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Probiotic bacteria *Lactobacillus rhamnosus* influences the blood profile in rainbow trout *Oncorhynchus mykiss* (Walbaum)

A. Panigrahi · V. Kiron · S. Satoh · T. Watanabe

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Abstract This paper reports the effect of feeding probiotic diets on blood profiles in rainbow trout. Two experiments were performed: in the first, fish of average weight 75 g were offered either a commercial feed or the same incorporated with 10^9 CFU g^{-1} of lactic acid bacteria *Lactobacillus rhamnosus* for 30 days; in the second study performed for a similar duration, fish of average weight 126 g were offered formulated diets that either contained the same bacteria in heat-killed or freeze-dried form (nearly 10^{11} CFU g^{-1}), or the basal diet without the bacteria. Blood samples were collected at different times after commencement of probiotic feeding to determine the total cholesterol, triglyceride contents, the plasma alkaline phosphatase activity, plasma protein and hematocrit value. The plasma cholesterol significantly increased upon probiotic feeding in the first experiment. A significant elevation ($P < 0.05$) of plasma cholesterol and triglyceride and alkaline phosphatase activity level was found in the freeze-dried probiotic fed groups at 20 and 30 days postfeeding. This was concomitant with the increased plasma protein and hematocrit values in FD group at 20 and 30 days. Likewise, the heat-killed probiotic fed group registered significantly high values of triglycerides, alkaline phosphatase activity, and plasma protein compared to the control diet fed groups after 20 days of feeding. Thus, alterations in the blood profiles could serve as supplementary information when examining the benefits of probiotics for fish.

Keywords Alkaline phosphatase · Cholesterol · Plasma protein · Probiotics · Rainbow trout · Triglycerides

A. Panigrahi · V. Kiron · S. Satoh · T. Watanabe
Department of Marine Biosciences, Tokyo University of Marine Science and Technology, Minato, Tokyo 108-8477, Japan

A. Panigrahi (✉)
Central Institute of Brackish Water Aquaculture, 75, Santhom High Road, R. A. Puram, Chennai 600-028, India
e-mail: apanigrahi2k@yahoo.com

Introduction

Probiotics is fast being recognized as an alternative therapy for health management, considering the encouraged restriction on antibiotics and the limitations of vaccination and chemotherapy. This technology already exists in human-health care and in animal husbandry (Fuller 1989, 1992; Gorbach 1990; Jonsson and Conway 1992), where probiotics as an alternative to antibiotics has been commercialized. The concept of probiotics in aquaculture has been clearly defined by Vershuere et al. (2000). Despite its wider acceptance in aquaculture, many questions remain unanswered about its influence on fish physiology. Recently, some studies have looked into

the Lactic acid bacteria (LAB)-induced immune response (Villamil et al. 2002; Nikoskelainen et al. 2003; Panigrahi et al. 2004) and disease resistance (Nikoskelainen et al. 2001) in rainbow trout and turbot. There is no report yet about the influence of LAB on the plasma components in fish.

Measurements of plasma constituent levels not only are easier compared to immunological parameters or pathogen-challenge trials for evaluating disease resistance, but also can be performed without killing the fish. Some significant studies (Maita et al. 1998a, b) reported that the levels of plasma lipid components, such as total cholesterol, free cholesterol and phospholipids, would be a good indicator of fish health condition based on correlation between these parameters and the mortality patterns following artificial or natural infection. Earlier, Watanabe et al. (1995) had described the relationship between the level of plasma lipid components and mortality due to infectious disease in yellowtail *Seriola quinqueradiata*. In a review, De Roos and Katan (2000) pointed out that the effect of probiotics on cholesterol metabolism in humans is uncertain. Nevertheless, studies in endothermic animals (Canzi et al. 2000; Pacini et al. 1989) revealed that LAB induce a hypercholesterolemic effect and have an influence on the levels of other plasma component. Triglycerides the other lipid component examined in this study is important as energy reserves and have been found to be influenced by probiotic supplementation, though its magnitude differ from case to case (Sindhu and Khetarpaul 2002). Further, the innate immune response to external stimuli is reflected in the altered plasma protein levels.

With this background, it is tempting to know whether there exists any pattern in the plasma component levels with respect to probiotic feeding that could be related to immune response in aquatic animals. Therefore, this study aims to assess the effect of feeding viable and non-viable forms of lactic acid bacteria *Lactobacillus rhamnosus* on blood profiles in rainbow trout.

