Studies on the effect of supplemental nucleotides to low fish meal and fish oil diet on the fatty acid of rainbow trout Oncorhynchus mykiss(低魚粉・魚油飼料への核酸添加がニジマスの脂肪酸組成に及ぼす影響に関する研究)

［課程博士・論文博士共通］

博士学位論文内容要旨
Abstract

専　攻 Major	APPLIED MARINE BIOSCIENCES	氏　名 Name	ASEP RIDWANUDIN
論文題目 Title	Studies on the effect of supplemental nucleotides to low fish meal and fish oil diet on the fatty acid of rainbow trout *Oncorhynchus mykiss* 低魚粉・魚油飼料への核酸添加がニジマスの脂肪酸組成に及ぼす影響に関する研究		

Replacement of fish meal and fish oil by plant protein and vegetable oil sources are main priorities to reduce utilization of those ingredients in aquafeed industry. To develop low fish meal and fish oil feed, numerous studies have reported that fish meal and fish oil could be totally or partially replaced by plant protein and vegetable oil sources without negative affect on growth of rainbow trout. However, changing fatty acid composition were commonly observed when inclusion of plant protein or vegetable oil sources in fish diet, particularly by decreasing the content of n-3 long-chain polyunsaturated fatty acid (LC-PUFA) composition such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic (DHA, 22:6n-3) in the tissue of fish. Similarly, although no significant in statistically, growth of fish fed with plant protein-based diet were lower than fish fed with fish meal-based diet. Therefore, in order to improve growth and also to increase fatty acid composition when fish fed with plant protein and/ vegetable oil-based diets, supplementation of nutritive compounds are required. Among available nutritive compounds, nucleotides are commonly used in aquaculture to improve growth of fish by increasing feed intake, and also modulate fatty acid synthesis in fish. The growth promotion of fish by dietary nucleotides have been recorded in various fish species, mainly when it's supplemented in high fish meal-based diet. However, the information regarding the effect of dietary nucleotides in low fish meal-based diet to promote growth of rainbow trout are still scarce. In addition, although dietary nucleotides altered fatty acid composition in the tissue of fish, modulation effect of dietary nucleotides on fatty acid synthesis are remain unclear. Therefore, the general objective of this study was to investigate the effect of nucleotides supplementation to low fish meal and fish oil-based diet on growth and fatty acid composition of rainbow trout.

In the first experiment, six isonitrogenous (42% crude protein) and isolipidic (18% crude lipid) diets were formulated. The control diet was a basal diet without supplementation of nucleotides, and five experimental diets were prepared by supplementing one of the five different nucleotides in the form of 5'-monophosphate (0.15%) i.e inosine (IMP), adenosine (AMP), guanosine (GMP), uridine (UMP) and cytidine (CMP) onto basal diet. Two hundred forty juvenile rainbow trout with an initial average body weight 9.8 g were randomly distributed into twelve aquaria. After 15 weeks of feeding period, growth performance and feed utilization of rainbow trout were not significantly different among dietary treatments. Hepatosomatic indices (HSI) of fish fed AMP, GMP and CMP diets were lower than the control diet. Plasma alanine aminotransferase activity decreased in fish fed IMP diet, whereas plasma aspartate aminotransferase and alkaline phosphatase activities, cholesterol, triglyceride, HDL-C and LDL-C remained unaffected by dietary nucleotides. Dietary GMP, UMP and CMP significantly increased hepatic 18:3n-3 and long chain homologue 18:4n-3 and 20:4n-3 contents.

Hepatic 18:2n-6 content showed also increase in fish fed GMP, UMP and CMP diets, but decreased in long chain homologue 20:3n-6 and 20:4n-6 contents. Decrease in 20:4n-6, 20:5n-3 and 22:6n-3 contents were also found in the muscle of fish fed IMP, GMP and CMP diets. These results clearly showed that there was no positive effect of dietary nucleotides on growth of fish, but dietary nucleotides particularly GMP, UMP and CMP altered polyunsaturated fatty acids composition of rainbow trout.

