JOURNAL OF THE
WORLD AQUACULTURE SOCIETY

Vol. 48, No. 2
April, 2017
doi: 10.1111/jwas.12336

The Effect of Kefir as a Dietary Supplement on Nonspecific Immune Response and Disease Resistance in Juvenile Rainbow Trout, *Oncorhynchus mykiss* (Walbaum 1792)

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Abstract

In this study, juvenile rainbow trout fed with commercial pellets containing kefir provided increased nonspecific immune response and improved disease resistance against lactococcosis and yersiniosis. Kefir was used as a feed supplement at 2, 5, and 10% inclusion rates and several nonspecific immune parameters were observed at day(s) 1, 7, 14, 21, 28, and 35 following the treatment. A total of four experimental groups, including control, was established. The various parameters including hematocrits, nitroblue tetrazolium positive neutrophils, total leukocytes, serum lysozyme activity, total serum protein, and immunoglobulin M (IgM) levels were examined. As a result of this study, kefir-fed fish had an increase in measured nonspecific immune parameters, especially in the group received the 10% kefir treatment. The challenged fish fed with kefir-supplemented diet showed a better survival rate against *Lactococcus garvieae* than *Yersinia ruckeri*. Kefir supplementation reduced fish mortality significantly against *L. garvieae*.

Aquaculture is an extensively expanding industry around the world, despite frequent outbreaks of bacterial diseases. Although some cases could be managed with antibiotics, the use of antibiotics in some cases has caused the proliferation of drug-resistant pathogens (Schmidt et al. 2001; Cabello 2006) and inhibition of aquatic animals' immune systems. These problems associated with the use of antibiotics (Rigos and Smith 2015) as well as some other therapeutic agents increased interest in possible alternatives to these agents. Probiotics have shown various health-promoting properties (Yan and Polk 2011; Kechagia et al. 2013) and are increasingly of interest in aquaculture (Ai et al. 2011). The first definition of probiotics made for terrestrial animals were "live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance" (Fuller 1989). However, this definition has been adapted to include many other sectors such as aquaculture. According to a broader definition adapted, a probiotic is defined as a live microbial supplement that is beneficial to the host by improving feed use, by modifying and/or improving the host and its ambient environment,

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and enhancing response to the disease by modifying both the host and environmental microbial community (Verschuere et al. 2000). Explicitly, the beneficial effects of probiotics include improvement of the feed value, the modulation of intestinal microflora, enzymatic contribution to digestion, inhibition of pathogenic microorganisms, growth-promoting factors, and the enhancement of immune responses. These have been demonstrated in a number of previous studies (Irianto and Austin 2002; Wang et al. 2008; Merrifield et al. 2010; Nayak 2010). Among the various benefits of probiotics, their immunomodulatory activity is an especially noteworthy specification for improving the overall health of the host. Although the list of probiotics used in aquaculture is expanding rapidly, the most common probiotics used in aquaculture belong to the lactic acid bacteria (LAB) group and *Bacillus* spp. (Wang et al. 2008; Muñoz-Atienza et al. 2013).

Kefir is a traditional product widely consumed in Eastern Europe, Southwest Asia, and many other regions in the world. Kefir grain is a natural source of probiotics and used as a natural starter culture for kefir making. *Lactobacillus kefiranofaciens*, *L. kefiri*, *L. parakefiri*, *L. acidophilus*, *L. helveticus*, *L. casei*, *L. bulgaricus*, *Bifidobacteria* spp., and yeasts such as *Saccharomyces* and *Kluyveromeyce*s are naturally embedded in the polysaccharide structure of kefir grains. Containing LAB and yeasts in a matrix of proteins, lipids, and sugars, kefir is rich in natural probiotics such as *Bifidobacterium* spp., *L. kefiranofaciens,* and *L. acidophilus* (Guzel-Seydim et al. 2011; Ulukoy et al. 2015). As a whole, these groups of bacteria have been reported to produce a wide range of positive effects, including stimulation of the immune system (Vinderola et al. 2005). Kefir has been reported to stimulate the immune system in both in vitro and in vivo studies (Furukawa et al. 1990; Osada et al. 1994). Several studies have found antibacterial, immunological, and antitumor effects of kefir on humans and some other animals (Furukawa et al. 1990; Ozcan et al. 2009). However, there are few studies on the effects of kefir on the nonspecific immune parameters and disease resistance of fish. The aim of this study was to determine the effects of dietary supplementation of kefir on disease resistance and nonspecific immune system parameters in rainbow trout, *Oncorhynchus mykiss*, juveniles.

