博士論文要約（Summary）

平成 22 年入学
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タイトル	Nutritional studies on the interactive effects of selected amino acids and lipid-related additives on marine fish 海産魚類におけるアミナ酸および油脂関連添加物の交互作用に関する栄養学的研究

キーワード（**amino acids**）　（**oxidized lipid**）　（**additives**）　（**marine fish**）

「序論及び目的」

The world aquaculture industry has grown dramatically in the past 50 years and was growing more rapidly than all other animal food-production sectors. Continued growth and intensification of aquaculture production depends upon the development of sustainable protein sources to replace fishmeal in aqua-feed, thus, many efforts have been contributed to this field in the last decade. It is notable that, although the aquaculture sector remains the largest user of fishmeal in the world, fishmeal use in aqua-feed has gradually fallen since 2006. It also suggested that, one of the reasons for this reduction is the increased use of more cost-effective dietary fishmeal replacer, which indicated that more and more substitutes will be contributed into aqua-feed.

Due to the reasonable amino acid (AA) profiles especially the abundant of functional amino acid concentrations, the replacement of fishmeal in aqua-feed is still facing many challenges. During the past 50 years, plenty of AA requirements studies were reported which may help to support the fishmeal substitutions by satisfying AA requirements, however most of them were only focused on the AA requirement individually. Only recent years, interactive effects of some chemically, functionally or structurally related amino acids were reported in animals, such as sulfur amino acids, branched-chain amino acids, alkaline amino acids, as well as functionally related amino acids, arginine and glutamine, which may help to modify the proper supplementation level and reveal the inner connection of these amino acids.

Besides their role as building blocks of protein and polypeptides, some AA are important regulators of key metabolic pathways that are necessary for maintenance, growth, reproduction, and immunity in organisms, therefore maximizing efficiency of food utilization, enhancing protein accretion, reducing adiposity, and improving health they are so called functional amino acid.

To investigate the interactions between amino acids is considered to be very important for understanding the functions and metabolisms of amino acids. In fish, interactive effect between amino acids has been addressed during the past decade, such as lysine and methionine, taurine and methionine, tryptophan and lysine, branched-chain amino acids as well as arginine and glutamine,

in which all of them discussed the importance of essential amino acids balance in aquatic feed.

Fish oil is easily oxidized during storage and transportation due to large amount of n-3 highly unsaturated fatty acids. Lipid oxidation is a series of chain reaction with molecular oxygen reacting with unsaturated lipids to form lipid peroxides resulting in organoleptic changes of flavor, texture and aroma of food and undesirable influences on fish metabolism by consuming oxidized dietary oil. Besides endogenous antioxidant defense system, fish can use exogenous anti-oxidative compounds to protect cells and tissues from oxidative damage which scavenge free radicals.

1. To determine the interactive effects of Arg and His on Japanese flounder.
2. To determine the interactive effects of Met and Trp on Japanese flounder.
3. To determine the interactions between two branched-chain amino acids (Leu and Val) in Japanese flounder.
4. To investigate the interactions of Tau and Gln on Japanese flounder.
5. To estimate the effects of additives on oxidative damage in fish.

「材料及び方法」

Preparation of the test diets

The preparation of pre-coated crystalline amino acids (CAA) and experimental diets, CAA mixtures were weighted separately and pre-coated by carboxymethyl cellulose (CMC) that cooked at 50℃ with distilled water in order to prevent leaching losses. Double coating method was used here with gelatinized κ-carrageenan

The original Pollack liver oil that contains no antioxidants was used as the source of making oxidized fish oil. The oil was oxidized by heating in a water bath at 70 ℃ with continuous aeration for 24 h. POV was monitored as the indicator of oil oxidative degree thus POV of the oil was determined by using a method of the Association of Official Analytical Chemists until the POV was 69.2 meq/kg oil.