Materials and methods

Bacterial strain and forms

The bacterium *L. rhamnosus* JCM 1136 was obtained from Japan Collection of Microorganisms (JCM), Riken, Japan in freeze-dried form. The culture was revived, sub-cultured, and the bacteria were harvested as described in our previous paper (Panigrahi et al. 2004). In first experiment (exp I), the live bacterial suspension (probiont pellet is diluted with sterilized diluents with sterile peptone water [NaCl, 0.85% and polypeptone, 0.1%]) was sprayed onto the feed, while in the second experiment (exp. II), the viable and non-viable forms of bacteria were used, being incorporated in the feed either as heat-inactivated form (HK) or freeze-dried form (FD). Initially, two identical suspensions of live bacteria were prepared from a stock culture. One of this was heat killed in a water bath at 75°C for 60 min with continuous stirring. The non-viability was confirmed by plating on MRS agar (De Man et al. 1960) and preserved at −80°C before incorporation in the feed. The other suspension was used to prepare the freeze-dried form by keeping it for 60 h at −20°C in a freeze-drier (REL 206, Kyowa Vacuum Tech., Tokyo, Japan). The dried powder was enumerated for bacterial number per gram of the product vacuum packed and preserved at −20°C until further use.

Feed formulation and probiotic supplementation

In exp. I, LAB was incorporated in the commercial rainbow trout feed (Nippon Formula Feed, Yokohama, Japan) by spraying the required amount of bacterial suspension into the feed while mixing gently in a drum mixer. The bacterial count was confirmed by spread plate method and found to be nearly 10^9 CFU g^{-1} of feed. The commercial feed sprayed with sterilized diluent with sterile peptone water (NaCl, 0.85% and polypeptone, 0.1%) served as the control diet (CO–C).

In exp. II, the diets were formulated with 50% defatted fishmeal (DFM) as the protein source and linseed oil as the lipid source. To reduce the microflora associated with the DFM, it was autoclaved and dried before use in diet preparation that followed sterile protocols. The diet also contained 10% fructooligosaccharides intended to serve as the prebiotic component. These non-digestible carbohydrates could influence the microflora composition and fermentation metabolites and contribute to local and systemic effects as in humans and animal models (Fishbein et al. 1998; Young 1998; Gibson and Roberfroid 1995). The ingredients (Table 1) were mixed mechanically

Table 1 Composition of basal diet used for rainbow trout in experiment II

Ingredients	Content (%)
Defatted fish meal	50
Dextrin	7
Starch	7
Fructooligosacharides	10
Gelatin	4
Linseed oil	10
Vitamin E[a]	0.01
Vitamin mix[b]	3
Mineral mix[b]	5
Choline chloride	0.4
Cellulose	3.59
Total	100

[a] DL-α-tocopheryl acetate, purity 50%

[b] Refer to Panigrahi et al. 2005 for detailed composition

(ACM-50 LAT, Aikohsha Mfg., Tokyo, Japan), and sterilized water was added prior to pelletizing (AEZ12M, Shimadzu, Kyoto, Japan). The pellets were dried in a freeze-drier and stored at −20°C until further use. This basal diet without the probiont served as the control (CO) diet. To produce the HK diet, the bacterial suspension, prepared as mentioned in the earlier section, was sprayed onto the basal diet and allowed to dry. The freeze-dried bacteria were included (by weight to get the identical density) in the third diet (FD) along with the basal ingredients during the diet preparation stage. The stock feed was kept at −20°C, and the daily requirement was kept at 4°C prior to feeding. The viability of the incorporated LAB was enumerated after incubation in Tryptic soy agar (TSA; BBL, Becton–Dickinson Cockeysville, USA) and MRS spread plates. The bacterial count was nearly 10^{11} CFU g^{-1} for the diet FD.

Experimental design

The feeding experiments were conducted in 60 l tanks arranged in a flow-through system, each treatment in triplicate. Rainbow trout that had been grown previously on commercial pellet rations from Nippon Formula Feed during early stages and Chubu Shiryo (Shizuoka, Japan) during their juvenile phase were stocked at 15 nos per tank. The initial average weight of fish in experiment I and II are, respectively, 75 ± 1.78 and 126 ± 2.95 g. The chemical composition of the commercial feed was 45% protein, 3.5% crude lipid, 3% crude fiber, 13% crude ash, 1.6% Ca and 1.2% P. In exp. I, the fish were fed on the commercial feed taken as control (CO) and probiont containing feed as test diet, whereas in exp. II, the fish were offered the basal diet for a 2-week period to get them weaned on their new diets, following that diets CO (basal diet prepared without any probiotic incorporation), HK and FD were fed, twice daily until satiation for a period of 30 days. The basal diet had similar chemical composition like that of the commercial feed. The water temperature during the term was 16 ± 1°C.

