Thesis for Degree of Master of fisheries science

Evaluation of the dietary fish meal analogue adding shrimp soluble extract in growing rainbow trout, *Oncorhynchus mykiss*

by

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육성기 무지개송어 사료내 새우가수분해물을 첨가한 어분대체품의 이용 가능성 평가

Advisors : Sungchul C. Bai

by

Hayun Jo

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A dissertation

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요 약 문

본 실험은 육성기 무지개송어 사료에 있어서 어분(Fish meal, FM)대체를 위하여 새우가수분해물(Shrimp Soluble Extract, SSE)을 2% 첨가한 어분대체품(Fish Meal Analogue, FMA)의 이용 가능성을 평가하고자 실시하였다. 평균무게 146 ± 3.8g (mean ± SD) 인 육성기 무지개송어를 대상으로 12 주간 사육실험을 실시하였다. 실험사료는 조단백질 43%, 조지방 25%로 동일하게 조정하였고, 어분단백질과 어분대체품의 비율을 다음과 같이 요약하였다; 대조구 (FM100% + FMA0%), SSE 구 (FM100% + FMA0% + SSE2%), FMA_{12} 구 (FM88% + FMA12% + SSE2%), FMA_{24} 구 (FM76% + FMA24% + SSE2%). 실험종료 후, 증체율(WG)과 일간성장률(SGR)에 있어서, SSE 와 FMA_{12} 실험구가 대조구와 FMA_{24} 실험구 보다 유의적으로 높은 것으로 나타났으며, 사료효율(FE)에 있어서는, 대조구, SSE 와 FMA_{12} 실험구가 FMA_{24} 실험구 보다 유의적으로 높은 것으로 나타났다. 단백질 전환효율에 있어서도 사료효율과 동일한 결과를 나타내었다. 비특이적 면역반응에 있어서, Superoxide dismutase (SOD)활성에 있어서는, SSE, FMA_{12} 와 FMA_{24} 실험구가 대조구보다 유의적으로 높은 것으로 나타났다. Lysozyme 의 경우, FMA_{12} 와 FMA_{24} 실험구가 대조구와 SSE 실험구 보다 유의적으로 높은 것으로 나타났다. 따라서 육성기 무지개 송어에 있어서, 새우가수분해물 (Shrimp Soluble Extract, SSE)을 2% 첨가한다면 어분대체품을 사용하여 12%정도의 어분을 대체 가능할 것으로 확인된다.

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Abstract

A 12-week of feeding trial was conducted to evaluate the effects of the dietary fish meal analogue adding 2% shrimp soluble extract in growing rainbow trout, *Oncorhynchus mykiss*. Fish averaging 146 ± 3.8g (mean±SD) were randomly distributed into 500 aquaria as groups of 20 fish and fed the experimental diets in triplicate at apparent satiation twice daily on dry matter basis. Fish were fed one of the 4 experimental diets; Control (FM100% + FMA0%), SSE (FM100% + FMA0% + SSE2%), FMA_{12} (FM88% + FMA12% + SSE2%), FMA_{24} (FM76% + FMA24% + SSE2%). After 12 weeks of the feeding trial, weight gain and specific growth rate of fish fed SSE and FMA_{12} diets were significantly higher than those of fish fed Control and FMA_{24} diet ($P<0.05$). Feed efficiency values of fish fed Control, SSE and FMA_{12} diets were significantly higher than those of fish fed FMA_{24} diet ($P<0.05$). Superoxide dismutase activity values of fish fed SSE, FMA_{12} and FMA_{24} diets were significantly higher than those of fish fed Control diet ($P<0.05$).

Lysozyme activity values of fish fed FMA_{12} and FMA_{24} diets were significantly higher than those of fish fed Control and SSE diets ($P<0.05$). Fish meal analogue can be used up to 12% fish meal adding 2% shrimp soluble extract as a substitute of fish meal in growing rainbow trout.

Ⅰ. Introduction

Aquaculture production has surpassed capture fisheries as the main source of seafood for human consumption, with farm-raised fish and shellfish production expected to exceed the total fisheries landings within the next ten years (OECD/FAO, 2015). The aquaculture industry is growing and simultaneously becoming more intensive and reliant on industrially compounded aquafeeds (Tacon et al., 2006). In the past few years, the aquaculture industry has seen an intensive rise in feed costs, particularly for carnivorous species due to competition for limited supplies of fish meal, which is considered the primary source of protein (Tacon and Metian, 2008). Furthermore, use of fishmeal in feeds of terrestrial livestock species is also limiting its availability for use in fish feeds (Naylor et al. 2009). Additionally, excessive use of fishmeal in aquatic feeds may cause a series of environmental problems due to the high content of phosphorus. Since fishmeal production is forecast to be unable to support the growth of the aquaculture sector, the search for alternative ingredients and protein sources and the optimization of dietary protein content is an important goal (NRC, 2011). Therefore, trout producers and feed manufacturers have looked to increase incorporation of low-cost alternative protein sources in feeds for trout.

In recent years, searching for alternative sources of affordable and high-quality ingredients that can replace fishmeal in aquatic feeds has received increasing attention. Several cost-effective alternative protein sources have been investigated as substitutes for fish meal in aquafeeds (Tacon et al., 2008). Many nutrition researchers have been studied on partial or total replacement of fish meal as fish meal analogues using plant protein sources (soybean meal, cotton seed meal, corn gluten meal, rapseed meal etc.) and animal by-products sources (meat and bone meal, meat meal, blood meal, feather meal, poultry by-product meal etc.) for low cost and stable supply of alternative protein sources. Meanwhile, research efforts are underway to identify an appropriate blend of products and other alternative feed ingredients that prevent nutritional deficiencies and ensure a proper supply of essential nutrients. This may further increase the replacement level of FM without detrimental effect on fish performance by restoring a proper balance of amino acid and increasing the palatability of the diet (Kader, 2012)

By development of the modern food processing technologies, interest has been directed towards the potentially marketable raw materials derived from fisheries by catch and seafood processing leftovers which have long been discarded as waste or processed into low market value products (Hsu et al., 2010). Furthermore, conversion and dietary utilization of these nutrient-rich feed stuffs in aquafeed can prevent environmental pollution and excessive expenses for their

disposal (Khosravi, 2015). In view of utilizing these nutrient rich by-products, enzymatic hydrolysis biotechniques have been developed to improve the functional and nutritional properties of the by-products through the conversion of native proteins into smaller biologically active peptides (Chalamaiah et al., 2012).

