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Influence of dietary vitamin C and bovine lactoferrin on blood chemistry and non-specific immune responses of Japanese eel, *Anguilla japonica*

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Abstract

A trial was conducted to determine the effect of ascorbyl-2-monophosphate Na/Ca (AMP-Na/Ca) and bovine lactoferrin (Lf) on blood chemistry and non-specific immune response of Japanese eel juveniles. Test diets with 32 or 762 mg AsA/kg diet, and with or without 500 mg Lf/kg diet were fed to juvenile Japanese eels (75.5±4.4 g) once a day for 3 weeks. A diet without AMP-Na/Ca and Lf supplements was employed simultaneously as a negative control. Liver AsA content of fish fed diets containing 762 mg AsA/kg was significantly ($P<0.05$) higher than fish fed diets containing 32 mg AsA/kg regardless of Lf level. Fish fed diets containing 762 mg AsA/kg showed lower serum glutamic oxaloacetic transaminase (GOT), lactate dehydrogenase (LDH), and serum total bilirubin (T-Bil) than fish fed diets containing 32 mg AsA/kg and the negative control group ($P<0.05$), respectively. Lf was not a significant factor on those parameters. Fish fed diets containing 762 mg AsA/kg showed significantly higher lysozyme activity (LA) of mucus and serum, and bactericidal activity of mucus than the fish fed the diet containing 32 mg AsA/kg. Lf was a significant factor on serum LA, and there was an interaction between AsA and Lf on serum bactericidal activity. These results demonstrated that AMP-Na/Ca is an effective AsA source and higher level in the diet improved blood chemistry and non-specific immune function of Japanese eel. Moreover, the highest serum LA and mucus bactericidal activity were found in eel fed the diet containing the highest AsA content with supplemental Lf.

Keywords: Japanese eel; Ascorbic acid; Lactoferrin; Bactericidal activity; Lysozyme activity

1. Introduction

Vitamin C (L-ascorbic acid, AsA) is an essential nutrient for normal growth and physiological function of most aquatic animal species (Lim and Lovell, 1978). AsA has also been shown to be an important nutrient correlating with fish immunity (Roberts et al., 1995; Ai et al., 2004; Lin and Shiau, 2005). The beneficial effects

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of vitamin C on various immunological parameters have been reported. Namely, dietary AsA enhanced serum bactericidal activity, phagocytic activity, antibody levels, serum complement activity and lysozyme activity (Li and Lovell, 1985; Navarre and Halver, 1989; Hardie et al., 1993; Verlhac et al., 1996; Ortuno et al., 1999; Zhou et al., 2002; Lin and Shiau, 2005). Whereas, other studies have reported that AsA did not affect certain immunological parameters (Thompson et al., 1993; Lygren et al., 1999).

Lactoferrin (Lf) is an iron-binding glycoprotein presented in some secretory fluids of mammals. It has various biological functions, such as iron absorption and transportation (Hagiwara et al., 1997) and bacteriostatic effects (Nemet and Simonovits, 1985), and is also known as an immunostimulant for certain aquatic animals. Orally administrated Lf enhanced phagocyte activity in rainbow trout (Sakai et al., 1993, 1995), number of granulocytes and lymphocytes in red sea bream (Kakuta et al., 1996), serum lysozyme level in Asian catfish (Kumari et al., 2003), mucus secretion in orange spotted grouper (Yokoyama et al., 2006), and bacterial clearance efficiency in giant freshwater prawn (Chand et al., 2006). However, Lygren et al. (1999) reported that dietary bovine lactoferrin did not affect non-specific immunity in Atlantic salmon.

The Japanese eel is a very important culture species in East Asia due to its high market value, desirable taste and recent supply shortage. However, information on the effect of vitamin C for Japanese eel is scarce. Ren et al. (2005) reported that vitamin C is required by Japanese eel and could enhance serum bactericidal activity when fed a diet containing crystalline vitamin C. However, no information is available on the effect of AsA derivatives as a vitamin C source. Furthermore, there is no information on the effect of dietary Lf on immunity of Japanese eel.

