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In vivo effects of adding singular or combined anti-oxidative vitamins and/or minerals to diets on the immune system of tilapia (*Oreochromis hybrids*) peripheral blood monocyte-derived, anterior kidney-derived, and spleen-derived macrophages

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Abstract

Macrophage function is an important factor for resistance to infection and anti-oxidative vitamins and minerals can affect how macrophages function in fish. We report the *in vivo* effect of adding singular or combined vitamins (A, C, and E) and/or minerals (Se, Zn, Cu, Mn, and Fe) in diets on the immune system of tilapia (*Oreochromis hybrids*) peripheral blood monocyte-derived, anterior kidney-derived, and spleen-derived macrophages. An optimal dose of vitamins and/or minerals in diets increased macrophage proliferation and protective activity, maintained macrophage viability, increased body weight and length, and increased lysozyme activity, however, at improper doses and combinations of vitamins or minerals a decrease was observed. Furthermore, vitamins and/or minerals at any doses and combinations in diets decreased superoxide and nitric oxide production. Therefore, appropriate doses and combinations of vitamins and/or minerals in diets may increase tilapia macrophages immunity.
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Keywords: Diet; *In vivo*; Macrophage; Minerals; Tilapia; Vitamins

1. Introduction

Many factors, including disease, pollutants, hormones, and diet may influence the fish immune system. Non-specific immune responses are very important for fish to defend against infectious agents. Macrophages play an important role in fish immune responses. Peripheral blood monocyte-derived macrophages (PBM), anterior kidney-derived macrophages (AKM), and spleen-derived macrophages (SM) primarily kill pathogens and are therefore key components of the cellular non-specific response. Two kinds of macrophages included fixed and dissociated forms were distinguished in fish. PBM was belonged to the dissociated form which possessed high phagocytosis ability. Furthermore, PBM might be the precursor cells that were able to differentiate as many kinds of phagocytes in other tissues. However, AKM and SM were belonged to the fixed form which also possessed high phagocytosis ability. Therefore, PBM, AKM, and SM were very important cells on fish immunity against pathogens (Brattgerd and Evensen, 1996; Robertsen, 1999; Tsujii and Seno, 1990). Vitamins and minerals are very important for fish growth and deficiency of vitamins and minerals may induce disease (Rukgauer et al., 2001; Lee and Shiau, 2002). We found that Vitamin A acetate (Vitamin A), L-ascorbic acid

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(Vitamin C), DL-α-tocopherol (Vitamin E), selenium (Se), zinc (Zn), copper (Cu), manganese (Mn), and iron (Fe) played a crucial role in non-specific immune responses of fish.

Vitamins are important essential nutrients for most animal species, however, both deficiencies and excesses in dietary intake can cause disease. For example, Vitamin A is a lipid-soluble vitamin. Animals deficient in Vitamin A are more susceptible to infections and stress-related disorders yet, an excess of Vitamin A supplement is a common cause of toxicity. Therefore, a suitable concentration of Vitamin A is important for life (Cuesta et al., 2004, 2003; Shiau and Su, 2003; Hilton, 1983). Vitamin C is also important and influential at various parameters of immune responses. In rainbow trout and grass shrimp, Vitamin C was recognized early as a required nutrient capable of altering phagocytic functions (Kitamura et al., 1965; Lee and Shiau, 2002). Dietary intake of Vitamin C can affect many functions including complement activity, phagocytosis in channel catfish and Atlantic salmon (Li and Lowell, 1985; Hardie et al., 1991; Sakamoto et al., 1981), antibody responses and bacterial killing in rainbow trout (Navarre and Halver, 1989; Blazer, 1992; Blazer and Wolke, 1984), and protecting neutrophils and lymphocytes from oxidative damage in higher vertebrates (human) (Panush and Delafuente, 1985; Anderson et al., 1990). Furthermore, Vitamin C is a powerful enhancer of non-heme iron absorption. The enhancement of iron absorption from vegetable meals is directly proportional to the quantity of Vitamin C present. Ascorbic acid may also be used to combat nutritional iron deficiency anemia (Lynch and Cook, 1980; Hardie et al., 1991), however it is costly and therefore provision is irregular. Vitamin E is also a lipid-soluble vitamin that is comprised of four tocopherols and four tocotrienols in nature. Among them, α-tocopherol possesses the highest Vitamin E activity. Vitamin E is also an essential anti-oxidant present in body tissues and is considered the first line of defense against lipid peroxidation because its quenching activity protects cell membranes from free radical injury (Sealey and Gatlin, 2002; Sies and Murphy, 1991; Le Grusse and Watier, 1993; Meydani et al., 1998). In juvenile hybrid striped bass, Vitamin E is also essential for non-specific immune responses and protects unsaturated fatty acids from oxidation (Sealey and Gatlin, 2002). Thus the dietary intake of these vitamins is crucial to fish health.

Metal ions such as Se, Zn, Cu, Mn, and Fe are essential for most organisms. However, high concentrations of these metal ions are known to be toxic for mammals and microorganisms. Essential trace elements are important parts of anti-oxidant enzymes as superoxide dismutase (SOD) and glutathione peroxidase (GPx) and may affect the anti-oxidant defense system. Se is a trace element with very high biological importance and anti-oxidative properties (Lauchli, 1993; Bronzetti et al., 2001). Zn, Cu, and Mn are structural elements in Cu, Zn-SOD and Mn-SOD responsible for the stabilization (Halliwell and Gutteridge, 1999; Magalova et al., 1997; Mahoney et al., 2006). Fe is an essential mineral in all higher animals including fish because of its importance in oxygen transport and cellular respiration (Shiau and Su, 2003; Lynch and Cook, 1980). Although fish can absorb soluble Fe across the gill membrane and intestinal mucosa, diet is considered the major source of iron for fish due to low concentrations of soluble Fe in natural waters (Shiau and Su, 2003). These minerals are therefore very important for fish health.

Some studies were discussed about the combination of vitamins and/or minerals included interaction of Vitamins C and E in juvenile hybrid striped bass (*Morone chrysops* (female) × *Morone saxatilis* (male)) (Sealey and Gatlin, 2002) and interaction of Vitamin C and Fe (Lynch and Cook, 1980). According those researches, vitamins and minerals had additive or reduced effects on fish immune responses. Therefore, we liked to understand the interaction and relationship of vitamins (A, C, and E) and minerals (Se, Zn, Cu, Mn, and Fe) in fish immunity. Furthermore, dietary anti-oxidative vitamin and mineral supplementation has confirmed the importance of fish immunity, where it enhances both humoral and cellular defenses (Meydani et al., 1998). But proportional addition of anti-oxidative vitamins and minerals remain unclear in tilapia immunity. Significance of the study is to examine the effects of combination of vitamins and/or vitamins and minerals. Therefore, this study will provide the fish industry and other researchers some insight on the relationship between anti-oxidation and macrophages in tilapia immunity.

2. Materials and methods

2.1. Fish preparation

Tilapia (*Oreochromis hybrids*) weighing 1–1.2 g were divided into groups and held in the laboratory fish farm. Each group consisted of 30 fish that were cultured in respective tanks. Fish were kept in a UV filtered re-circulating system with aerated fresh water (25–28 °C) for 2 months prior to data collection. The control group was fed twice daily with diets including no additional

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