Crustacean protein hydrolysates have long been used in aquafeeds as potential protein source (Plascencia et al., 2002) or as dietary supplements in small amounts for improvement of diet palatability (Kolkovski et al., 2000).

Processing of shrimp for human consumption has led to the production of a huge amount of by-products accounting for 50~70% of the processed materials. These waste materials contain valuable components such as protein, chitin and astaxanthin (Bataille et al., 1983; Shahidi et al., 1999; Tacon et al., 2006) making them as potential ingredients for aquafeeds. Recovery of the shrimp wastes by enzymatic hydrolysis results in the formation of biologically active peptides with pharmaceutical and growth-stimulating properties (Gildberg et al., 2001).

Shrimp soluble extract is an important feed attractant substance. The product is made from the extraction and hydrolysis of shrimp, provides essential nutrients, and creates an extremely attractive smell and taste for animals such as cattle, poultry, shrimp, and fish. The natural nutrient elements in fresh shrimps provides natural protein full of amino-acids, therefore the active peptides that are

highly digestible and absorbable for animals creating an easy to consume product, which enhances breeding productivity.

Rainbow trout, *Oncorhynchus mykiss* has become the iconic freshwater aquaculture species around the world. The global aquaculture production of rainbow trout has grown rapidly and reached 814,068 tons in 2013 (FAO, 2015). The rainbow trout is usually reared in enclosed spaces and efforts have been made to increase productivity per unit space (Sakai, 1999). A massive expansion along with the intensification has led to sudden increase in the annual production of rainbow trout in the Republic of Korea and reached 3,304 ton in 2014 (KOSTAT, 2015).

II. Materials and Methods

Experimental Diets

Shrimp soluble extract source tested in this study were provided by VNF Company (Vietnam Food Joint Stock Company), Ca Mau, Vietnam. Shrimp Soluble Extract production process is divided into the following key stages. Shrimp heads collected from seafood processors are processed to eliminate extraneous matter, then cut and pressed to obtain liquid extract. The shell leftovers are used to produce chitin and glucosamine. This liquid extract will go through centrifugal processing to acquire its purest form. The extract is then sent to chemical processing area where the protein will be broken into peptides and digestible single protein (amino acid) such as: aspartic acid, threonine, serine,

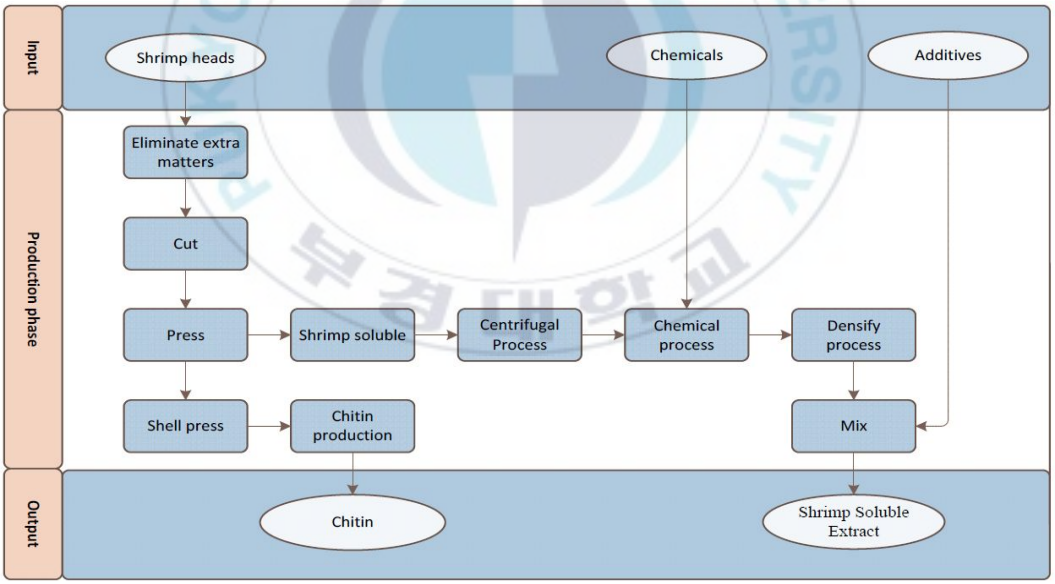

Fig 1. Shrimp soluble extract production process

glutamic acid, proline, glycine, alanine, valine, cystein, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, arginine. Many among these amino acids are widely used as growth stimulants for livestock and aquaculture (aspartic acid, threonine, methionine, lysine). Chemically processed extract is densified to create products that will meet various levels of quality standards according to customer demands. Densified soluble is mixed and added with flavor-preservation additives, to maintain the product's unique scent. Proximate composition and amino acid profile of the shrimp soluble extract have been provided in Tables 1, 2 respectively.

Dietary formulation and proximate composition of the basal diet are shown in Table 3 and amino acid composition of the four experimental diets are shown in Table 4. Four experimental diets were formulated to be isonitrogenous and isoenergetic and to contain 43% crude protein. The basal diet without fish meal analogue and shrimp soluble extract supplementation was used as a Control, and three other diets were prepared by supplementing shrimp soluble extract (FM100% + FMA0% + SSE2%), FMA_{12} (FM88% + FMA12% + SSE2%), FMA_{24} (FM76% + FMA24% + SSE2%). Fish meal, chicken meal, meat and bone meal, soybean meal and squid liver powder were used as the protein sources, soybean oil and fish oil as the lipid source, and wheat flour as the carbohydrate source in the experimental diets (Table 3). Fish meal analogue were used for ingredients of

poultry by-product meal, seasoning residue, wheat gluten meal, blood meal, lysine, methionine and phosphate.