Sample preparation and analysis of blood components

Besides, the respective initial samples, in exp. I a sample was taken on day 30, while in exp. II they were collected on day 10, 20 and 30. A total of nine fish were taken from each treatment three from each tank randomly at one point of time after a 24 h starvation period, and blood was drawn (approx. 3–4 ml) from the caudal vein of individual fish after anaesthetization with 300 ppm 2-phenoxyethanol (Wako Pure Chemical Industries Ltd., Tokyo, Japan). Plasma samples were collected after spinning down the heparinized blood at 1,500 × *g* for 5 min at 4°C and stored at −80°C prior to analysis.

The plasma samples were thawed, and the total cholesterol and triglycerides contents (mg dl^{-1}) and alkaline phosphatase activity (IU l^{-1}) were determined using an auto analyzer (ARKRAY SPOTCHEM™ EZ SP-4430, Shiga, Japan) after calibration with the magnetic card attached to reagent strip and employing the corresponding strips (ARKRAY) based on dry chemical analysis.

Plasma protein was determined by Biuret method using standard Bovine serum albumin (BSA) and other reagents (Sigma Chemicals, Tokyo, Japan). The plasma sample was diluted 100 times with 0.85% NaCl. and color developed with the biuret reagents, which react with the peptide bond, was measured in the spectrophotometer relating to the standard graph.

An aliquot of the heparinized whole blood was used for hematocrit determination. The blood samples were drawn into microhematocrit tubes that were

centrifuged at 12,000×*g* for 5 min using a high-speed microcentrifuge (MC 150, Tomy, Tokyo, Japan).

Statistical analysis

Data are presented as mean ± SD of the number of fish per treatment. For Exp I, Student's *t*-test was conducted using SPSS 17 (SPSS Inc., Chicago, USA). The data of Exp. II were subjected to multiple comparisons through repeated measures (ANOVA; SPSS 17.0 software, SPSS Inc., Chicago, USA) to determine the significant variation between treatments. All tests used a significance level of $P = 0.05$.

Results

In experiment I, after 30 days of probiotic feeding, the total cholesterol was elevated significantly compared to fish receiving the control diet as shown in the Table 2. However, the triglyceride contents and alkaline phosphatase activity did not show any significant increase compared to that of the control (Co). These findings were followed up in exp. II where the bacterial viability was also taken into consideration.

Total cholesterol

Total cholesterol levels of the probiotic fed groups did not show any significant difference at 10 days (175 ± 37 (control) to 205 ± 93 and 193 ± 40 mg dl^{-1} in HK and FD group, respectively). At 20 and 30 days, the FD group showed significantly higher levels ($P < 0.05$) as 204 ± 27 and 230 ± 50 mg dl^{-1} compared to the control, whereas the HK group showed non-significant increment up to 188 ± 52 and 215 ± 43 mg dl^{-1} compared to that of the control group (Fig. 1).

Table 2 Plasma biochemical parameters from rainbow trout in experiment I 30 days after being fed with the probiotic diet ($n = 9$)

Treatments	Total cholesterol (mg dl^{-1})	Triglycerides (mg dl^{-1})	Alkaline phosphatase (IU l^{-1})
Control diet	282 ± 42[a]	281 ± 140[a]	250 ± 75[a]
Probiotic diet	319 ± 35[b]	337 ± 115[a]	302 ± 119[a]

Unlike superscript letters in a column indicate significant difference ($P < 0.05$) between the diets

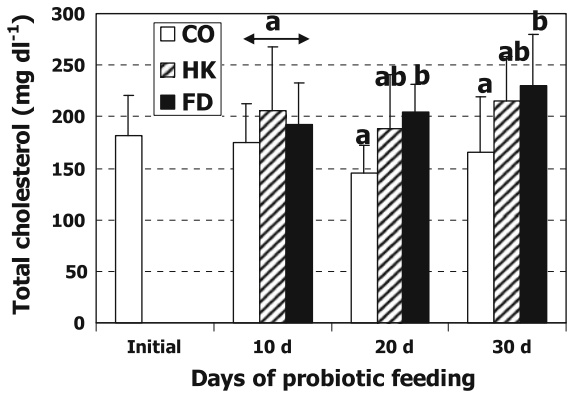

Fig. 1 Plasma total cholesterol levels in rainbow trout fed on diets with either heat-killed or freeze-dried LAB or the control diet at 10, 20 and 30 days. *CO*, control feeding with no probiotic fed group; *HK*, heat-killed form of probiotic fed group; and *FD*, freeze-dried probiotic fed groups. Data shown as mean with standard deviation as error bars; *different letters* on the bars indicate significant difference ($P < 0.05$) between groups ($n = 6$)

Triglycerides

Plasma triglyceride levels at 10 days were 218 ± 58 mg dl^{-1} in the FD group, whereas in the control it was 170 ± 69 mg dl^{-1} and rose significantly ($P < 0.05$) to 231 ± 21 and 285 ± 55 mg dl^{-1} at 20 and 30 days, respectively. The HK group registered a significant increase in the triglyceride value 20 days that was not sustained until 30 days (Fig. 2).

