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Quality Control of Cultured Fish by Feed Supplements

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Abstract：The importance of algae, chitin and laurate that are widely ingested by a variety of wild fish was confirmed as feed supplement in cultured fish. The substance was supplemented to the diets of ayu, red seabream and black seabream at the same level found in the stomach content of wild fish. The dietary supplements commonly improved physiological condition such as acceleration of lipolysis activity and protein assimilation, depression of adipocytes, resistance to air-dipping, and activation of liver function, without depression of growth.

Key words : dietary supplements, fish quality, lipid metabolism, protein metabolism, vitality

Advancements in fish nutrition have progressed considerably with regard to growth performance. However, much concern has arisen over the quality of fish flesh and health in aquaculture. In response, a variety of feed supplements have been investigated with respect to their effects on physiological condition and the quality of cultured fish.

Materials and Methods

Fish and rearing condition

Prior to the experiment, organisms in the stomach contents of wild fish were analyzed. The supplemented levels of ingredients in the experimental diets were decided based on the stomach contents in wild fish. Fish used for the feeding experiment were 0-year ayu (*Plecoglossus altivelis*), black seabream (*Acanthopagrus schlegelii*) and red seabream (*Pagrus major*). Groups of experimental fish were fed either a control diet (not supplemented) or the control diet supplemented with algae (<5% in dry diet), chitin (10% in wet diet) or lauric acid (0.5% of ethyl laurate in dry diet).

Biological measurements

All the fish in each replicate treatment group were weighed individually. The whole muscle, liver and adipocyte were obtained from 20 fish of each group. Adipocyte diameter was histologically measured in 50 cells per fish.

Vitality

For comparing liver function, fish were dipped in water containing anesthetic at a concentration of 0.1% for 50 sec. Then the fish were transferred to oxygen-saturated fresh seawater and recovery time was recorded. For air-dipping resistance, fish were exposed to air-dipping for 5 min and returned to oxygen-saturated seawater. Recovery time from was recorded.

Biochemical measurements

The feed, muscle, liver and adipocytes in the intraperitoneal cavity were frozen immediately after sacrifice of the fish and kept in a freezer. Lipid was extracted with methanol-chloroform. The lipid class composition and fatty acid composition were analyzed by an Iatroscan and a gas chromatograph.

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Results and Discussion

Micro- and macro-algae

A variety of wild fishes ingest algae, including some primarily carnivorous species. The primary foods of wild ayu are benthic blue-green algae and diatoms in rivers. *Chlorella*-extract as a feed supplement affects physiological characteristics such as lipid metabolism, disease resistance, carcass quality and vitality in ayu and yellowtail (*Seriola quinqueradiata*). In the giant prawn (*Macrobrachium rosenbergii*), dietary *Spirulina* elevated growth performance and feed utilization over ranges of supplementation of 5–20% of the diet.

Although lipid content in the intraperitoneal fat body of cultured ayu was almost 98%, *Chlorella*-extract supplementation reduced the value to 66.7%. *In vitro* lipolysis activity was superior in the *Chlorella*-extract (1%) fed group, suggesting activation of lipid utilization to energy prior to muscle protein consumption. *Chlorella*-extract suppressed body weight loss caused by starvation in ayu, because the reserved lipid decreased remarkably and consumption of muscle protein might have been suppressed.

The *Chlorella*-extract distinctly improved tolerance of hypoxic conditions and liver function. The preventive action exerted by *Chlorella*-extract might involve some internal barrier to infectious disease, such as an inflammatory response and an increase in the number of phagocytes, rather than an immediate effect on the disease as an antibiotic substance.

Spirulina supplementation correlated with marked increases in some key substances for β-oxidation of fatty acids, including hepatic free carnitine and long-chain acylcarnitine, and carnitine palmitoyltransferase activity. Red seabream fed a diet supplemented with *Spirulina* at a 2% level exhibited elevated protein synthesis, and the stroma (connective tissue) fraction was significantly increased. The muscle protein composition measured by solubility approached that of wild fish. *Spirulina* increased total muscle collagen; the collagen fractions soluble at 20℃ and 70℃ decreased, but the insoluble collagen increased.

The addition of a very small amount of algal meal has produced a significant increase in the growth and feed utilization of a variety of fish such as, red seabream, Japanese flounder (*Paralichthys olivaceus*), yellowtail (*Seriola quinqueradiata*), ayu, rockfish (*Sebastes schlegeli*), nibbler (*Girella punctata*), and snakehead (*Channa striatus*). The optimum feed efficiency and protein efficiency were attained in black seabream when the supplementation level of *Ulva* meal was 2.5–5.0% of the diet. Body weight loss of black seabream during wintering was minimized with supplementation of *Ulva* meal. Growth of Japanese flounder was maximized with *Ulva* at 2% of the diet. Algae also affect other growth indicators when used as dietary supplements.