Procedures for diet preparation and storage were followed as previously described by Bai & Kim (1997). Briefly, after thoroughly mixing the dry ingredients and fish oil with filtered tap water, experimental diets were pelleted using a extruder machine (Twin extruder, ATX-Ⅱ, Fesco, Korea) without heating using a 4.7 mm diameter module. After processing, all diets were kept at -20°C in refrigerator until use.

Table 1. Composition of the shrimp soluble extract for growing rainbow trout (% of wet sample)

Ingredients	%
Moisture	68.0
Crude protein	19.0
Crude lipid	3.10
Crude ash	2.90
Digestible protein/total protein	90.0
Calcium	0.49
Phosphorous	0.20

Table 2. Free amino acid contents of the shrimp soluble extract (% of wet sample)

Amino acids	%
Indispensable	
Arginine	0.05
Histidine	0.04
Isoleucine	0.19
Leucine	0.34
Lysine	0.20
Methionine	0.11
Phenylalanine	0.16
Threonine	0.04
Tryptophan	0.00
Valine	0.29
Dispensable	
Taurine	0.24
Aspartic acid	0.02
Glutamic acid	0.14
Serine	0.02
Proline	0.11
Glycine	0.26
Alanine	0.72
Tyrosine	0.08
$\sum$ FAA[1]	3.65

[1] $\sum$ FAA : total free amino acids

Table 3. Composition of the basal experimental diet for growing rainbow trout (% of dry matter basis)

Ingredients	%
Fish meal [1]	42.0
Fish meal analogue[2]	0.00
Chicken meal	5.00
Wheat meal	6.97
Wheat flour	6.00
Meat and bone meal	5.00
Soybean oil[1]	12.1
Fish oil (cod liver)	7.26
Etc[3]	15.4
Shrimp soluble extract	0.00
Proximate analysis (% of DM basis)	
Moisture	6.14
Crude protein	44.1
Crude lipid	23.7
Crude ash	13.2

[1] Norse LT-94®, low-temperature dried fish meal, Norsildmel, Bergen, Norway

[2] Poultry by-product meal, seasoning residue, wheat gluten meal, blood meal, lysine, methionine and phosphate

[3] Seasoning residue, soybean meal, corn gluten meal, squid liver powder, vitamin and mineral premix

Table 4. Amino acid contents of four experimental diets for growing rainbow trout (% of dry matter basis)

	Diets			
	Cont	SSE	FMA_{12}	FMA_{24}
Aspartic acid	4.51	4.54	4.49	4.56
Threonine	1.95	1.90	1.87	1.92
Serine	1.91	1.89	1.87	1.93
Glutamic acid	6.60	6.61	6.69	6.81
Proline	2.37	2.34	2.40	2.47
Glycine	2.98	2.99	3.05	3.09
Alanine	2.98	2.98	2.99	3.11
Valine	2.37	2.39	2.41	2.62
Isoleucine	1.98	1.96	1.96	2.04
Leucine	3.60	3.56	3.58	3.80
Tyrocine	1.24	1.22	1.23	1.32
Phenylalanine	1.97	1.98	1.98	2.11
Histidine	1.64	1.67	1.65	1.69
Lysine	3.07	3.04	3.16	3.21
Arginine	2.65	2.62	2.61	2.70
Cystein	0.70	0.69	0.70	0.69
Methionine	0.83	0.81	0.85	0.79
Total	43.4	43.2	43.5	44.8

Experimental fish and feeding trial

The feeding trial was carried out at the Feeds and Foods Nutrition Research Center, Pukyong National University, Busan, South Korea. Growing rainbow trout were obtained from Sangju in the Republic of Korea. Prior to the start of the feeding trial, all the fish were fed the basal diet for one week to become acclimatized to the experimental conditions and facilities. At the start of the experiment, 20 rainbow trout with an initial weight averaging 146 ± 3.8 g (mean ± SD) were randomly distributed into each of the 15 tanks. Each tank was then randomly assigned to one of three replicates of four dietary treatments. Fish were fed 2 times daily (9:00 and 16:00 hrs) for twelve weeks at apparent satiation of wet BW/day. Total fish weight in each tank was determined every one month and the feeding rate was adjusted accordingly. The feeding trial was conducted by using the running system with 15 tanks (500 L) receiving freshwater at the rate of 3 L/min from the center tank. Supplemental aeration was provided to maintain the dissolved oxygen near saturation, and also water temperature and pH during the experiment were maintained at 16 ± 0.5℃ and 7.5 ± 0.3, respectively. Photoperiod of 12h light: 12h dark was used throughout the experimental period.

Throught the experimental period, temperature, dissolved oxygen concentration total ammonia and pH (YSI Model 85, YSI Incorporated, Yellow Springs, OH, USA) were measured daily at 08:00 hours in the tanks.

Sample collection and analysis

At the end of the feeding trial, the total number and weight of fish in each tank were determined for calculation of weight gain, feed efficiency, specific growth rate, protein efficiency ratio, survival rate, hematosomatic index, visceralsomatic index and condition factor. Five fish per tank were randomly selected, blood samples were collected from the caudal vein with heparinized syringes for measurement with whole blood, plasma was separated by centrifugation at 5000 × *g* for 10 min and stored at −70°C for determination of blood biochemical parameters including aspartate transaminase, alanine transferase, plasma glucose, triglyceride, total protein and total cholesterol. Another set of blood samples of the same fish were taken without heparin and allowed to clot at room temperature for 30 min. Then, the serum was separated by centrifugation at 5000 × *g* for 10 min and stored at −70°C for the analysis of non-specific immune responses including lysozyme and superoxide dismutase activities. Three additional fish from each tank were used to analyze whole-body proximate composition. Proximate composition analyses of the experimental diets and fish bodies performed by the standard methods of AOAC (1995). Samples of diets and fish were dried at 105℃ to a constant weight to determine their moisture content. Ash content was determined by incineration at 550℃. Protein was determined using the kjeldahl method (N x 6.25) after acid digestion, and crude lipid was ascertained by soxhlet

extraction using the soxhlet system 1046 (Tacator AB, Sweden) after freeze-drying the samples for 20h. Amino acid analysis of edible protion was performed by ninhydrin method (Sykam Amino Acid Analyzer S433, Sykam; Eresing, Germany).