Alkaline phosphatase

The alkaline phosphatase activity in the FD group recorded a significant ($P < 0.05$) increase at 20 and 30 days, the levels being 140 ± 31 and 188 ± 48 IU l^{-1} compared to the corresponding values for control 83 ± 21 and 115 ± 62 IU l^{-1}, respectively. This elevation was more evident on day 30. The activity of the enzyme for the HK group registered significant increase at 20 days compared to the control group (Fig. 3).

Plasma protein

At 10 days, there was no variation in plasma protein level from the treatments compared to that of the

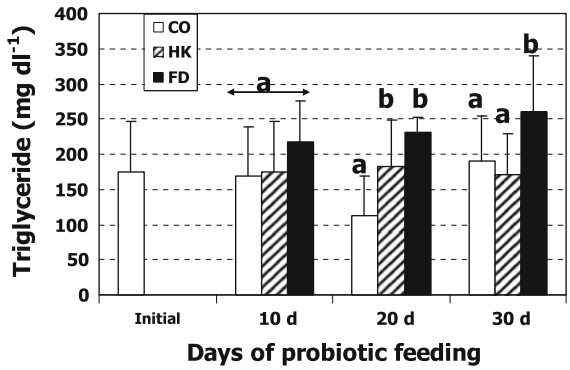

Fig. 2 Plasma triglyceride levels in rainbow trout fed on diets with either heat-killed or freeze-dried LAB or the control diet at 10, 20 and 30 days. *CO*, control feeding with no probiotic fed group; *HK*, heat-killed form of probiotic fed group; and *FD*, freeze-dried probiotic fed groups. Data shown as mean with standard deviation as error bars; *different letters* on the bars indicate significant difference ($P < 0.05$) between groups ($n = 6$)

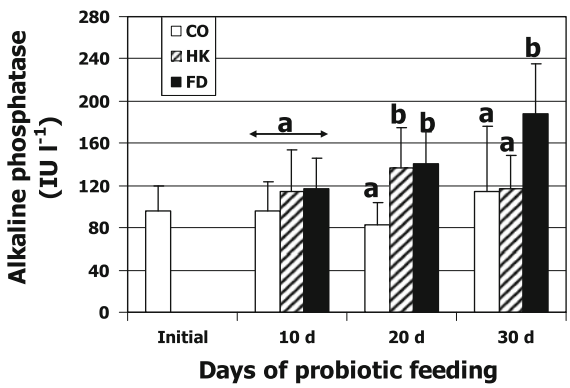

Fig. 3 Alkaline phosphatase activity in rainbow trout fed on diets with either heat-killed or freeze-dried LAB or the control diet at 10, 20 and 30 days. *CO*, control feeding with no probiotic fed group; *HK*, heat-killed form of probiotic fed group; and *FD*, freeze-dried probiotic fed groups. Data shown as mean with standard deviation as error bars; *different letters* on the bars indicate significant difference ($P < 0.05$) between groups ($n = 6$)

control. However, both the HK and FD groups recorded a significantly ($P < 0.05$) higher level of plasma protein (42 ± 9, 44 ± 1 mg ml^{-1}) than that of the control group (37 ± 7 mg ml^{-1}) at 20 days. Subsequently at 30 days FD group registered a significantly higher ($P < 0.05$) level, whereas HK group did not show any significant increment in plasma protein than that of the control group (Fig. 4).

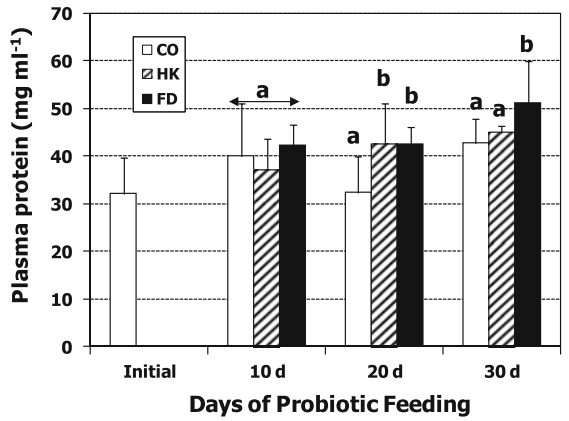

Fig. 4 Plasma protein levels in rainbow trout fed on diets with either heat-killed or freeze-dried LAB or the control diet at 10, 20 and 30 days. *CO*, control feeding with no probiotic fed group; *HK*, heat-killed form of probiotic fed group; and *FD*, freeze-dried probiotic fed groups. Data shown as mean with standard deviation as error bars; *different letters* on the bars indicate significant difference ($P < 0.05$) between groups ($n = 6$)