Feeding trials

All of the test fish were obtained from local hatchery (Matsumoto Suisan, Miyazaki, Japan), and transported to Kamoike Marine Production Laboratory, Faculty of Fisheries. The fish were acclimated to the laboratory conditions for two week and fed with commercial diet (Higashimaru Co. Ltd, Kagoshima, Japan) during this period. The feeding trials were conducted under natural temperature filtered sea water and with consistent salinities through the trials. Black polyethylene tanks with flow-through sea water (2.0 L/min/tank) where each tank was equipped with continuous aeration. The tanks were maintained under a 12/12 h, light/dark regime by fluorescent lamp. Fish in each tank were fed twice (08:30 and 16:30 h) a day by manually, apparent satiation. Tanks were cleaned with siphon daily and the uneaten diets were collected one hour after each feeding to determine feed intake daily basis.

Sample collection

At the end of the feeding trial, all experimental fish were fasted for 24 h. Individual body weight of fish were measured, the growth parameters were calculated accordingly. Three fish from each

tank were randomly collected, washed with distilled water and then stored at -80℃ for fish whole body proximate analysis. Skin mucus was collected from body surface of the three fish from each tank by using a small piece of sterilized cotton. Sample was put into a handmade set of two centrifugal tubes, the upper tube had a small filter, with which the mucus in the cotton will be collected in the lower tube while centrifuged. The sets of the double-tube were centrifuged at 3000×g for 5min under 4℃, the supernatant in the under tube was transferred into anther centrifugal tube and kept at -80℃. Heparinized syringes were used to collect blood from the caudal vein of five fish in each replicate tank and pooled. Partial of the heparinized whole blood was used to analyze the hematocrit and hemoglobin levels. On the other hand, plasma was obtained by centrifugation at 3000×g for 15min under 4℃, and then stored at -80℃. Liver was dissected out from the fish above, weight individually to calculate the hepatosomatic index, and then pooled by each replicate tank and stored at -80℃. The white muscle of fish was dissected, pooled, freeze dried and then stored at -80℃ for further analysis.

「結果」

Experiment 1 Interactive effects and optimum supplementation level of arginine and histidine in Japanese flounder *Paralichthys olivaceus*

The most encouraging result of growth performance (final body weight, body weight gain and special growth rate) was found in AH-HM group, and significantly higher than other groups ($P < 0.05$) except AH-HL ($P > 0.05$), meanwhile interactions of arginine and histidine were found on the growth parameters. In addition, positive results of the nutrient utilization (feed conversion ratio and protein efficiency ratio) were also observed in high Arg groups. The hematocrit, hemoglobin, glucose, total cholesterol, total bilirubin and mucus bactericidal activity of experimental fish were not significantly affected by the various treatments. Low dietary arginine together with low dietary histidine negatively affected the oxidative stess and lysozyme activity of Japanese flounder. Various dietary arginine and histidine levels significantly affected the concentrations of muscle free amino acids. According to the results, fish fed with 2.70 g arginine/100g diet and 1.56 g histidine/100g diet provided better growth performance and physiological status than other groups.

Experiment 2 Interactive effects and optimum supplementation level of methionine and tryptophan in Japanese flounder *Paralichthys olivaceus*

Statistically significant ($P < 0.05$) interactions of methionine and tryptophan were found on the final body weight, body weight gain, plasma glucose, glutamyl oxaloacetic transaminase, muscle free tryptophan and fresh water tolerance of test fish. Fish fed the diet with higher methionine supplementation showed significantly better performances of several parameters such as feed intake, feed conversion ratio, protein efficiency ratio, whole body protein content, hemoglobin, glutamyl-pyruvate transminase, LT_{50} and muscle methionine content. Furthermore, muscle free tryptophan and taurine levels increased significantly in higher methionine supplementation groups. The highest growth parameters and most sound blood parameters were found in fish fed with MH-TH (Methionine 1.8% of dry diet and Tryptophan 0.5% of dry diet).