Hematological parameters

Hematocrit was determined on three individual fish randomly selected pre aquarium by the microhematocrit method (Brown 1980), and hemoglobin was measured with the same fish by cyan-methemoglobin procedure using Drabkin's solution. Hemoglobin standard prepared from human blood (Sigma Chemical Co., St Louis, MO, USA) was used.

The plasma levels of plasma glucose, total protein, total cholesterol and activities of AST and ALT were measured by a chemical analyzer (Fuji DRI-CHEM 3500i, Fuji Photo Film, Ltd., Tokyo, Japan).

Non-specific immune responses

A turbidometric assay was used for determination of serum lysozyme level by the method described by Hultmark (1980) with slight modifications. Briefly, Micrococcus lysodeikticus (0.75 mg ml^{-1}) was suspended in sodium phosphate buffer (0.1 M, pH 6.4), 200 μl of suspension was placed in each well of 96-well plates, and 20 μl serum was added subsequently. The reduction in absorbance of

the samples was recorded at 570 nm after incubation at room temperature for 0 and 30 min 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.

SOD activity was measured by the percentage reaction inhibition rate of enzyme with WST-1 (Water Soluble Tetrazolium dye) substrate and xanthine oxidase using a SOD Assay Kit (Sigma-Aldrich, 19160) according to the manufacturer's instructions. Each endpoint assay was monitored by absorbance at 450 nm (the absorbance wavelength for the colored product of WST-1 reaction with superoxide) after 20 min of reaction time at 37 °C. The percent inhibition was normalized by mg protein and presented as SOD activity units.

Color measurement

Instrumental colorimetric analyses were performed with a Hunterlab Tristimulus Colorimeter (Model D25 Optical Sensor; Hunter Associates Laboratory, Fairfax, VA). Fish were thawed, manually skinned and filleted, then approximately 400 g of fillet from each treatment group were pooled and minced for 5–10 s with a Cuisinart® food processor. A 40 g subsample was taken for instrumental colorimetric analyses and the remainder was used for sensory analyses. The minced, raw fillet was densely packed into the glass sample cup (6 cm diameter) to a height of approximately 2 cm. Measurements were recorded

using the L*a*b scale (Hunter, 1987), in which L* indicates lightness, a* indicates redness, and b* indicates yellowness of the sample. Total color difference (ΔE) was calculated for each of the test samples vs. the commercial control using the equation $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$ (Hunter, 1987).

Statistical Analysis

All data were analyzed by one-way ANOVA (Statistic 3.1; Analytical Software, St. Paul, MN, USA) to test the effects of the dietary treatments. When a significant treatment effect was observed, an LSD test was used to compare means. Treatment effects were considered at $P<0.05$ level of significance.

III. Results

The results of water quality parameters monitored are shown in Table 5. The measured water quality in all experimental groups remained within recommended levels for rainbow trout culture.

Growth performance and of growing rainbow trout fed different experimental diets are shown in Table 6. At the end of 12 weeks of feeding trial, weight gain of fish fed SSE and FMA_{12} diets was significantly higher than those of fish fed control and FMA_{24} diets ($P<0.05$). Also, Feed efficiency and protein efficiency ratio of fish fed control, SSE and FMA_{12} diets were significantly higher than those of fish fed FMA_{24} diet. Furthermore, Specific growth rate of fish fed SSE and FMA_{12} diets was significantly higher than those of fish fed control and FMA_{24} diets. However, there were no significant differences in survival rate of fish fed all the diets.

There were no significant differences in proximate composition among fish fed all the experimental diets (Table 7).

Table 8 shows hematological parameters of growing rainbow trout fed different experimental diets. Alanine transferase of fish fed control, SSE and FMA_{12} diets were significantly lower than those of fish fed FMA_{24} diet ($P<0.05$). However, there were no significant differences in hematocrit, hemoglobin, alanine transferase, glucose, total protein and total cholesterol contents of fish fed experimental diets ($P>0.05$).

Non-specific immune responses are shown in table 9. Superoxide dismutase activity values of fish fed FMA_{12} and FMA_{24} diets were significantly higher than those of fish fed control diet ($P<0.05$). Lysozyme activity values of fish fed SSE, FMA_{12} and FMA_{24} diets were significantly higher than those of fish fed control diet.

Color measurement of muscle from growing rainbow trout fed different experimental diets are shown in table 10. There were no significant differences among them ($P>0.05$).

Table 5. Average water quality parameters of growing rainbow trout fed the experimental diets for 12 weeks

	Diets				Pooled SEM[2]
	Cont	SSE	FMA_{12}	FMA_{24}	
Temperature(℃)	15.9	15.9	16.0	15.9	0.03
DO (mg/L)[1]	7.8	7.9	8.0	7.9	0.02
pH	7.4	7.4	7.5	7.3	0.02
Total ammonia (%)	0.48	0.48	0.47	0.47	0.01

[1]DO : dissolved oxygen

[2]Pooled standard error of mean : SD/√n

Table 6. Growth performance of growing rainbow trout fed the experimental diets for 12 weeks

	Diets				Pooled SEM[9]
	Cont	SSE	FMA_{12}	FMA_{24}	
WG (%)[2]	154[b]	166[a]	166[a]	144[b]	3.13
FE (%)[3]	85.9[a]	87.3[a]	87.4[a]	80.1[b]	1.08
SGR (%/day)[4]	1.35[b]	1.42[a]	1.42[a]	1.31[b]	0.02
PER[5]	0.40[a]	0.41[a]	0.39[a]	0.37[b]	0.01
HSI (%)[6]	1.04	1.14	1.13	1.01	0.02
VSI (%)[7]	9.86	10.6	10.3	9.68	0.17
CF[8]	1.31	1.36	1.36	1.35	0.02