The present study was conducted to clarify the efficiency of AMP-Na/Ca as an AsA source, and the possible interaction between dietary bovine Lf and vitamin C on Japanese eel, focusing on several non-specific immunity and blood parameters.

2. Materials and methods

2.1. Experimental design and test diets

A 2×2 factorial design was employed to evaluate interaction effects of dietary AsA and Lf. Our previous study showed that two levels of AsA (27 and 645 mg/kg diet) could improve growth and blood chemistry for Japanese eel juveniles respectively (Ren et al., 2005). 400 mg/kg Lf enhanced the stress resistance for Japanese flounder juveniles (Yokoyama et al., 2005). Thus, four test diets containing two levels of AsA (32 and 762 mg/kg diet) and Lf (0 and 500 mg/kg diet) were formulated for Japanese eel (Table 1) according to Aoe (1980). Major protein and lipid sources were white and brown fishmeals, and squid oil, respectively. α-Starch was mixed to the diets as a carbohydrate source as well as a binder. A diet without AMP-Na/Ca and Lf supplementation was used as a negative control. A derivative of AsA (AMP-Na/Ca, 35% AsA activity, DSM Nutrition Japan K.K. Tokyo, Japan) and bovine Lf (97.2% purity, Lot No. 951110, Morinaga Milk Industry Co., Ltd, Tokyo, Japan) were used in this study. The quantification of AMP-Na/Ca in the test diet was conducted using high performance liquid chromatography (HPLC) by DSM Nutrition, Japan K.K. The content of Lf in the test diets was determined by sandwich enzyme-linked immunosorbent assay (ELISA) as described in a previous study (Yokoyama et al., 2005). All dry ingredients were mixed well and stored at −28 °C until diet preparation. Tap water was added to the mixed ingredients at 120% and a ball-shaped dough was made before feeding. Diet preparation and feeding protocol

Table 1
Basal ratio of test diet for Japanese eel

Ingredients	g/kg dry diet
White fish meal (defatted) [a]	295
Brown fish meal (defatted) [a]	300
α-Starch [b]	200
Vitamin mixture (vitamin C free) [c]	20
Mineral mixture [d]	30
Squid oil [e]	140
Attractants [f]	10
α-Cellulose + AsA derivative [g] + bovine lactoferrin [h]	5
Total	1000

[a] Nippon Suisan Co.Ltd., Japan.
[b] Kanto Chemicals, Tokyo, Japan.
[c] Vitamin mix (mg/20g): β-Carotene, 9.63; Vitamin D_3, 0.97; Menadione $NaHSO_3 \cdot 3H_2O$, 4.58; DL-α-tocopherol acetate, 38.50; Thiamin-nitrate, 5.78; Riboflavin, 19.24; Pyridoxine-HCl, 4.58; Cyanocobalamin, 0.01; Biotin, 0.58; Inositol, 384.91; Nicotinic acid, 76.97; Ca pantothenate, 26.95; Folic acid, 1.44; Choline chloride, 786.93; *p*-aminobenzonic acid, 38.33; α-Cellulose, 192.45.
[d] Mineral mix (mg/30 g): NaCl, 107.79; $MgSO_4 \cdot 7H_2O$, 380.02; $NaHPO_4 \cdot 2H_2O$, 241.91; KH_2PO_4, 665.20; $Ca(H_2PO_4) \cdot 2H_2O$, 376.70; Fe Citrate, 82.38; Ca Lactate, 907.10; $Al(OH)_3$, 0.52; $ZnSO_4 \cdot 7H_2O$, 9.90; $CuSO_4$, 0.28; $MnSO_4 \cdot 7H_2O$, 2.22; $Ca(IO_3)_2$, 0.42; $CoSO_4 \cdot 7H_2O$, 2.77.
[e] Oriental Yeast, Chiba, Japan.
[f] Alanine, 5 g; Betaine, 5 g.
[g] L-ascorbyl-2-monophosphate-Na/Ca (DSM Nutrition Japan K.K.).
[h] Lot No. 951110. Morinaga milk industry Co. Ltd.