Experiment 3 Interactive effects and optimum supplementation level of leucine and valine in

Japanese flounder *Paralichthys olivaceus*

Interactive effects of leucine and valine were found on growth parameters (final body weight, body weight gain and special growth rate) of Japanese flounder. Not only antagonism was observed in high dietary Leu level groups, but also the stimulative effect of increased dietary Val in low Leu level groups. Significantly interaction was also found on feed conversion ratio of Japanese flounder fed with test diets, however the fish whole body proximate compositions were not altered by the various diets. In trial 2, dietary Leu and Val dominant the plasma free Leu and free Val concentrations, but no antagonism was found. Interactions were also found on plasma LDH and GPT of test fish, and it was showed that for fish fed with high dietary Val level in low Leu (1.6% of diet) diet can protect the cells from oxidative stress.

Experiment 4 Interactive effects and optimum supplementation level of taurine and glutamine in Japanese flounder *Paralichthys olivaceus*

No interactive effect is shown in the selected parameters of Japanese flounder in this study. The growth parameters (final body weight, body weight gain and special growth rate) and FI were significantly affected by both Tau and Gln, meanwhile, plasma glucose, GOT, GPT and triglycerides of Japanese flounder were significantly decreased when fed with high level of dietary Tau. Other blood parameters were not significantly altered by various diets. The Japanese flounder muscle free Tau concentration directly reflected the dietary Tau level, which was not affected by Gln. Interestingly, fish fed with both high level of Tau and Gln showed a higher oxidant resistance, which was illustrated the important roles of Tau and Gln in fish oxidative stress protection.

Experiment 5 Effects of two additives on growth performances, hematology and air dive tolerance of red sea bream *Pagrus major* fed with oxidized fish oil

After the feeding trial, the growth performances, hematology, air dive tolerance fatty acid compositions of fish whole body were used for estimating the anti-oxidative effects of the two additives on red sea bream. Dietary oxidized lipid did not significantly affect the growth performances of red sea bream in 30 days. However, it significantly decreased the hemoglobin of red sea bream, and the hemoglobin level was increased when added either rosemary acid or lactoferrin in the diets. Fish whole body fatty acid compositions were not altered by the various treatments.

「結論及び考察」

1. Interactive effects of Arg and His existed on growth parameters of Japanese flounder. Increased His levels promoted the growth performance when dietary Arg is low.
2. Low dietary Arg (1.7%) together with low dietary His (1.0%) increased the plasma GOT and GPT, but decreased lysozyme activity, the phenomenons were mitigated by either increase dietary Arg or His levels.
3. The optimal ration of Arg and His in Japanese flounder low fishmeal diet are 2.70 g/100g diet and 1.56 g/100g diet, separately.
4. Interactions of methionine and tryptophan were found on growth performance, plasma glucose, GOT, muscle free tryptophan and fresh water tolerance of test fish.

5. Fish fed the diet with higher methionine supplementation showed significantly better performance, blood parameters and LT_{50}
6. The highest growth parameters and most sound blood parameters were found in fish fed with MH-TH (Methionine 1.8% of dry diet and Tryptophan 0.5% of dry diet).
7. Interactive effects of Leu and Val were found on growth parameters and FCR of Japanese flounder. Not only antagonism was observed in high dietary Leu groups, but also the stimulative effect of increased dietary Val in low Leu diets.
8. No antagonism was found on plasma free Leu or Val concentrations.
9. Interactions were also found on plasma LDH and GPT of test fish, and it was showed that for fish fed with high dietary Val level in low Leu diet can protect the cells from oxidative stress.
10. No interactive effect is shown on Japanese flounder in this study. But both Tau and Gln significantly affected the growth parameters and FI.
11. Plasma glucose, GOT, GPT and triglycerides of experimental fish were significantly decreased when fed with high level of dietary Tau indicated that Tau can protect the liver function of Japanese flounder.
12. Japanese flounder fed with both high level of Tau and Gln showed a higher oxidant resistance, which was illustrated the important roles of Tau and Gln in fish oxidative stress protection.
13. Dietary oxidized lipid did not significantly affect the growth performances of red sea bream in 30 days. However, it significantly decreased the hemoglobin of red sea bream, and the hemoglobin level was increased when added rosemary acid or lactoferrin in the diets.