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

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

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

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

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

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

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

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

[9]Pooled standard error of mean : SD/√n

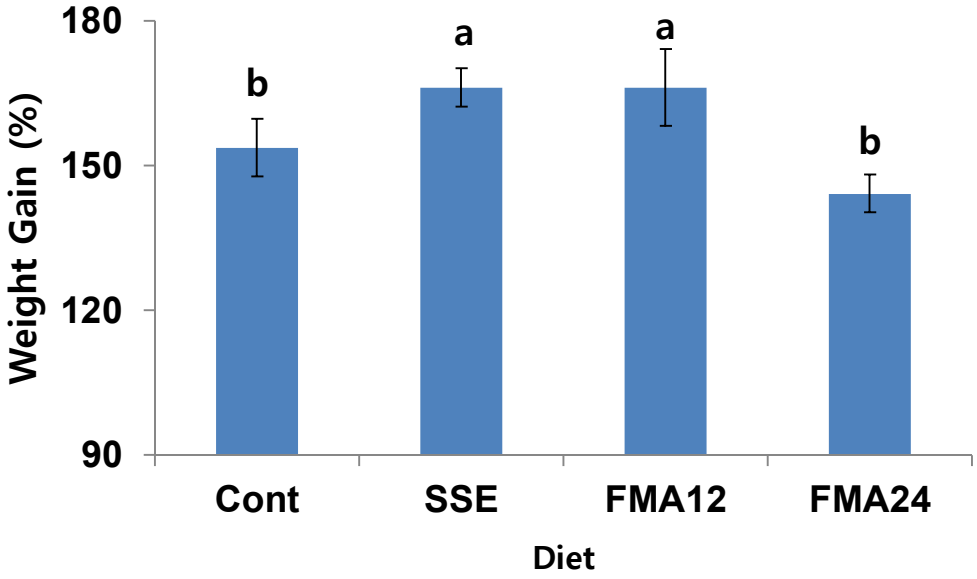

Fig 2. Weight gain of growing rainbow trout fed the experimental diets for 12 weeks

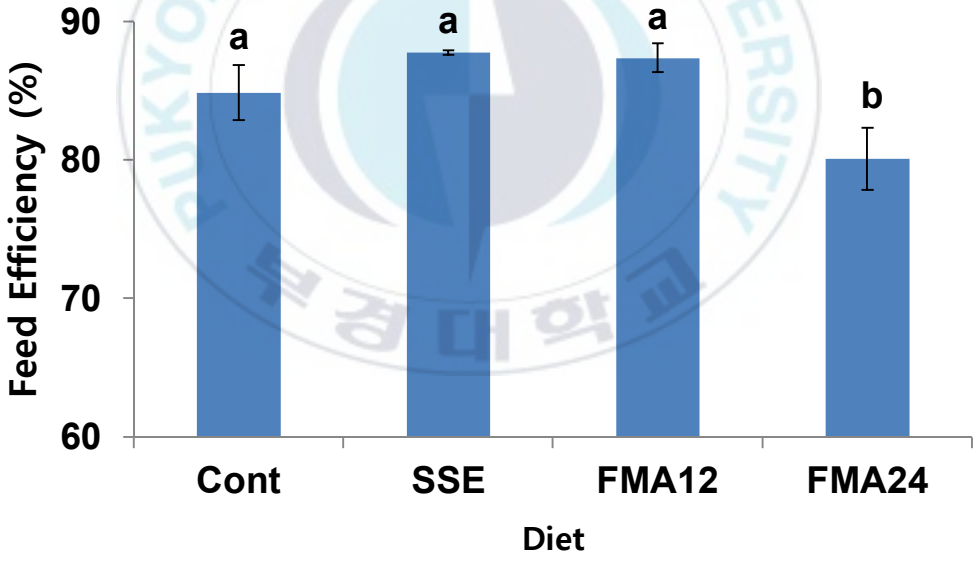

Fig 3. Feed efficiency of growing rainbow trout fed the experimental diets for 12 weeks

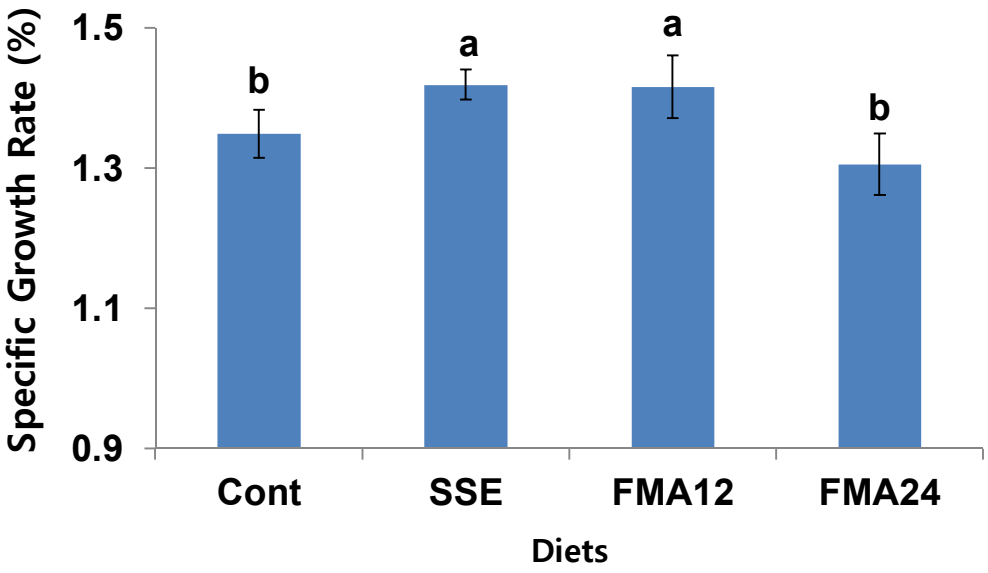

Fig 4. Specific growth rate of growing rainbow trout fed the experimental diets for 12 weeks