Materials and Methods

Kefir

Kefir grains were obtained from Suleyman Demirel University, Department of Food Engineering, Isparta in Turkey. In the laboratory, kefir grains were inoculated (2%, w/v) into the pasteurized milk and fermented at +24C for 22 h to produce kefir. At the end of the fermentation (pH 4.6) the grains were retrieved by sieving and kefir was stored at +4C for 1 d.

Experimental Diets

Commercial rainbow trout feed (crude protein 45%, crude lipid 20%, digestible energy 4325 kcal/kg) was used as the basal diet for the supplementation of kefir. The feed was ground into a fine powder by using a 320-μm mesh and homogenized with 0% (control, without kefir), 2, 5, or 10% kefir (dry w/w). Then, 40% water was added in order to homogenize and form a paste of the feed to facilitate pellet preparation. The feeds were then pressure pelleted with a meat grinder (2-mm die) and dried at room temperature to moisture content less than 10% for 24 h. The pelleted feeds were then ground with a mortar and pestle. They were sieved through a 2-mm mesh and stored in airtight plastic bags. Prepared feed samples were stored at +4C until used.

Experimental Design

Healthy rainbow trout (mean initial weight of 56.2 ± 6.6 g) were obtained from a commercial fish farm in Isparta, Turkey. The fish were kept in 400-L tanks and acclimated for 2 wk. They were fed twice daily with a commercial diet during this period. During the experimental period, the water quality was maintained at 12C, dissolved oxygen 7.54 mg/L, pH 7.2, and a flow rate of 1–1.5 L/min with continuous aeration. The experimental fish were divided randomly into four triplicate groups with 85 fish in each. They

were fed with the experimental and control diets three times daily for 35 d at 3% of their body weight/d. The blood samples were collected on days 1, 7, 14, 28, and 35 of the experiment.

Blood and Serum

Fish blood samples were drawn with a syringe from caudal vein at days 1, 7, 14, 21, 28, and 35. Five fish from each group were randomly selected and anesthetized with phenoxyethanol (0.01 mg/L). A portion of the blood was directly put into an Eppendorf tube, kept at 4C overnight, and then centrifuged at 3500 *g* for 15 min before serum was collected with a pipette. The serum samples were stored at −20C until assayed. A portion of the blood was taken with a heparinized syringe for the other tests.

Hematocrit Levels

Blood samples from each fish were taken into two heparinized capillary tubes. Hematocrit levels (% red blood cells) were determined after centrifugation in a microhematocrit centrifuge at 12,000 g for 5 min. Percent hematocrit values were inferred using a hematocrit reader scale and the mean value of hematocrit values of sampled blood was recorded for each fish (Kim et al. 2014).

Count of Nitroblue Tetrazolium (NBT)–Positive Cells

NBT stain (Sigma Aldrich, N-6876, Munich, Germany) was used to determine the respiratory burst activity by following a modified method described by Anderson et al. (1992). Briefly, 50 μL of blood was dropped onto a coverslip and incubated in a humid atmosphere for 30 min at 25C. NBT (Sigma-Aldrich, St. Louis, MO, USA) solution (0.2%) was freshly prepared in sterile saline solution at 0.85% (w/v). The coverslip was gently washed in 0.067 mM sodium phosphate buffer (pH 6.4) to remove the red blood cells. A drop of 0.2% NBT solution was placed onto a microscope slide and the coverslip placed face down on the NBT solution. The cells were incubated in NBT solution for 30 min at 25C. NBT-positive cells, which appeared dark blue under the microscope (×40 magnification), were counted. Five coverslips were examined from each blood sample and five random microscopic fields were counted on each slide. The 25 fields were averaged and the mean and SE of values per field of fish were calculated.

