学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

Study on diets for Japanese medaka fish to reduce cadmium toxicity
（カドミウム毒性を低減するためのメダカの食餌に関する研究）

Emerging evidences show increasing trends of aquatic environmental pollution which undermine pristine water conditions and the support to fish and aquatic lives. Aquatic environments are not only pickers of anthropogenic contaminations, but also serve as their final destination. Cadmium (Cd) is listed as a priority heavy metal pollutant of aquatic environments. It is non-biodegradable, persistent, cumulative, and toxic to biological tissues. Fishes are designated as 'sentinel species' of aquatic environmental pollution due to their high vulnerability to Cd and other aquatic contaminants. Various water treatment methods usually accompany water pollution cases, but little or no attention is given to surviving fish. Moreover, Cd induced effects can be protracted, defying the protection by endogenous antioxidants and defense mechanism in fish. Such scenarios potentially lead to impaired productivity and decline in fish population, Cd biomagnification along successive links in aquatic food chains, and increased Cd exposure risk on fish consumers. This therefore necessitates the need for recuperation in surviving fish from Cd exposure. The context of this study identifies two major categories of effects on exposed fish as follows; i) accumulation effects; and ii) toxicity effects. These categories constitute chapters 2 and 3, respectively in this dissertation. Various plants and plant derived compounds have been widely researched for their pharmacological and remediating potentials to environmental toxicants. Based on their immune stimulating and rich antioxidative merits, garlic, propolis, and wakame were selected for this study. The experimental model, Japanese medaka (*Oryzias latipes*) was also used for this study based on its numerous research qualities – it is small, cost-effective, and easy to breed in large numbers, prolific, and can be confined easily. Also, most experimental tools for the analysis of gene function can be applied to the species. As yet however, its welfare and recovery dynamics from Cd and other environmentally relevant heavy metals is yet to be addressed. More so, as pollution by heavy metals is almost inescapable to fish, it becomes crucial to examine its recuperation dynamics. Therefore, the main purpose of this study was to assess the effects of garlic,

propolis, and wakame supplemented diets on recuperation in Cd exposed Japanese medaka fish. Specifically, two aims of the study were i) to assess ability of experimental diets to improve Cd depuration in tissues of exposed Japanese medaka fish; ii) to assess ability of experimental diets to ameliorate Cd induced toxicity within tissues of exposed Japanese medaka fish. To achieve these aims, two set of investigations were carried out as chapters two and three, respectively.

The specific objectives of chapter two were to comparatively assess i) Cd bioconcentration, and ii) Cd depuration in exposed medaka fish juveniles under three dietary regimes. Fish were exposed to 0.3 mg Cd/L in water for 21 days under a control diet. Surviving fish were further depurated for 21 days and fed garlic (GD at 4%), propolis (PD at 4%), wakame (WD at 5%) supplemented diets, or control diet (MD). Fish gills, liver, and muscles were excised and assessed weekly for Cd bioconcentration and depuration by atomic absorption spectroscopy (AAS); and metallothionein induction (MT) by enzyme linked immunosorbent assay (ELISA). Dietary performance trial results showed fish biometrics and condition indices in GD, PD, and WD compared favorably with those of MD. There was increased Cd bioconcentration and MT induction following Cd exposure. Conversely, treatments with GD, PD, and WD showed a significant decrease in Cd bioconcentration across tissues and an upregulation in MT expression. Therefore, dietary supplementation supported Cd depuration and potentially reduced bioconcentration margin and associated health risks in the order GD>PD>WD>MD. The specific objectives of chapter three were to comparatively assess i) Cd induced toxicity and ii) Cd toxicity amelioration under three dietary regimes. Experimental protocols were similar with chapter 2. Gill, liver, and muscle tissues were assessed weekly for the antioxidants superoxide dismutase (SOD) and total glutathione (GSH), and also lipid peroxidation (LPO). Results showed reduced antioxidant activity by significantly increasing LPO and reducing SOD activity and GSH contents in gill and muscle tissues upon Cd exposure. In contrast, GD, PD, and WD diets significantly reduced LPO, while significantly increasing contents of GSH and SOD activity, in addition to reducing mortality and improving survival. Condition indices in GD, PD, and WD groups were also significantly higher than those in MD groups. Dietary supplementation therefore significantly increased recuperation and tissue functions in fish, in the order GD>PD>WD>MD. In summary, the experimental diets and their respective percentage inclusions in this study are relevant in improving fish health status following incidences of Cd pollution and water treatment, especially in aquaculture settings. The diet materials are relatively cheap, affordable, and readily accessible to fish famers all year round, and are strongly recommended to fish farmers. In future studies, in vitro studies with major bioactive compounds from respective experimental diets exploring the molecular mechanics of amelioration are suggested.