The second experiment investigated the effect of pyrimidine nucleotides supplementation on growth and fatty acid composition of juvenile rainbow trout. Six isonitrogenous (44% crude protein) and isolipidic (18% crude lipid) diets were formulated with two different levels of fish oil. High fish oil (HFO) group contain 12% fish oil, while low fish oil (LFO) group contains 4% fish oil, 6% linseed oil and 2% rapeseed oil. Uridine 5'-monophosphate disodium salt (UMP) or cytidine 5'-monophosphate disodium salt (CMP) were supplemented at 0.15%, respectively. For control, CMP or UMP were not supplemented to HFO and LFO diets, respectively (HFO-Cont. and LFO-Cont). Rainbow trout with an initial average body weight of 13.5 g were randomly placed into twelve tanks and offered one of the six diets for 12 weeks. At the end of trial, growth and feed utilization of rainbow trout were not significantly influenced either by fish oil or pyrimidine nucleotides supplementation. However, viscerosomatic index (VSI) in fish fed UMP diets were higher compared to control diet either in HFO or LFO group. In addition, fatty acid composition in the liver were significantly influenced by fish oil, but not by pyrimidine nucleotides supplementation. Moreover, fish oil also affected on the fatty acid composition in the muscle of fish. Interestingly, pyrimidine nucleotides supplementation affected on some fatty acid compositions including 20:4n-6 (arachidonic acid, ARA) and 22:6n-3 (docosahexaenoic acid, DHA) in the muscle. The content of 20:4n-6 in the fish fed UMP diets were higher than control diets either in HFO or LFO diet group. Moreover, fatty acid content of 22:6n-3 in HFO diets group tended to decrease, while increasing trend was observed in LFO diets group. These results showed that dietary pyrimidine nucleotides has also no positive effect on growth of fish. However, supplementation of pyrimidine nucleotides including UMP and CMP has a potential to increase the important fatty acids contents such as 20:4n-6 and 22:6n-3 in the muscle of rainbow trout particularly when it's supplemented in low fish oil-based diet.

The third experiment investigated the effect of purine and mixture nucleotides to low fish meal and fish oil based diet on growth and long-chain polyunsaturated fatty acid composition of juvenile rainbow trout. Inosine 5'-monophosphate (IMP), adenosine 5'-monophosphate (AMP), guanosine 5'-monophosphate (GMP) and mixed-nucleotides (MIX) that consists mixture of IMP, AMP, GMP, uridine 5'-monophosphate (UMP) and cytidine 5'-monophosphate (CMP) (ratio 1:1:1:1:1) disodium salt forms were supplemented at a level 0.15% into basal diet containing 44% crude protein (isonitrogenous) and 19% crude lipid (isolipidic), respectively. Control diet was also used by devoid of nucleotides. Rainbow trout with initial average body weight of 8.4 g were randomly distributed into ten aquaria at a stocking density 20 fish per aquarium. The results of twelve weeks feeding trial showed that there were no significant effect of dietary purine and mixed-nucleotides on growth and feed utilization of rainbow trout. Similarly, fatty acid composition in the muscle were not affected by dietary purine and mixed-nucleotides. However, the contents of 22:6n-3 (DHA) and total n-3 PUFA in the liver decreased by dietary GMP and mixed-nucleotides. Dietary GMP and mixed-nucleotides also down-regulated the expression of mRNA Δ5 desaturase, Elovl2, SREBP-1 and LXR genes in the liver of rainbow trout. These results showed that although dietary purine and mixed-nucleotides has no positive affect

on growth of rainbow trout, dietary purine mainly GMP and mixed-nucleotides altered fatty acid composition in the liver by decreasing the content of 22:6n-3 (DHA), and also by down-regulation the expression of fatty acid synthesis regulator genes.

These results suggest that supplementation of 0.15% nucleotides either singularly or in combination to low fish meal and fish oil based-diet did not affect growth and feed utilization of rainbow trout. However, dietary nucleotides decreased 22:6n-3 (DHA) and total n-3 PUFA contents in the liver of fish as the effect of the modulation of dietary GMP and mixed-nucleotides on the regulator of fatty acid synthesis (SREPB-1 and LXR) to down-regulated the expression of mRNA Δ5 desaturase and Elovl2 elongase genes. On the other hand, dietary pyrimidine nucleotides including UMP and CMP improve the quality of fatty acid composition by increasing 22:6n-3 (DHA) content in the muscle of fish, mainly when fish fed with low intake of fish oil.