学位論文の要約

With growing use of plant protein and lipid sources in fish feed, growth retardation due to limited feed consumption and helath problem have been reported. Along with strict control of use of antibiotics and drug for food animals, increasing attention ha sbeen paid for research on fuctional nutrients in fish feed ingrediets. One of the ingredients is amino acid with growth promotion and immunostimulant fuction. With its versatile nature and important function in the immune system, supplemental level of arginine should be explored as a solution for increasing the health status of cultured fish to mitigate the adverse effect of climate change in aquaculture industries. Although most of the beneficial and adverse effects of arginine supplementation in the human and terrestrial animal have been evaluated before, the responses of arginine supplementation on fishes are not fully comprehended due to limited research conducted on it. In order to figure out fate of dietary arginine in fish body, plasma dynamic of arginine metablites has to be determined as well as growth and biological performance of fish fed arginine supplemented diets. Here Fauzi IA conducted a series of experiments to investigae arginine catabolism in raibow trout fed diets supplemented with different levels of arginine during postprandial. He also examined cytokine gene expression.

The first research is aimed to evaluate the effect of supplemental dietary arginine levels on growth performance, plasma amino acids, and genes expression of enzymes that catabolize arginine in rainbow trout. For feeding trial, basal diet was formulated with 50.3% protein and 17.8% lipid. to fulfill all essential amino acid requirements, crystalline amino acid was also used in this research. To simulate grade arginine level, 2% and 4% of l-arginine was used. Upon amino acid analysis, the diets were confirmed to contain 1.47 (CTRL), 3.89 (3.89A) and 5.64 % of arginine (5.64A). Feeding trial was conducted for 9 weeks using juvenile rainbow trout with average weight of 62.5 g. After 9 weeks, fish were then fasted for 3 days and subjected for postprandial plasma amino acid study. Positive correlation between dietary arginine level with plasma arginine and ornithine were found while no increase of plasma arginine level found in all postprandial time of CTRL. Highest citrulline level was found in CTRL while high supplementation of arginine decreases plasma citrulline production. There is no difference was found in plasma glutamic acid among treatments. Increase of plasma urea was only observed in arginine supplemented groups and there is no plasma urea difference found between those treatments. There was also no significant difference found in growth performance and total amino acid content in muscle of all treatments. However, higher protein

content was found in the muscle of 3.89A compared to CTRL. No indication of arginine-lysine antagonism found in this study based on plasma lysine dynamics and growth performance among treatments.

Since the first experiment suggests that rainbow trout fed diet supplemented with arginine shows higher plasma ornithine (a precursor of polyamine), and lower plasma citrulline after 18 hours postprandial. However, supplemental arginine is also reported to increase arginine degradation through urea cycle and consequently reduce arginine availability. Thus, to avoid excessive arginine degradation and to better understand the role of citrulline, two more researches were conducted to evaluate the effect of dietary supplementation of ornithine and citrulline on resistance of rainbow trout, *Oncorhynchus mykiss* against *Vibrio anguillarum*, while in the same time observe postprandial amino acid dynamics and growth performance of rainbow trout fed by those amino acids. For the second experiment, 20 juvenile rainbow trout (average size 34.1 g) were reared in 60 L aquaria with a recirculating system at 15^{o} C. Dietary treatment was consisted of control diet (CTRL) with 48 % protein and 16 % lipid level, while treatment diets were made by supplementing control diet with 1% l-ornithine (ORN), 1% l-citrulline (CIT), and combination of 1% of l-ornithine and 1% of l-citrulline (ORN-CIT). Fish were fed twice daily for six day a week until apparent satiation. To evaluate the effect of short and long feeding period on immune system, feeding trial was conducted twice: 15 days and 30 days. However, growth performance was only evaluated in the fish that was fed for 30 days. After 15 days and 30 days feeding, fish were injected intraperitoneally with *Vibrio anguillarum* that was diluted with phosphate-buffered saline at 3.0 x 10^{6} CFU per fish. At 1 day post injection, blood was collected for plasma amino acid analysis and RNA was also extracted from kidney for quantitative-real-time PCR analysis of inducible nitric oxide (iNOS), interleukin-1-betta (Il-1β), and arginase. There is no significant difference in growth performance and feed efficiency upon 30 days of feeding. Furthermore, pre-feeding with these supplemental amino acids did not affect survival upon challenge with *Vibrio anguillarum* in both feeding regime. However significant differences were found in the expression of iNOS and Il-1β in kidney in the case of 15 days feeding regime, and in iNOS, Il-1β, and arginase in kidney in the case of 30 days feeding regime. Moreover, it was also shown in the postprandial plasma amino acid analysis that CIT treatment produce higher plasma arginine compared other treatments.

Since the second experiment found that supplementation with citrulline can increase plasma arginine level, the third experiment was aimed at comparing dietary supplementation with citrulline, ornithine, and arginine. Juvenile rainbow trout with average size of 9.1 gram

was reared with the same condition with second experiment. Dietary treatment was consisted of control diet (CTRL) which was formulated to have 47 % protein and 15 % lipid level, while treatment diets were made by supplementing control diet with 2% l-arginine (ARG), l-ornithine (ORN), and l-citrulline (CIT) at the expense of cellulose. Feeding was conducted in similar ways with the previous experiment for 30 days period. After 30 days, fish were also injected intraperitoneally with *Vibrio anguillarum* that was diluted with phosphate-buffered saline at 3.0 x 10^6 CFU per fish. After 24 hours post injection, blood was collected and RNA was extracted from kidney for plasma amino acid and quantitative-real-time PCR analysis respectively. A better growth performance was found in ARG compared to CIT, while there is no significant difference found between CTRL with other treatments. Survival analysis after disease challenge showed an improve resistant in CIT treatment compared to CTRL and the gene analysis and significantly higher expression level of iNOS was observed in CIT then CTRL. Moreover, postprandial plasma analysis showed a similar level of plasma arginine between ARG and CIT treatment and both treatments were significantly higher than CTRL. Thus, based on the results of this study, it can be concluded that citrulline supplementation improves rainbow trout's resistance against *Vibrio anguillarum*.

As a general conclusion, dietary supplementation with 2% citrulline for at least 30 days can improve resistance against *Vibrio anguillarum* of rainbow trout. This is the first-time l-ornithine and l-citrulline were used as dietary supplement for rainbow trout and it is the first time citrulline supplementation was shown to increase plasma arginine level in rainbow trout. The present results suggest that arginine supplementaion is beneficial to improve immune status and trelance to *Vibrio anguillarum* infection of rainbow trout and high potential of citrulline as an arginine source in fish feed.