論文要約（４（２）関係）

博士論文要約（Summary）

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タイトル	Nutritional study on the growth performance and body composition of red sea bream and yellowtail using recirculating aquaculture system **(循環式飼育システムを用いたマダイ及びブリの成長と体組成に関する栄養学的研究)**

キーワード（ Nutritional requirement of plant protein, lipid and vitamin E）（Red sea bream; Yellowtail）（Recirauclating aquaculture system）

「序論及び目的」

Marine fish culture in raceways, ponds and net cages in open water adversely affects the surrounding environment with effluents productions from the culture species. To sustain the aquaculture development, the most advanced technology is known as a closed recirculating aquaculture systems (RAS), which can mitigate most of the potential environmental impacts by dramatically reducing the volume of water discharged. However, RAS need a continuous supply of clean water and dissolved oxygen for the optimum growth of culture species by using with a foam separation unit, a nitrification unit and a denitrification unit.

In carnivorous fish, fish meal (FM) is a main protein source in aquafeeds due to its high level of protein, excellent amino acid balance, low carbohydrate level, high digestibility and few antinutritional factors. However, limitation of marine products has resulted to increase an investigation of alternative protein and oil sources for aquafeeds to sustain the aquaculture development. It is well documented that high inclusion level of fish oil have highly susceptible to oxidation, however, palm olein can reduce lipid

oxidation due to the high inclusion of tocopherols and tocotrienols. The use of fish meal (FM) and fish oil (FO) can be substituted up to 50% with alternate protein in combination of SBM, CGM, and MM, and lipids such as palm oil and beef tallow, respectively, for yellowtail, and the combination of fish oil and other lipids would be useful in lowering in *vivo* peroxidation. At present, a partial replacement of plant protein (15-30%) in fish feed is widespread through the world, but it is not currently possible to completely eliminate the use of FM and fish oil without negative impact on their nutritional profile. Many different kind of plant protein sources can be utilized to replace fish meal include soybean meal, lupine seed meal, corn gluten meal and rapeseed/canola meal, those are either locally available or traded on the world market. Replacing of FM with different levels of plant proteins is possible for red sea bream in net cage culture.

Dietary lipid is a large group of organic compound, an important in energy production processes of animal tissues, a source of an essential fatty acids (EFA), transfer and absorption of fat soluble vitamins, promote maturation, increase growth and contribute to the palatability of feeds. Fish oil is a by-product of fish meal production, it have been widely used in marine fish diets as a source of energy and essential fatty acids (EFA) for the optimal growth and health of farmed fish. Previous studies have been shown that, too much dietary lipid may decrease weight gain and may increase lipid deposition which can effect on carcass composition of the experimental fish. Normally, commercial yellowtail feeds include about of 48% crude protein and 20% crude lipid; 67-71% crude protein and 9 to 13% crude lipid. The fatty acid composition of tissue lipids are influenced by the fatty acid composition of dietary lipid /and linear correlations exist between individual fatty acids in tissue total lipid and their concentrations in dietary lipid.

Vitamin is an organic compound and most species require vitamin in trace amounts from an exogenous source for development of normal tissue, for growth, health,

maintenance, and reproduction. Vitamin E (α-tocopherol) is one of the micronutrient vitamins in which fat soluble vitamin. The concentration of vitamin E in plasma, tissue and liver were increased by a dietary supplementation of vitamin E in red drum and juvenile grouper fish. The increasing level of tissue vitamin E had negatively response to TBARS content of juvenile hybrid tilapia. The requirement level of vitamin E for optimum growth has been demonstrated in a number of fish, such as 99 mg/kg for mrigal, *Cirrhinus mrigala*, ≥100 mg/kg for rohu, *Labeo rohita*, 300 mg/kg for common carp, *Cyprinus carpio*.