Hematocrit value

The hematocrit values at 10 and 20 days did not show any significant difference between the control and probiotic fed groups. However, on the 30th day, the FD groups had significantly higher values compared to that of the CO. The HK group also had a hematocrit value over that of the control by day 20, but not significant statistically (Fig. 5).

Discussion

Maita et al. (2002) had established that the levels of plasma constituents would be a good indicator of the health condition of fish. The first experiment of the present study demonstrated that the plasma total cholesterol increased after feeding probiotic bacteria *L. rhamnosus* incorporated diet for 30 days. The triglyceride levels and alkaline phosphatase activities marginally increased in the probiotic fed group compared to the control though not statistically significant. The second experiment also revealed that the level of blood lipid metabolites and the activity of the enzyme alkaline phosphatase in the fish fed viable probiotic diets (FD) were significantly higher than those that received no probiotic supplements (CO) at 20 and 30 days. The group receiving the non-viable

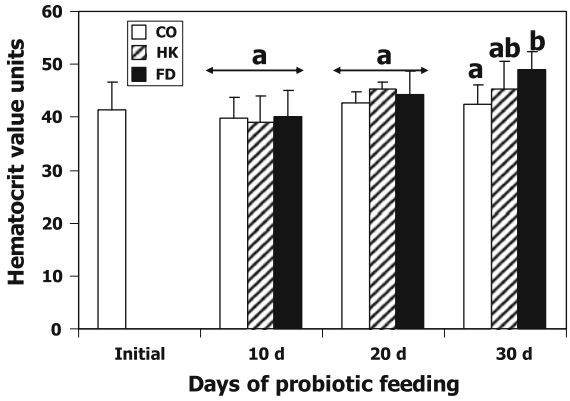

Fig. 5 Hematocrit value in rainbow trout fed on diets with either heat-killed or freeze-dried LAB or the control diet at 10, 20 and 30 days. *CO*, control feeding with no probiotic fed group; *HK*, heat-killed form of probiotic fed group; and *FD*, freeze-dried probiotic fed groups. Data shown as mean with standard deviation as error bars; *different letters* on the bars indicate significant difference ($P < 0.05$) between groups ($n = 9$)

heat-killed LAB (HK) had all these values are between the control and viable LAB fed groups. Though both these experiments were independent of each other, viable probionts were found to have pronounced influence on the biochemical and hematological profiles. The freeze-dried form (FD) diet fed groups seems to be better compared to that of the non-viable (HK) form. The HK diet is not enumerable postheat killing. However, in case of equal volume live spray diet, leaching to the surrounding media could be one factor, but considering the order of leaching compared to the high level of incorporation and the demand feeding regime followed, it is negligible.

The plasma cholesterol levels were elevated by the probiotic feeding in fish in contrast to most of the findings in endothermic animals. Although not without exception, results over several years from animal and human studies suggest a moderate cholesterol-lowering action of dairy products fermented with strains of lactic acid bacteria and bifidobacteria (Periera and Gibson 2002). Mechanistically, probiotic bacteria ferment food-derived indigestible carbohydrate to produce short chain fatty acids in the gut, which can then cause a decrease in the systemic levels of blood lipids by inhibiting hepatic cholesterol synthesis and/or redistributing cholesterol from plasma to the liver. Furthermore, some bacteria may interfere with cholesterol absorption from the gut by deconjugating bile salts, and thereby affecting cholesterol metabolism or by directly assimilating cholesterol. An elevation in the total cholesterol noticed in response to viable probiotic feeding in the present study may be due to the influence of LAB on the cholesterol metabolism. Tortuero et al. (1997) reported a significant increase in the HDL cholesterol fraction in rats in response to a concurrent administration of raffinose plus LAB. De Rodas et al. (1996) claimed that the assimilation of cholesterol and the deconjugation of bile salts had a lowering effect on serum cholesterol levels through interference with the enterohepatic cycle. Overall, the effect of probiotics on cholesterol mechanism is inconclusive (reviewed by de Roos and Katan 2000). However, an increase in the plasma cholesterol is correlated with a better health status in fish as reported by some studies described below.

