Studies on the effect of supplemental nucleotides to low fish meal and fish oil diet on the fatty acid of rainbow trout Oncorhynchus mykiss

［課程博士・論文博士共通］

Doctoral Dissertation Summary

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		

Dissertation research topics:

I. Effect of nucleotides supplementation to low-fish meal feed on long-chain polyunsaturated fatty acid composition of juvenile rainbow trout *Oncorhynchus mykiss*.

   (This part was published in Aquaculture Research (2019) 50(8), 2218-2230 with doi: 10.1111/are.14103; Publisher: John Wiley & Sons Ltd.)

II. Effect of pyrimidine nucleotides supplementation on growth and long-chain polyunsaturated fatty acid composition of juvenile rainbow trout *Oncorhynchus mykiss*.

III. Effect of purine and mixed-nucleotides supplementation on growth and long-chain polyunsaturated fatty acid composition of juvenile rainbow trout *Oncorhynchus mykiss*.

Purpose:

The comprehensive studies on the effects of nucleotides supplementation to low fish meal and fish oil-based diet on growth and fatty acid composition of juvenile rainbow trout *Oncorhynchus mykiss* were presented in this dissertation through;

I. Investigate the effect of nucleotides supplementation including IMP, AMP, GMP, UMP and CMP to low-fish meal based diet on growth and fatty acid composition of rainbow trout *Oncorhynchus mykiss*.

II. Examine the effect of pyrimidine nucleotides supplementation including UMP and CMP in different levels of fish oil on growth and fatty acid composition of rainbow trout *Oncorhynchus mykiss*.

III. Investigate the effect of purine (IMP, AMP and GMP) and mixed-nucleotides (IMP:AMP:GMP:UMP:CMP ratio 1:1:1:1:1) to low fish meal and fish oil-based diet on growth and fatty acid composition of rainbow trout *Oncorhynchus mykiss*.

Methodology/Experiments/Analysis:

I. 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. At the end of experiment period, five fish from each tank were randomly selected to calculate HSI and VSI. Muscle without skin from these fish were also

removed from the backbone for proximate analysis. In order to analyze proximate composition of whole fish body, another three fish from each tank were also randomly sampled. Plasma sample of five fish from each tank were also taken for analyses of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), cholesterol, triglyceride, high density lipoprotein-cholesterol (HDL-C) and low density lipoprotein-cholesterol (LDL-C).

II. Six low fish meal diets were formulated to be iso-nitrogenous (44% crude protein) and iso-lipidic (18% crude lipid) as the experimental diets. Different inclusion level of fish oil in the experimental diets were designed as high and low fish oil diet groups. High fish meal (HFO) diet contain 12% of fish oil as the sole of lipid source, whereas low fish oil (LFO) diet consists of different level of oil sources including 4% fish oil, 6% linseed oil and 2% rapeseed oil. Two purified pyrimidine nucleotides including uridine 5'-monophosphate (UMP) and cytidine 5'-monophosphate (CMP) disodium salt forms from Tokyo Chemical Industry, Tokyo, Japan were then supplemented at 0.15% onto each group, respectively. The remaining two diets were served as control diets (HFO-Cont. and LFO-Cont.) without supplementation of pyrimidine nucleotides. At the beginning of feeding trial, 12 groups of rainbow trout with an initial average body weight 13.5±0.2 g were randomly distributed into 60-L experimental tanks at a stocking density of 20 fish in each tank. For determination of HIS and VSI, liver and viscera of five fish from each tank were removed and weighed, respectively. Liver samples were then pooled and frozen for later crude lipid and fatty acid analysis. Following this, muscle samples from these fish were carefully dissected from the backbone for subsequent proximate analysis. An additional three fish were also used for determination of proximate composition of whole fish body.

III. Five low fish meal and fish oil-based diets were formulated to be iso-nitrogenous (44% crude protein) and iso-lipidic (18% crude lipid) by supplementation of 0.15% purified purine and mixed-nucleotides in the form of 5'-monophosphate disodium salt. Purine nucleotides including inosine (IMP), adenosine (AMP) and guanosine (GMP), while mixed-nucleotides (MIX) consist of combination of inosine (IMP), adenosine (AMP), guanosine (GMP), uridine (UMP) and cytidine (CMP) at ratio 1:1:1:1:1:1. Control diet was a basal diet without supplementation of nucleotides. At the start of feeding trial, two hundred homogenous size of rainbow trout with an initial average body weight 8.4±0.2 g were randomly distributed into ten experimental tanks at a stocking density of 20 fish in each tank. Five fish from each tank were removed and weighed for calculation of HSI and VSI, respectively. Then, liver samples were pooled for later crude lipid and fatty acid analysis. At the same time, muscle samples from these fish were carefully dissected from the backbone for subsequent proximate analysis. Three other fish from each tank were also used for determination of proximate composition. In addition, another three fish from each tank was also collected to remove liver for gene expression analysis.

Results:

I. After 15 weeks of feeding period, growth performance and feed utilization of rainbow trout were not significantly different among dietary treatments. 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.

II. After 12 weeks of feeding trial, growth and feed utilization of rainbow trout were not influenced either by fish oil or pyrimidine nucleotides supplementation. However, 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 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 diets. Moreover, fatty acid content of 22:6n-3 in HFO diets tended to decrease, while increasing trend was observed in LFO diets.

III. 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. There were also no differences among dietary treatments in HIS and VSI of fish. Similarly, there were no differences in crude lipid content in the liver and also on crude lipid, crude protein, ash and dry matter contents in the muscle and whole fish body among dietary treatments. 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 *elovl2*, *SREBP-1* and *LXR* genes in the liver of rainbow trout.

Conclusion:

I. The results 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.

II. The findings 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.

III. The 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 of rainbow trout by decreasing the content of 22:6n-3 (DHA), and also by down-regulation the expression of fatty acid synthesis regulator genes.