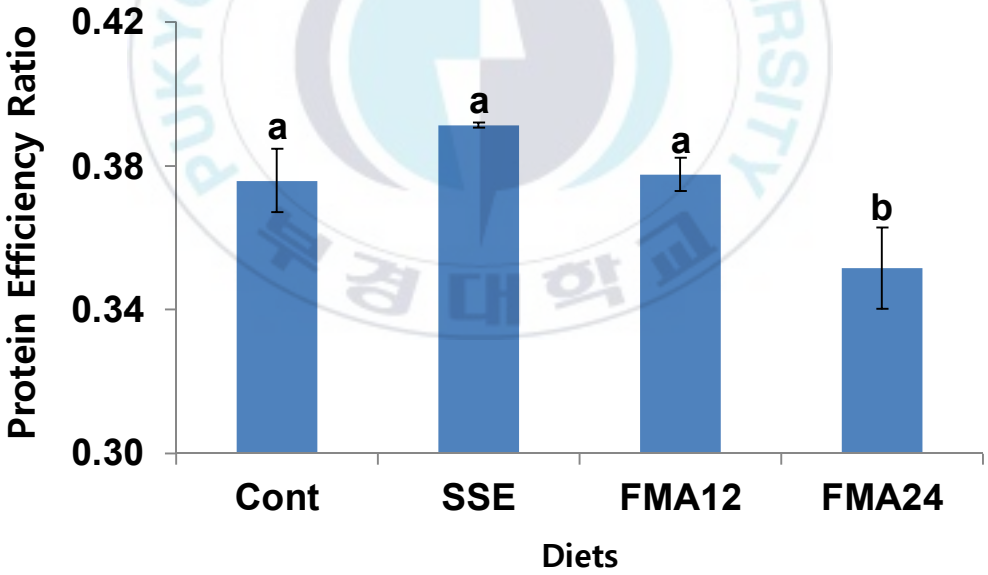

Fig 5. Protein Efficiency Ratio of growing rainbow trout fed the experimental diets for 12 weeks

Table 7. Whole-body proximate composition of growing rainbow trout fed the experimental diets for 12 weeks (%, dry matter basis)[1]

	Diets				Pooled SEM[2]
	Cont	SSE	FMA_{12}	FMA_{24}	
Moisture(%)	66.1	65.9	66.8	67.1	0.39
Crude Protein(%)	48.0	47.9	48.4	48.3	0.09
Crude Lipid(%)	42.0	43.4	41.4	43.5	0.33
Crude Ash(%)	4.73	4.57	4.78	4.93	0.45

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

[2] Pooled standard error of mean : $SD/\sqrt{n}$

Table 8. Hematological parameters of growing rainbow trout fed the experimental diets for 12 weeks[1]

	Diets				Pooled SEM[6]
	Cont	SSE	FMA_{12}	FMA_{24}	
PCV (%)[2]	38.3	38.0	36.5	37.7	0.42
Hb (g/dl)[3]	8.3	8.2	8.0	8.1	0.06
AST (U/L)[4]	27.9^{b}	26.4^{b}	25.6^{b}	37.5^{a}	1.56
ALT (U/L)[5]	17.7	17.0	18.0	19.0	0.50
Glucose (mg/dl)	72.7	70.0	69.4	69.5	0.86
T-Protein (g/dl)	4.7	4.6	4.8	4.9	0.09
T-Cholesterol (mg/dl)	241	244	240	252	2.75

