Effects of various concentrations of garlic powder and garlic extract in the diets on growth, serum chemistry and immune response of juvenile olive flounder Paralichthys olivaceus

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Effects of various concentrations of garlic powder and garlic extract in the diets on growth, serum chemistry and immune response of olive flounder were determined. Thirty-five juvenile fish averaging 5.1 g were randomly distributed into 21 of 180 L flow-through tanks. Seven experimental diets with various concentrations of garlic powder (GP) and garlic extract (GE) were prepared in triplicate: GP-0 without garlic supplementation, GP-0.5, GP-1, GP-2, GP-3 and GP-5 diets containing garlic powder at the concentrations of 0.5, 1, 2, 3 and 5%, respectively at the expense of wheat flour and finally, GE-0.4 diet containing 0.4% garlic extract were prepared. At the end of the 8-week feeding trial, serum chemistry of fish was measured. In addition, twenty fish from each tank were artificially infected with *E. tarda* for the following 96 h to monitor cumulative mortality. Weight gain of fish fed GP-0 diet was higher than that of fish fed GP-1, GP-2, GP-3 and GP-5 diets. No difference in serum criteria (total protein, glucose, glutamate oxaloacetate transaminase, cholesterol and triglyceride levels) of olive flounder was found among the experimental diets except for glutamate pyruvate transaminase. Lysozyme activity of fish fed GP-0, GP-1, GP-3 and GE-0.4 diets was higher than that of fish fed GP-5 diet. The highest cumulative mortality was 93.3% in fish fed GP-0 diet at 96 h after *E. tarda* infection, followed by GP-3, GP-1, GP-5, GP-2, GP-0.5 and GE-0.4 diets. In considering these results, dietary inclusion of garlic powder and garlic extract has no distinctive positive effect on improvement in growth, serum chemistry and immune response of olive flounder in this experimental conditions, therefore, its application should be carefully considered.

Key words : Olive flounder Paralichthys olivaceus, Garlic powder, Garlic extract, Lysozyme activity, *E. tarda*

Since olive flounder *Paralichthys olivaceus* has been one of the most important marine finfish for aquaculture in Korea, many feeding trials to determine dietary nutrients requirement (Lee et al., 2000; Alam et al., 2002; Lee et al., 2002), optimum feeding ratio/frequency (Lee et al., 1999; Cho et al., 2006b), alternative protein sources for fishmeal in the diets (Sato and Kikuchi, 1997; Kikuchi, 1999) for fish have been reported.

In addition, dietary additives, such as herbal medicine (Kim et al., 1998, 2000), culture broth of lactic acid bacteria in herb (*Acanthopanax koreanum*) extract (Jhon et al., 2009), green tea (Cho et al., 2006a, 2007), chitosan (Cha et al., 2008), extract of mushroom mycelium
(Phellinus linteus and Coriolus militaris) (Kim et al., 2006a), microalgae, Chlorella ellipsoidea (Kim et al., 2002) and macroalgae, Hizikiafusiformis (Pham et al., 2006), wood vinegar (Lee et al., 2008) and glucan (Kim et al., 2006b) were effective to improve performance and/or immune response of olive flounder. However, mortality of fish resulting from outbreak of disease frequently occurs during year-round and it eventually lowers fish production. Therefore, development of new additive for aquafeed is still highly needed.

The use of natural resources as dietary additives has several advantages, such as food safety for human consumption and minimizing the risk of side-effects. Garlic (Allium sativum) containing allicin, allyl cysteine, ajoene, allin and related components (Afzal et al., 2000; Chung, 2006) has been known to have antibacterial, antimicrobial, anti-inflammatory, antioxidant and/or antitumourigenic effects (Afzal et al., 2000; Shin and Kim, 2004; Chung, 2006; Wilson and Demmig-Adams, 2007). It was also known to lower cholesterol of men (Steiner et al., 1996) and hyperlipidemia in rats (Kang et al., 2008). Therefore, garlic seems to have high potential for aquafeed as an immunostimulant as well.

Dietary inclusion of fermented garlic powder was effective against Vibrio anguillarum and Streptococcus iniae infection, but not for Edwardsiella tarda (Kim et al., 2010). In above study, however, fish were grown and challenge test were performed in low temperature (16-18°C). Since those disease occurs frequently in olive flounder at high temperature (over 20°C), more studies in high temperature are needed. In addition, Lee et al. (2010) reported that injection of garlic extract effectively enhanced nonspecific immunity and resistance against E. tarda and S. iniae when fish were grown at 20-22°C.

In this study, therefore, effects of various concentrations of garlic powder and garlic extract in the diets on growth, serum chemistry and immune response of juvenile olive flounder were determined.

Materials and Methods

Experimental conditions

Juvenile olive flounder were purchased from a private hatchery (Taean, Chungcheongnam Do, Korea), transferred to the laboratory and acclimated for 2 weeks before an initiation of the feeding trial. During the acclimation period, fish were fed with commercial extruded pellet containing 54% crude protein and 11% crude lipid twice a day. Thirty-five juvenile fish averaging 5.1 g were randomly distributed into 21 of 180 L flow-through tanks (water volume: 150 L) and water flow rate of tank was 6.8 L/min. The water source was the sand-filtered natural seawater and aeration supplied to each tank. Water temperature ranged from 18.4 to 24.1°C (mean±SD: 21.5±1.16°C) and photoperiod followed natural condition.

Preparation of garlic powder and extract for the experimental diets Garlic powder (crude protein: 19.0%, crude lipid: 0.8% and ash: 3.1%) and garlic extract (Edentownfnb, Incheon, Korea) were used as the additives. Seven experimental diets with various concentrations of garlic powder (GP) and garlic extract (GE) were prepared in triplicate: GP-0 without garlic supplementation, which was used as control diet, GP-0.5, GP-1, GP-2, GP-3 and GP-5 diets containing garlic powder at the concentrations of 0.5, 1, 2, 3 and 5%,