Maita et al. (1998b) in an important study attempted to correlate the plasma component levels and resistance to bacterial infection and stated that decrease in the plasma lipid components of cultured fish could indicate a reduction in fish health condition. The mortality among rainbow trout having low plasma total cholesterol level was significantly higher than that among fish having high levels of it when challenged with *Vibrio anguillarum*. Similarly, the mortality among yellowtail having low-cholesterol levels was significantly higher than that among fish with lower levels when challenged with *Lactococcus garvieae*. The levels of plasma cholesterol and phospholipids were decreased (Maita et al. 1998b) when mortality due to *L. garvieae* infection in yellowtail was increased by low-ambient dissolved oxygen (Fukuda et al. 1997). It was suggested that plasma lipid component levels in cultured fish are reduced when fish are affected by other factors that could eventually lower their disease resistance.

Harikrishnan et al. (2003) while studying the hematological and biochemical parameters in common carp, *Cyprinus carpio*, following herbal treatment with *Azadirachta indica* leaf extract for *Aeromonas hydrophila* infection, observed a significant decrease in serum cholesterol levels over a period of 30 days, while the treated fish registered a slight increase by day 10 that had almost doubled by day 30. The present finding of a hypercholesterolemic effect of probiotic feeding of viable form in rainbow trout is in agreement with the earlier mentioned

reports, suggesting that the elevation of these plasma components may be an indication of the healthy status. Regarding the influence of duration of feeding, the effect of probiotic diet with viable form was observed after 10 days and continued to improve until day 30; however, statistically there was no interaction between factors, diet and time.

Maita et al. (1998a) reported a decrease in plasma lipid components like total cholesterol, free cholesterol, triglycerides and phospholipids in yellowtail *Seriola quinqueradiata* fed with non-fishmeal diet compared to that fed with fishmeal based diets which are more resistant to disease when challenged. Chatzifotis et al. (2004) reported a higher total protein, total fat, triglycerides and cholesterol in common dentex *Dentex dentex* (Linnaeus) at spawning and spermiation when they are more resistant to diseases. The levels of plasma lipid components were lowered by the effect of inanition (MacDonal and Milligan 1992), a low-calorie diet, anemia (Maita et al. 1996) and dysfunction of liver. In the present study, elevated triglyceride levels upon probiotic feeding of viable form FD group showed a similar trend as that of the total cholesterol. Since there is no qualitative difference between the tests diets except the supplementation of probiotics, the reason may not be anywhere close to inanition, anemia or low-calorie diet. Among other reasons, it may be possible that these probiotics may influence the lipid digestibility that is reflected in the plasma lipids.

In this study, the plasma alkaline phosphatase activity recorded is within the normal range reported by Hille (1982) from nine independent studies for rainbow trout (98–261 IU l^{-1}). In contrast to the observations in terrestrial vertebrates (Boyd et al. 1983) that the elevated enzyme activity indicate low dietary phosphorus intake or frank deficiency, in fish the mineral deficiency correlated with low-alkaline phosphatase activity in plasma or serum (Sugiura et al. 2004). The increase in plasma enzyme activity upon viable probiotic addition observed in our study might reflect the up-regulation of this enzyme resulting from the increased availability of certain minerals or their retention.

Several bacterial moieties including peptidoglycan have been shown to act as biological response modifiers (Standiford et al. 1994). In the present study, the plasma protein of the probiotic fed groups irrespective of their viability was higher than the control group at 20 days, however, at 30 days, only a viable group showed a significantly higher level of plasma protein compared to that of the CO and HK groups. Total protein and lysozyme levels are measurable humoral components of the non-specific defense mechanism, and the elevated levels of the former may imply a better health status in fish. In animal models and human beings, the molecular effect of LAB include the stimulation of immunoglobulin A, production of antioxidants, various nutrients, neonutrients, growth and clotting factors (Quan and Gill 2002; Bengmark 2001). This change in the plasma protein is also thought to be an adaptive response that contributes to regaining homeostasis after tissue injury or infection (Gerwick et al. 2002). It has also been reported that the total protein in plasma was significantly higher in rainbow trout fed with the low dose of glucan before stress (Jeney et al. 1997). Following stress and in the recovery period, the total protein levels were reduced in all groups, independent of the glucan status in the fish. Very low levels of plasma total protein have significance in relation to infectious disease, kidney damage and nutritional imbalance in fish (Wedemeyer and McLeay 1981).