Lysozyme Activity

Lysozyme activity was detected by using the lysoplate technique. For this method, 0.60 mg/mL *Micrococcus luteus* was cast in a 1% agarose gel (Oxoid, LP0011, Hampshire, United Kingdom) with 50 mM phosphate buffer (pH 6.2). Wells (3 mm in diameter) were punctured in the agar layer, then 25 μL of the serum samples and standards were applied. The plates were incubated at 25C for 20 h, after which the diameter of the zones of inhibition were measured. The results for standards were plotted on semilogarithmic graph paper and sample values extrapolated from this standard curve (Grinde et al. 1988).

Total Leukocyte Count

Blood samples were taken from five fish per treatment group and total leukocyte counts were determined in a Neubauer counting chamber as described by Schaperclaus et al. (1991). The blood sample was diluted in a leukocyte pipet with Natt-Herrick solution. Duplicate counts were done from each blood sample.

Serum Total Protein

Serum total protein was determined by the Bradford method. Briefly, standard concentrations of bovine serum albumin (Sigma-Aldrich A 2153, Munich, Germany) in phospate-buffered saline (PBS) ranging from 0.5 to 1.0 mg/mL were prepared. Then, a standard curve was constructed by plotting the absorbance values of known protein concentrations at 595-nm wavelenght (A_{595}) using a spectrophotometer (Sharifuzzaman and Austin 2009). Serum samples (100 μL of 100-fold dilutions in PBS) were put into Eppendorf tubes, mixed with 1 mL of Bradford reagent (Sigma-Aldrich, B6916, Munich, Germany), vortexed and incubated for 2 min at room temperature. The A_{595} values were measured using disposable plastic cuvettes and

recorded. Solutions containing 100 μL of PBS and 1 mL of Bradford reagent served as blanks. Serum total protein concentrations were calculated based on the constructed standard curve.

Serum Immunoglobulin M (IgM)

Total immunoglobulin M (IgM) levels in fish serum were determined using enzyme-linked immunosorbent assay (ELISA) using a fish Immunoglobulin M (IgM) ELISA Kit (Cusabio Biotech Co. Ltd., CSB-E12045Fh, MD, USA) following the manufacturer's instructions.

Bacterial Challenges

Resistance of juvenile rainbow trout against *Lactococcus garvieae* and *Yersinia ruckeri* was tested by challenging both kefir-fed fish and control group fish to these pathogens on day 35. Before the trial, the LD_{50} of each pathogen was determined in a separate group of naive fish. The two experimental groups, consisting of 50 fish/treatment, were challenged with *L. garvieae* (4.69×10^7 cfu/mL) and *Y. ruckeri* (6.0×10^6 cfu/mL), respectively. The pathogens were administered by intraperitoneal (ip) injection. Mortalities were recorded daily over 2 wk and all dead fish and survivors examined bacteriologically to determine the presence of the pathogens. The relative percent survival (RPS) was calculated according to Amend (1981).

$$\text{RPS} = (1 - \%\ \text{mortality in experiment group} / \%\ \text{mortality in control}) \times 100$$

Statistical Analysis

All data were analyzed by one-way ANOVA using the general linear model. Duncan's Multiple Range Test was used to compare treatment means. Differences were considered significant at $P < 0.05$. All statistical analyses were carried out using SPSS Software (Version 17.0, IBM SPSS Inc., NY, USA).

