2020	155	0.76	26.61	2.05	26.61
	6	13	1815	140	0.76	23.91	1.84	19.01
	12	13	1604	123	0.76	21.13	1.63	4.83
D3 FMA_{20}	3	13	1729	133	0.77	22.46	1.73	12.21
	7	6	837	140	0.77	10.87	1.81	6.11
	11	13	1724	133	0.77	22.39	1.72	14.49
D4 FMA_{30}	4	13	1794	138	0.86	20.89	1.61	9.74
	10	13	1709	131	0.86	19.90	1.53	10.70
	13	13	1490	115	0.86	17.35	1.33	5.53
D5 FMA_{40}	5	13	1494	115	0.85	17.64	1.36	11.33
	8	13	1755	135	0.85	20.72	1.59	8.59
	14	13	1458	112	0.85	17.21	1.32	2.74
D = Diets, **FMA** = Fishmeal Analogue, **DM** = Dry Matter, **BW** = Body Weight								

Data at the 10th Week Final Weight (g) & Feeding (g) &Mortality Feeding Rate: 1–1.5%								
Diets	**Tank No**	**No. of Fish**	**Final (Total Weight)**	**Final (Individual Weight)**	**DM**	**Final (Total Feed Intake)**	**Final (Individual Feeding)**	**Final Mortality**
D1 FMA_0	1	12	1956.4	163.03	0.82	955.63	79.64	1
	9	10	1699.5	169.95	0.82	842.37	84.24	3
	15	13	2115.8	162.75	0.82	988.42	76.03	0
D2 FMA_{10}	2	13	2092.4	160.96	0.76	1020.10	78.47	0
	6	12	2049.9	170.82	0.76	1071.76	89.31	1
	12	13	2089.5	160.73	0.76	1009.59	77.66	0
D3 FMA_{20}	3	13	2092.5	160.96	0.77	1084.53	83.43	0
	7	6	1200.5	200.08	0.77	515.11	85.85	7*
	11	13	2169.0	166.85	0.77	1094.75	84.21	0
D4 FMA_{30}	4	13	2077.9	159.84	0.86	966.84	74.37	0
	10	13	1964.6	151.12	0.86	783.56	60.27	0
	13	13	1956.8	150.52	0.86	857.64	65.97	0
D5 FMA_{40}	5	12	1780.0	148.34	0.85	770.76	64.23	1
	8	13	1963.5	151.04	0.85	850.14	65.40	0
	14	13	1956.1	150.47	0.85	789.68	60.74	0
D = Diets, **FMA** = Fishmeal Analogue, **DM** = Dry Matter, **BW**=Body Weight,* lost fish								

(b) Weights and Lengths of Liver and Viscera

Diets	Tank No	Weight (g)	Length (cm)	Liver (g)	Intestine (g)
D1 FMA_0	1	174.60	47.00	1.52	1.46
		152.80	49.00	1.76	1.91
	9	142.20	47.20	1.09	1.09
		180.20	48.00	2.11	1.57
	15	176.70	50.30	1.47	4.52
		191.10	50.20	2.23	4.63
D2 FMA_{10}	2	138.30	44.00	1.40	1.31
		172.80	51.00	1.64	3.33
	6	166.70	50.00	1.90	2.22
		213.40	52.50	1.99	3.16
	12	166.60	48.80	1.39	2.98
		164.20	46.20	1.78	2.00
D3 FMA_{20}	3	102.60	45.00	1.99	2.09
		151.50	51.00	1.61	2.33
	7	148.60	47.00	1.61	1.69
		122.90	44.00	1.51	1.86
	11	205.00	52.30	1.88	2.76
		163.50	48.50	1.48	2.12
D4 FMA_{30}	4	168.70	48.50	1.54	2.09
		129.50	48.00	1.41	1.50
	10	191.00	48.00	1.79	2.64
		152.80	44.00	1.48	2.80
	13	147.00	48.10	1.13	1.63
		105.50	34.30	0.95	1.52
D5 FMA_{40}	5	126.00	48.00	1.51	1.31
		129.80	47.00	1.49	1.24
	8	43.70	50.00	2.19	2.48
		186.40	51.90	1.61	2.83
	14	110.40	46.40	1.30	2.11
		118.30	45.30	1.56	2.04