Supplementation with *Ascophyllum, Porphyra,* and *Ulva* at 3–5% in prepared diets elevated muscle RNA/DNA ratio (protein synthetic activity) and suppressed acid protease activity (protein catabolism) in red seabream, providing biochemical proof of growth. The effect on growth was due to an acceleration of nutrient absorption by dietary algae.

The addition of a small amount of algal meal to the fish diet can exert considerable influences on carcass quality. While micro-algae generally decrease accumulation of lipid in the muscle, macro-algae often induce an increase in muscle lipid. While muscle lipid of the control group of young yellowtail was 1.6%, supplementation of *Laminaria digitata* at 1% increased muscle lipid to 4.8%. Similar tendencies for increased muscle lipid occurred with supplementation of *Undaria pinnatifida* and *Ascophyllum nodosum* in the diet of red seabream and with *Ulva* in black seabream. Sensory evaluation of fish meat showed that supplementation of macro-algae in the diet generally improved taste and quality as a whole. Under imbalanced nutritional conditions and inadequate feeding regimes (overfeeding, inadequate feeding frequency, etc.), muscle protein may be consumed in place of lipid reserves. Fish reared under such conditions cannot endure food shortages.

Supplementation of *Ulva* meal to a red seabream diet or *Laminaria* meal to a yellowtail diet increased consumption of lipid reserves and suppressed muscle protein consumption. Wild red seabream and black seabream ingest algae in nature, and the proportion of algae ingested increases before wintering. The

ingestion of algae before wintering could help to activate lipid reserves for energy during the winter period.

Stressors during fish rearing such as high rearing density, low dissolved oxygen, water pollution, and nutritional imbalances can depress disease resistance. Resistance to low-oxygen levels was higher in algae-fed red seabream, black seabream, and rockfish. Many algal species are likely to improve vitality in a similar way, because they contain a wide array of macronutrients, micronutrients, and other components.

Supplementation of *Ulva* meal to a prepared diet at 5% elevated phagocytosis in black seabream. Effects of similar supplementation with *Ulva* were found on numbers of lymphocytes and granulocytes, agglutinin titer, and hemolytic and bactericidal activities in red seabream. In addition, bactericidal activity after immunization was enhanced. A nutritional disease involving retardation of growth and high mortality in young yellowtail is caused by continuously feeding on sardine (*Sardinops melanocticta*). The disease was reduced by supplementation of 0.5% of *Laminaria digitata*. Simultaneous addition of a vitamin mixture enhanced the effect, suggesting a synergistic effect of algae and certain vitamins. Atlantic salmon (*Salmo salar*) fed on a diet supplemented with alginate had high survival and complete hemolytic activity after challenging by *Aeromonas salmonicida*.

Chitin

Crustaceans are important and often the major food organisms of larval fish and contain considerable amount of chitin in the digesta (2.3–9.3% in dry weight). The growth, feed efficiency and survival rate were improved by adding dietary chitin (10% in a purified moist diet). The relative intestine length is an indicator of the diversified use of food and nutrients. The intestine in the chitin group was significantly longer than in the control group. The long intestine presumably extended evacuation time of food in the gut by increasing surface area. Consequently, growth performance would be improved. The dietary chitin decreased the lipid content and size of lipid droplets in the liver. Low lipid reserves was mainly due to the reduction of the adipocytes accompanied by a reduction in the adipocyte diameter. Reduction of the liver oil globules and the adipocyte size might suggest suppression of lipogenesis and activation of lipolysis. Suppression of body weight loss by starvation in the chitin group could be due to sparing the consumption of muscle protein.

Dietary chitin significantly reduced the impact of air-dipping and improved the recovery time from the succumbed condition. During the treatment, 50% of the fish died in the control group, while the chitin group recovered completely.

Lauric acid

A small amount of lauric acid was found in the stomach content of wild black seabream. Although dietary laurate did not affect growth, survival, or feed efficiency, muscle ratio was significantly increased when it was present. In addition, hepatosomatic index, adipocyte ratio, and adipocyte diameter were significantly reduced by laurate supplementation. Hepatic lipid was significantly lower; almost half that of the control group. Dietary laurate markedly depressed triglycerides, although free fatty acids and phospholipids were hardly influenced.

Dietary laurate significantly reduced the impact of air-dipping and quickened the recovery time. In the liver function test, the survival from the anesthetic condition was only 40% in the control group, but fish of the laurate group recovered completely.

Feeding laurate led to a proportional decrease of monoenes and slight increase in n-3 fatty acids in the hepatopancreas. While lauric acid was not found in the organs of the control group, lauric acid and DHA slightly increased in the heart, brain, and eye in the laurate group. Dietary laurate might account for the absorption and/or incorporation of DHA into the fish body.

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