Results

The effects of kefir as a dietary supplement on nonspecific immune parameters of rainbow trout are presented in Table 1. Zone of inhibition measurements in lysoplate technique indicated that the lysozyme activity was significantly increased in the serum samples of the fish fed with feed supplemented with 5 and 10% kefir until day 14 after initial treatment. No significant lysozyme activity was detected with different treatment groups. The respiratory burst activities measured by NBT-positive number of cells in 10% kefir-fed group, at days 1 and 28, and 2% kefir-fed group at day 35 was significantly higher ($P < 0.05$). This activity did not show significant difference in the other treatment groups. The comparisons made with the control group revealed that the serum total protein measurements were statistically significant on days 1, 7, and 28 in groups fed with kefir-supplemented feed. The group fed with 10% kefir had the highest serum total protein content on day 28 after initial feeding. The total number of leukocytes was significantly higher in groups fed with kefir-supplemented feed, compared with the total number of leukocytes of control groups ($P < 0.05$). However, hematocrit levels in fish fed with kefir-supplemented feed did not reveal any differences across other treatment groups ($P > 0.05$).

ELISA test results revealed an increase in the serum IgM levels in all treatment groups (Table 2). Compared with the control group, the increase was statistically significant in groups fed with feed containing both 5 and 10% kefir ($P < 0.05$) after 2 wk feeding period until end of the trial.

Among all measured nonspecific immune parameters, NBT(+) cells, total leukocyte count, serum total protein, and IgM levels in groups fed with 10% kefir-supplemented feed revealed an increase, suggesting an enhancement of nonspecific immunity in the studied group of fish. A high serum total protein measured in the serum samples obtained from kefir-fed fish, which supported the increasing of immunoglobulin level.

Fish fed with the kefir-supplemented feed were challenged with *L. garvieae* and *Y. ruckeri* at day 35. Challenge results indicated that the fish fed with kefir-supplemented preparations had better survival rates against *L. garvieae* than *Y. ruckeri*

TABLE 1. *Serum lysozyme activity (mg/mL), hematocrit level (%), NBT(+) neutrophil counts/microscopic field, total leukocyte counts ($\times 10^3$ cells/μL), serum total protein (mg/mL) in blood samples of juvenile rainbow trout fed with different rates of kefir.*[1]