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

[2] PCV (%) : Hematocrit

[3] Hb (g/dL): Hemoglobin

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

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

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

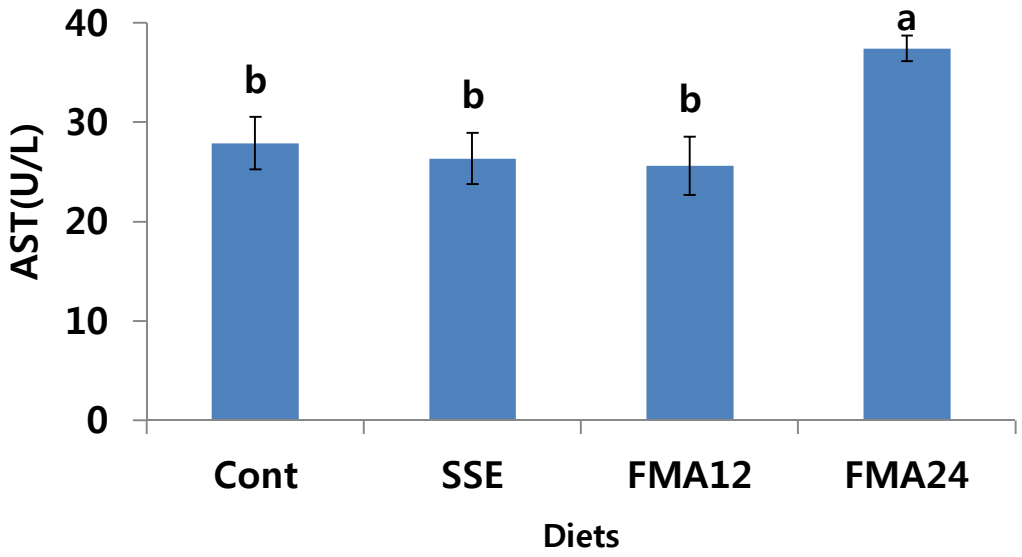

Fig 6. AST of growing rainbow trout fed the experimental diets for 12 weeks

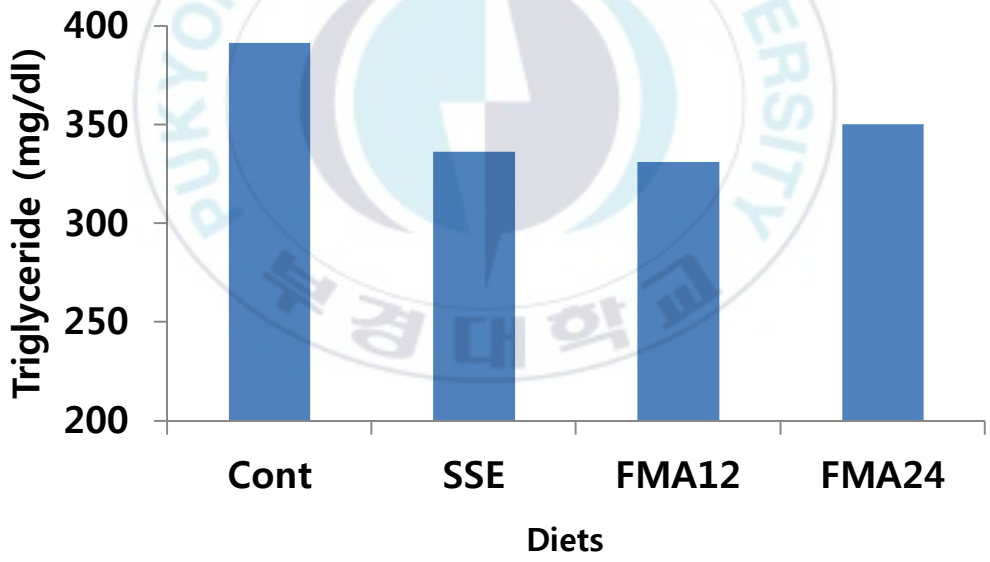

Fig 7. Triglyceride of growing rainbow trout fed the experimental diets for 12 weeks

Table 9. Non-specific immune responses of growing rainbow trout fed the experimental diets for 12 weeks[1]

	Diets				Pooled SEM[3]
	Cont	SSE	FMA_{12}	FMA_{24}	
SOD[2]	71.4^{b}	74.3^{a}	75.3^{a}	75.0^{a}	0.86
Lysozyme (U/ml)	1.09^{b}	1.39^{ab}	1.49^{a}	1.44^{a}	1.56

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

[2]SOD (% inhibition): Superoxide dismutase

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

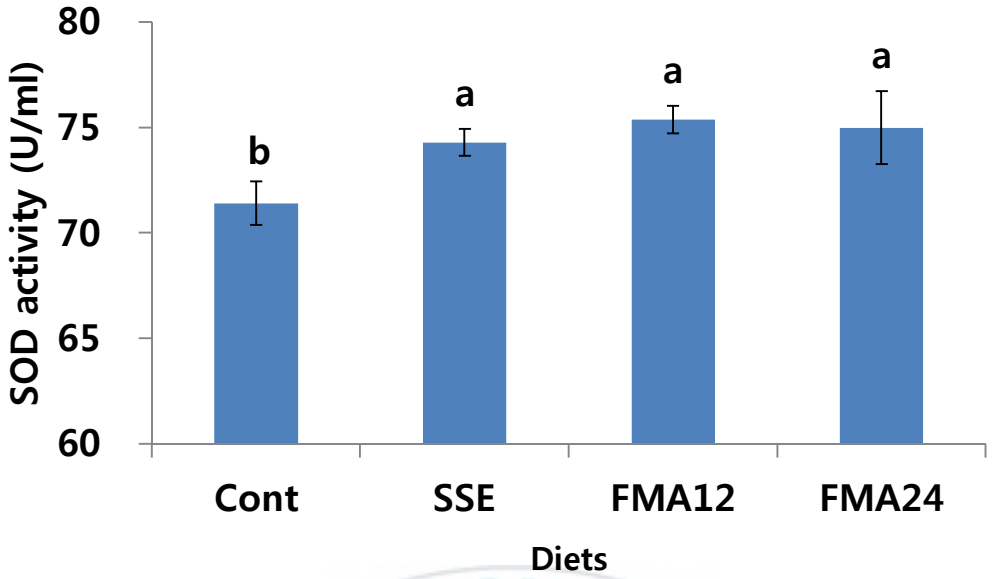

Fig 8. Superoxide dismutase activity of growing rainbow trout fed experimental diets for 12 weeks

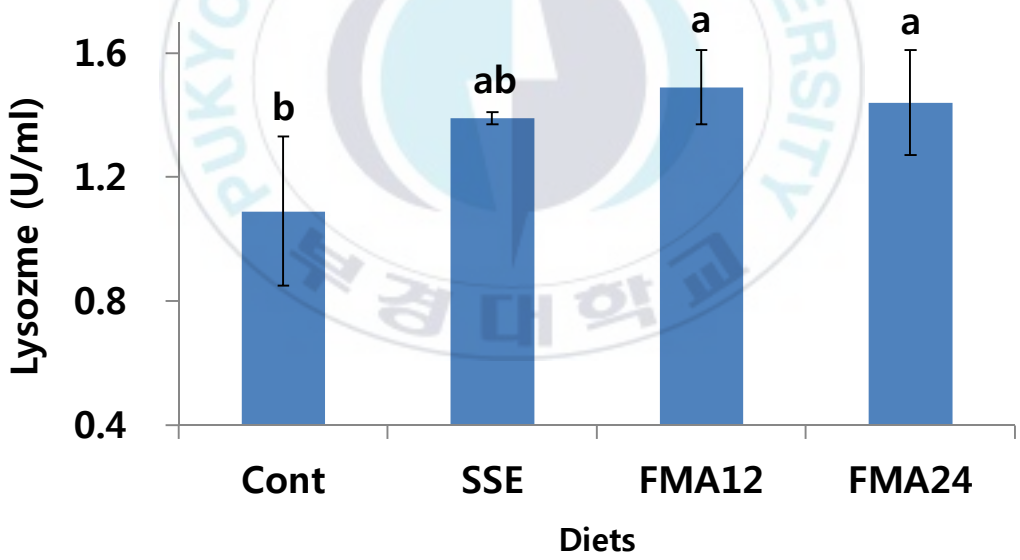

Fig 9. Lysozyme activity of growing rainbow trout fed experimental diets for 12 weeks