Concurrent to the plasma component levels, the hematocrit values increased with *L. rhamnosus* feeding at 30 days, implying improved health status. In yet another study describing the consequences of feeding a diet lacking fishmeal, Maita (2003) linked poor health and low-hematocrit values in fish. Additionally, we have already reported (Panigrahi et al. 2004, 2005) that the viable LAB is better than non-viable bacteria in inducing non-specific immune responses like phagocytosis and serum alternate complement activity in rainbow trout. Probiotic cultures have been shown in a variety of test systems to stimulate certain cellular and humoral functions of the immune system (Villamil et al. 2002; Gill 1998). In a study on human volunteers (Jahreis et al. 2002), probiotic bacteria were shown to influence blood lipids and immunological parameters, further supporting our observations on fish. Other studies have shown improved survival of pathogen-infected laboratory animals consuming probiotic cultures when compared to animals consuming a control diet. Results accumulated so far suggest that probiotics may indeed provide additional protection to its consumer, farmed fish included. The changes in the blood profiles, such as those presented in this report, would serve as supplementary information when examining the benefits of the probiotics in fish.

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References

Bengmark S (2001) Nutritional modulation of acute and chronic phase responses. Nutrition 17:489–495

Boyd RD, Hall D, Wu JF (1983) Plasma alkaline phosphatase as a criterion for determining biological availability of phosphorus for swine. J Anim Sci 57:396–401

Canzi E, Zanchi R, Camaschella P, Cresci A, Greppi GF, Orpianesi C et al (2000) Modulation by lactic acid bacteria of the intestinal ecosystem and plasma cholesterol in rabbits fed a casein diet. Nutr Res 20:1329–1340

Chatzifotis S, Muje P, Pavlidis M, Agren J, Paalavuo M, Molsa H (2004) Evolution of tissue composition and serum metabolites during gonadal development in the common dentex (*Dentex dentex*). Aquaculture 236:557–573

De Man JC, Rogosa M, Sharpe ME (1960) A medium for the cultivation of lactobacilli. J Appl Bacteriol 23:130–135

De Rodas BZ, Gilliland SE, Maxwell CV (1996) Hypocholesterolemic action of *Lactobacillus acidophilus* ATCC 43121 and calcium in swine with hypercholesterolemia induced by diet. J Dairy Sci 79:2121–2128

De Roos NM, Katan MB (2000) Effect of probiotic bacteria on diarrhea, lipid metabolism, carcinogenesis. A review of papers published between 1988 to 1998. Am J Clin Nutr 71:405–411

Fishbein L, Kaplan M, Gough M (1998) Fructooligosaccharides: a review. Vet Hum Toxicol 30(2):104–107

Fukuda Y, Maita M, Satoh K, Okamoto N (1997) Influence of dissolved oxygen concentration during pre and post infection on the mortality induced by artificial infection with *Enterococcus seriolicida* in yellow tail. Fish Pathol 32:129–130

Fuller R (1989) Probiotics in man and animals. J Appl Bacteriol 66:365–378

Fuller R (1992) Probiotics: the scientific basis. Chapman & Hall, London

Gerwick L, Steinhauer R, Lapatra S, Sandell T, Ortuno J, Hajiseyedjavadi N et al (2002) The acute phase response of rainbow trout (*Oncorhynchus mykiss*) plasma proteins to viral, bacterial and fungal inflammatory agents. Fish Shellfish Immunol 12:229–242

Gibson GR, Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 125:1401–1412

Gill HS (1998) Stimulation of the immune system by lactic culture. Int Dairy J 8:535–544

Gorbach SL (1990) Lactic acid bacteria and human health. Ann Med 22:7–41

Harikrishnan R, NishaRani M, Balasundaram C (2003) Hematological and biochemical parameters in common carp, *Cyprinus carpio*, following herbal treatment for *Aeromonas hydrophila* infection. Aquaculture 221:41–50

Hille S (1982) A literature review of the blood chemistry of rainbow trout *Salmo gairdneri* Rich. J Fish Biol 20:535–569

Jahreis G, Vogelsang H, Kiessling G, Schubert R, Hammes WP (2002) Influence of probiotic sausage (*Lactobacillus paracasei*) on blood lipids and immunological parameters of healthy volunteers. Food Res Int 35:133–138

Jeney G, Galeotti M, Volpatti D, Jeney Z, Douglas PA (1997) Prevention of stress in rainbow trout (*Oncorhynchus mykiss*) fed diets containing different doses of glucan. Aquaculture 154:1–15

Jonsson E, Conway P (1992) Probiotics for pigs. In: Fuller R (ed) Probiotics. The scientific basis. Chapman and Hall, London, pp 259–316

MacDonal DG, Milligan CL (1992) Chemical properties of blood. In: Hoar WS, Randall DJ, Farrell AP (eds) Fish physiology. Academic Press, San Diego, pp 56–133