In aquaculture, dietary requirements of energy, protein and amino acids, lipids, minerals, and vitamins are affected by species, size, feed intake, energy density in the diet, interactions of dietary nutrients, and physiological sate of the animals as well as environmental factors. Fish must be fed adequate quantities of diets to meet all their nutrient requirements, as diets have strong effects on stress tolerance and health. Therefore, we must know specie's nutritional requirements and meet those requirements with balanced diet formulation and appropriate feeding practices. In Japan, Japanese flounder and tiger puffer can be produced intensively in closed recirculation system with a high survival rate. The red sea bream and yellowtail are both commercially important cultured warm water marine fish in Japan, however, no information is available on the nutritional requirement for those species cultured under the recirculating aquaculture system. For that the aim of this study was to determine the requirement level of lipid, an alternative plant protein sources and vitmin E when the fish cultured in RAS.

「材料及び方法」

All experiments were carried out in closed recirculating aquaculture system (kamoike Aquaculture Research Centre, ARC), Kagoshima, Japan. In this system, adequate water quality was maintained through drum filter (80 Series filter, FAIVRE Sarl, France) and biological filter (Floating plastic filter media). Dissolved oxygen was maintained by providing with electric blower (SF 1.0, EMERSON$_{TM}$ ST. Louis, MO.

USA). Water flow was kept to each rearing tank with pump (TC 50-160; APEX Pump, Bristol UK). Light and dark cycle of 12:12 h was maintained with fluorescent lights (172 LX). 1000 L fiber tank was used for red sea bream and 7000 L was reared for yellowtail.

Fish were acclimated to their new surroundings for one week and were fed a commercial Skreeting diet. After that, fish were randomly distributed into the respective tank for each dietary group. The experimental diets were produced from Skreeting Co., Ltd., Fukuoka, Japan, expect the vitamin E experimental diets. Each diet was assigned to three replicate groups of fish. All groups of fish were fed their respective diet at 09:30 and 17:00 h, 6 days per week and feed consumption was recorded daily.

At the end of the experiment, fish (n=3) were caught from each of the replicate tank. Blood sample was taken from the caudal venin with heparinized syringe, measured hematocrit and hemoglobin, then centrifuged (3000 × g for 15 min at 4°C) to obtain the plasma for liver function and oxidative condition analysis. Viscera and liver samples were dissected from the same fish, and then measured the weigh for calculation of somatic indexes. Then liver and muscle samples were withdrawn to analyze proximate composition, amino acids, fatty acids, vitamin E and thiobarbituric acid reactive substances composition. Finally, all samples were kept at -80°C until analysis.

Proximate composition (moisture, ash, crude protein and total lipid) of experimental diets and muscle samples were performed by the standard methods of AOAC (1990). Total amino acid (TAA) and free amino acid (FAA) concentration in diet and muscle were analyzed by high performance liquid chromatography (HPLC, Shimadzu Crop. Tokyo, Japan) according to Teshima et al. (1986). Fatty acids from total lipid were converted to fatty acid methyl esters (FAME) by acid-catalyzed transesterification with 1ml boron trifluoride (14% BF_3-MeOH) at 100 ºC for 1 h according to the method of Querijero et al. (1997). Vitamin E content in feed, muscles and livers were analyzed by reversed phase high performance liquid chromatography

(HPLC) with fluorescence detector according to the method of Gao et al. (2012a). The measurement of (TBARS) was carried out using the method of Yagi (1987). The level of hematocrit was determined by the method of Kawadzu (1981). Plasma liver functions, total protein and triglyceride were determined with an automated analyzer (SPOTCHEM^{TM} EZ model SP-4430, Arkray Inc. Kyoto, Japan. And, Biological antioxidant potential (BAP) and reactive oxygen metabolites (d-ROMs) were also measured from blood plasma with an automated analyzer (FRAS4, Diacron International s.r.l., Grosseto, Italy) following Kader et al. (2010).