Table 10. Color measurement of growing rainbow trout fed experimental diets for 12 weeks[1]

	Diets				Pooled SEM[3]
	Cont	SSE	FMA_{12}	FMA_{24}	
L (lightness)	57.5	61.3	58.6	58.5	1.03
a (redness)	24.9	23.8	24.5	26.4	0.61
b (yellowness)	37.5	32.6	27.2	36.0	0.82
ΔE^2	23.3	24.0	24.9	28.5	0.98

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

[2]Overall color difference $(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$

[3] Pooled standard error of mean : $SD/\sqrt{n}$

IV. Discussion and Conclusion

Increasing production costs in aquaculture industries have triggered the interest to utilize low-cost food processing by-products in aquafeed. It has several important advantages, these being to diminish dependence on expensive protein source such as fish meal and to eliminate the need for costly waste management programme (Kader et al., 2011). In the present study, Fish meal analogue was evaluated as Fish meal substitute in terms of growth performance, hematological performance, non-specific immune responses of growing rainbow trout. In addition, dietary supplementation of shrimp soluble extract significantly improved growth performance, feed utilization and non-specific immune responses. Shrimp soluble extract used in the present study mainly consists of small peptides and free amino acids (Table 2). Similarly, Khosravi et al. (2015) showed that supplementing feed additives including shrimp hydrolysate can improved on weight gain, specific growth rate, feed conversion ratio in red sea bream. Several authors have reported that dietary supplementation of protein hydrolysates at low inclusion levels has positive impact on growth performance and health condition of fish (Aksnes et al., 2006; Liang et al., 2006; Kotzamanis et al., 2007; Zheng et al., 2012). In the current study, enhanced growth performance by the inclusion of the shrimp soluble extract might be explained by the presence of certain free amino acid and bioactive peptides derived from hydrolysis processes which most likely are essential for promoting the biological performance of fish (Aksnes et al., 2006).

Hematological parameters are useful indicators for evaluating the physiological and health status of fish (Maita et al., 2007). In the present study, AST of fish fed FMA_{24} diet was significantly higher than those of fish fed Con, SSE and FMA_{12} diets. Increment of AST level in this study may indicate nutritional or environmental problems of the high fishmeal replacement of fish fed FMA_{24} diet. The other measured hematological parameters in this study were not affected by the dietary treatments, and the values were within the reported ranges for growing rainbow trout (Talas et al., 2009; Trullàs et al., 2016)

Non-specific immune responses are acknowledged to be the indicator of fish health and thereby its ability of fend off disease. Lysozyme is a hydrolytic enzyme that degrades bacterial cell walls by attacking peptidoglycans, thereby restricting the bacterial growth, encouraging phagocytosis and ultimately enhancing the immune system of an animal, including fish (Bae et al., 2008). In the present experiment, FMA_{12} and FMA24 diets was observed to significantly improve the lysozyme activity over control diet. The family of antioxidant enzymes, called the superoxide dismutase (SOD) catalyses the dismutation of the highly reactive (O_2) to the less reactive H_2O_2 and functions in the main antioxidant defense pathways in response to oxidative stress (Fridovich et al., 1995). The present experiment showed the fish fed diets adding 2% shrimp soluble extract improve SOD activities in compare with the fish fed Control diet. Overall observation for immune response suggested, 2% shrimp soluble extract could be effective on

nonspecific enzymatic activity. Similar observations have been reported in various previous studies for dietary additives such as shrimp hydrolysate (Khosravi et al., 2015), fish protein hydrolysate (Murray et al. 2003) and ultra-filtered fish hydrolysate (Zheng et al., 2013).

Whole-body composition of fish was not significantly affected by FMA or shrimp soluble extract in this study which is in accordance with the results of Oliva-Teles et al. (1999). Also, Aksnes et al. (2006) found no beneficial effect of fish hydrolysate on whole-body protein and ash in rainbow trout. Zheng et al. (2012) reported that dietary inclusion of higher dose of ultrafiltered fraction of fish hydrolysate was able to effectively increase the whole-body protein content in Japanese flounder whereas a lower dose failed. These findings show that amount of protein hydrolysate and fish species should be applied to observe any significant effects on whole-body composition.

In conclusion, fish meal analogue can be used up to 12% fish meal adding 2% shrimp soluble extract as a substitute of fish meal in growing rainbow trout. With fish meal prices on the rise, it remains a matter of time until fish meal analogue adding shrimp soluble extract can be economically produced in a lot of quantities at a competitive price and become a common feed ingredient.

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VI. Appendix

Initial data

	Tank No	Total Weight	Number	Individual weight	Total feeding	Individual feeding
CON	4	2985	20	149.3	247	12.3
	7	2962	20	148.1	259	12.9
	15	2900	20	145.0	273	13.0
SSE	2	2826	20	141.3	329	16.5
	6	2916	20	145.8	326	16.3
	12	2801	20	140.1	298	14.9
FMA_{12}	1	2859	20	143.0	311	15.6
	10	2818	20	140.9	294	14.7
	13	3008	20	150.4	311	15.5
FMA_{24}	3	2896	20	144.8	329	16.4
	8	2964	20	148.2	308	15.4
	14	3043	20	152.2	318	15.9

12th weeks data

	Tank No	Total Weight	Number	Individual weight	Total feeding	Individual feeding
CON	4	7432	20	371.6	5176	258.8
	7	7716	20	385.8	5423	271.1
	15	7301	20	365.1	5234	261.7
SSE	2	7629	20	381.5	5483	274.2
	6	7642	20	382.1	5471	273.6
	12	7472	20	373.6	5316	265.8
FMA_{12}	1	7869	20	393.5	5736	286.8
	10	7334	20	366.7	5229	261.4
	13	7916	20	395.8	5547	277.3
FMA_{24}	3	7200	20	360.0	5393	269.6
	8	7146	20	357.3	5383	269.2
	14	7392	20	369.6	5252	262.6