Maita M (2003) Plasma cholesterol as an index for evaluating health of farmed fish. World Aquaculture Society Book of Abstracts, Salvador, p 444

Maita M, Ushio H, Okamoto N (1996) The effect of anemia artificially induced by bleeding on blood constituents, concentration of ATP-related compounds and Na+-K+ ATPase activity in gill and kidney of coho salmon. Fish Pathol 31:237–238

Maita M, Aoki H, Yamagata Y, Satoh S, Okamoto M, Watanabe T (1998a) Plasma biochemistry and disease resistance in yellowtail fed a non-fish meal diet. Fish Pathol 33:59–63

Maita M, Aoki H, Satoh K, Fukuda Y, Lee H, Winton JR et al (1998b) Correlation between plasma component levels of cultured fish and resistance to bacterial infection. Fish Pathol 33:129–133

Maita M, Satoh K, Satoh S, Kiron V, Watanabe T (2002) Effects of non-fish meal diet on hematological parameters, disease resistance and lipid profiles of liver and erythrocytes in yellow tail. World Aquaculture Society Book of Abstracts, Beijing, p 475

Nikoskelainen S, Ouwehand AC, Bylund G, Salminen S (2001) Protection of rainbow trout (*Oncorhynchus mykiss*) from furunculosis by *Lactobacillus rhamnosus*. Aquaculture 198:229–236

Nikoskelainen S, Ouwehand AC, Bylund G, Salminen S, Lilius E (2003) Immune enhancement in rainbow trout (*Oncorhynchus mykiss*) by potential probiotic bacteria (*Lactobacillus rhamnosus*). Fish Shellfish Immunol 15:443–452

Pacini N, Ferrari A, Zanchi R, Corti M, Greppi GF (1989) The effect of protein dietary sources on serum cholesterol and bile acid microbiological transformation in rabbits. Nutr Rep Int 39:851–859

Panigrahi A, Kiron V, Kobayashi T, Puangkaew J, Satoh S, Sugita H (2004) Immune responses in rainbow trout *Oncorhynchus mykiss* induced by a potential probiotic bacteria *Lactobacillus rhamnosus* JCM 1136. Vet Immunol Immunopathol 102:379–388

Panigrahi A, Kiron V, Puangkaew J, Kobayashi T, Satoh S, Sugita H (2005) The viability of probiotic bacteria as a

factor influencing the immune response in rainbow trout *Oncorhynchus mykiss*. Aquaculture 243:241–254

Periera IAD, Gibson RG (2002) Effect of consumption of probiotics and prebiotics on serum lipid levels in humans. Crit Rev Biochem Mol Biol 37:259–281

Quan S, Gill HS (2002) Immune protection mediated by the probiotic *Lactobacillus rhamnosus* HN001 (DR 20) against *Escherichia coli* 0157:H7 infection in mice. FEMS Immunol Med Microbiol 34:59–64

Sindhu SC, Khetarpaul N (2002) Effect of feeding probiotic fermented indigenous food mixture on serum cholesterol levels in mice. Nutr Res 23:1071–1080

Standiford TJ, Arenberg DA, Danforth JM, Kunkel SL, VanOtteren GM, Strieter RM (1994) Lipoteichoic acid induces secretion of interleukin-8 from human blood monocyte: a cellular and molecular analysis. Infect Immun 62:119–125

Sugiura SH, Hardy RW, Roberts RJ (2004) The pathology of phosphorus deficiency in fish—a review. J Fish Dis 27:255–265

Tortuero F, Fernandez E, Ruperez P, Moreno M (1997) Raffinose and lactic acid bacteria influence caecal fermentation and serum cholesterol in rats. Nutr Res 17:41–49

Vershuere L, Rombaut G, Sorgeloos P, Verstraete W (2000) Probiotic bacteria as biological control agents in Aquaculture. Microbiol Mol Biol Rev 64:655–671

Villamil L, Tafalla C, Figueras A, Novoa B (2002) Evaluation of immunomodulatory effect of lactic acid bacteria in turbot (*Scophthalmus maximus*). Clin Diagn Lab Immunol 9:1318–1323

Watanabe T, Aoki H, Viyakaran V, Maita M, Yamagata Y, Satoh S et al (1995) Combined use of alternative protein sources as a partial replacement for fish meal in a newly developed soft dry pellet for yellowtail. Suisan zoshoku 43:511–520

Wedemeyer GA, Mcleay DJ (1981) Methods for determining the tolerance of fishes to environmental stressors. In: Pickering AD (ed) Stress and fish. Academic Press, London, pp 246–275

Young J (1998) European market developments in prebiotic- and probiotic-containing foodstuffs. Br J Nutr 80(4):231–233