「結果」

In study 1, five diets (FM40T, FM25T, FM20T, FM15T, FM15) containing 52% crude protein and 20% lipid were used to examine the effect of replacing fish meal with plant protein mixture on red sea bream (18 g). After 54 days, feed intake, specific growth rate and increase rate of biomass significantly increased in FM40T and FM25T. FM15 significantly increased the liver lipid contents, GOT (Glutamyl oxaloacetic transaminase) and GPT (Glutamic pyruvate transaminase). FM15T supplemented diet had a higher tolerance against an oxidative stress.

In study 2, three diets (FM34, FM25 and FM15) contained 48 to 77% plant proteins, 70% fish oil and 30% palm olein in 37% crude protein and 28% lipid were used to examine the effects of fishmeal and fish oil replacements with plant sources for yellowtail (2236 g). After 96 days, feed consumption was significantly declined in FM15 diet. Muscle monounsaturated and highly unsaturated fatty acids were significantly increased in the diet of FM34 and FM25.

Study 3 examined the effects of three lipid levels (LF13, MF16, and HF19) on juvenile yellowtail (100 g). After 60days, HF diet significantly increased muscle lipid. HF and MF diet significantly decreased linoleic acid contents and increased docosahexaenoic acid (DHA) contents in both liver and muscle tissues. HF and MF diet significantly reduced n-6 HUFA in liver and muscle (P>0.05). The muscle n-3 HUFA

contents significantly increased in HF and MF diets, and compositions of n-3 HUFA were significantly higher than that of the liver.

In study 4, juvenile red sea bream (112 g) were fed diets (0, 100, 200, 400 mg VE/kg diet) for 30 days. Tissues VE was significantly higher in 400 mg VE/kg diet, followed by 200 and 100 mg VE/kg diet, respectively. Thiobarbituric acid reactive substances (TBARs) were inversely related (P<0.05) with VE levels. Dietary VE significantly increased the profile of DHA and n-3 HUFA. Hemoglobin significantly increased but plasma triglyceride, total cholesterol and oxidative stress decreased as VE increased.

「結論及び考察」

FM40T and FM25T diet could be recommended for the optimal growth and health of red sea bream fish. Substitution of fish meal and fish oil did not affect the growth performances, but increased the muscle HUFA in FM34 and FM25 for yellowtail. High fat diet improved the tissue fatty acid and health conditions. And, vitamin E 200mg/kg diet reduced the tissue lipid oxidation and improved the health conditions. Over all conclusions, alternative protein and lipid replacement levels in red sea bream and yellowtail using this RAS are similar to those which reported with net cages and flow-through systems previously.

References

AOAC (1990) Official Methods of Analysis of the Association of Official Analytical Chemists, 15th ed. Association of Official Analytical Chemists, Arlington, VA, USA, pp. 1298.

Gao, J., S. Koshio, M. Ishikawa, S. Yokoyama, M. A. Kader and A. Laining (2012a) Interaction between vitamin C and E on oxidative stress in tissues and blood of red sea bream (*Pagrus major*). Aquacult. Sci., 60, 119-126.

Kader, M. A., S. Koshio, M. Ishikawa, S. Yokoyama and M. Bulbul (2010) Supplemental effects of some crude ingredients in improving nutritive values of low fishmeal diets for red sea bream, *Pagrus major*. Aquaculture, 308, 136-144.

Kawadzu, H. (1981) Ketsueki kensaho. In: N. Egami, editor. Jiken doubutsu toshite no gyorui-kiso jikenho to dokusei shaken. Soft Science Press, Tokyo, Japan. 228-241 pp (in Japanese).

Querijero, B. V. L., S. Teshima, S. Koshio and M. Ishikawa (1997) Utilization of monounsaturated fatty acid (18:1n-9, oleic acid) by freshwater prawn *Macrobrachium rosenbergii* (de Man) juveniles. Aquaculture Nutrition, 3, 127-139.

Teshima, S. I., A. Kanazawa and M. Yamashita (1986) Dietary value of several proteins and supplemental amino acids for larvae of the prawn, *Penaeus japonicus*. Aquaculture, 51, 225–235.

Yagi, K. (1987) Lipid peroxides and human diseases. Chem. Phys. Lipids, 45, 337-351.