		Days					
	Groups	1	7	14	21	28	35
Serum lysozyme activity (mg/mL)	2%	14.23 ± 0.43^{bB}	20.46 ± 0.46^{aA}	15.83 ± 1.87^{bB}	15.66 ± 1.52^{aB}	16.26 ± 0.54^{aB}	13.93 ± 1.50^{aB}
	5%	15.99 ± 0.19^{aCD}	20.80 ± 0.20^{aA}	18.35 ± 1.11^{abB}	18.06 ± 1.00^{aBC}	16.40 ± 0.50^{aBCD}	15.13 ± 0.46^{aD}
	10%	16.08 ± 0.36^{aC}	19.73 ± 1.17^{aAB}	20.33 ± 0.63^{aA}	16.66 ± 1.56^{aBC}	16.73 ± 0.94^{aBC}	14.66 ± 0.73^{aC}
	Control	14.11 ± 0.48^{bC}	20.13 ± 0.26^{aA}	18.63 ± 0.72^{abAB}	16.00 ± 0.80^{aBC}	16.66 ± 1.45^{aBC}	16.20 ± 0.50^{aBC}
Hematocrit level (%)	2%	39.53 ± 0.89^{bAB}	37.61 ± 1.16^{BC}	39.71 ± 0.84^{abAB}	41.07 ± 1.31^{aA}	35.86 ± 1.21^{C}	38.00 ± 0.71^{aABC}
	5%	39.21 ± 0.72^{bA}	37.13 ± 1.24^{AB}	37.64 ± 0.89^{bAB}	35.53 ± 0.77^{bB}	35.85 ± 1.12^{B}	35.40 ± 0.97^{bB}
	10%	38.13 ± 0.77^{b}	36.41 ± 1.30	37.23 ± 1.03^{b}	36.66 ± 0.97^{b}	35.21 ± 1.02	36.33 ± 0.74^{ab}
	Control	42.80 ± 0.96^{aA}	37.76 ± 0.50^{B}	41.75 ± 1.30^{aA}	37.86 ± 0.86^{bB}	35.13 ± 1.12^{B}	35.20 ± 0.60^{bB}
NBT (+) neutrophil counts	2%	7.28 ± 1.25^{abB}	5.28 ± 1.44^{aAB}	5.55 ± 1.17^{aAB}	3.03 ± 0.37^{AB}	1.82 ± 0.45^{bB}	13.96 ± 3.93^{aA}
	5%	3.94 ± 0.70^{bAB}	5.06 ± 0.65^{aA}	3.98 ± 0.95^{abAB}	4.08 ± 0.72^{AB}	2.17 ± 0.41^{bBC}	1.25 ± 0.09^{bC}
	10%	11.21 ± 2.43^{aA}	4.20 ± 0.94^{abB}	5.69 ± 1.37^{aAB}	3.54 ± 0.63^{B}	3.70 ± 0.37^{aB}	1.70 ± 0.18^{bB}
	Control	3.00 ± 0.66^{b}	2.05 ± 0.22^{b}	1.86 ± 0.48^{b}	2.73 ± 0.56	1.79 ± 0.18^{b}	1.68 ± 0.31^{b}
Total leukocyte counts ($\times 10^3$ cells/μL)	2%	48.50 ± 3.89^{aBC}	41.00 ± 1.91^{aC}	46.88 ± 3.15^{abC}	59.26 ± 5.34^{abAB}	61.08 ± 4.26^{aA}	60.50 ± 3.54^{aA}
	5%	47.70 ± 5.14^{aB}	36.40 ± 2.07^{abC}	67.22 ± 3.56^{aA}	72.36 ± 3.95^{aA}	50.50 ± 3.70^{aB}	66.31 ± 4.53^{aA}
	10%	50.25 ± 2.05^{aC}	38.53 ± 2.75^{abD}	71.38 ± 3.68^{aA}	64.27 ± 5.99^{abAB}	57.38 ± 3.85^{aBC}	59.91 ± 2.50^{aBC}
	Control	35.31 ± 1.82^{bB}	33.17 ± 1.96^{bB}	48.46 ± 2.08^{bA}	53.25 ± 4.24^{bA}	37.50 ± 3.19^{bB}	40.50 ± 1.69^{bB}
Serum total protein (mg/mL)	2%	46.64 ± 1.81^{abBC}	42.70 ± 0.70^{cC}	56.83 ± 2.15^{A}	49.02 ± 1.34^{B}	48.01 ± 1.47^{bB}	46.95 ± 2.07^{BC}
	5%	41.02 ± 3.26^{abC}	52.68 ± 3.49^{aAB}	56.42 ± 1.91^{A}	53.49 ± 3.30^{AB}	51.12 ± 2.64^{bAB}	46.34 ± 2.56^{BC}
	10%	47.90 ± 2.59^{aB}	52.63 ± 5.03^{aB}	54.53 ± 2.98^{AB}	51.02 ± 1.22^{B}	66.57 ± 11.38^{aA}	49.93 ± 2.70^{B}
	Control	40.37 ± 1.52^{bB}	43.77 ± 1.57^{abB}	54.86 ± 3.80^{A}	48.21 ± 2.29^{AB}	40.66 ± 2.32^{bB}	42.41 ± 2.66^{B}

[1]The differences between the averages shown in the different lower case letters in the same column and the different capital letters in the same line were statistically significant ($P < 0.05$).

TABLE 2. *IgM levels (μg/mL) in serum samples of rainbow trout fed with different rates of kefir.*[1]

	Days					
Groups	1	7	14	21	28	35
2%	70.00 ± 6.7^{Aa}	95.43 ± 5.1^{Aa}	118.28 ± 7.8^{Ba}	140.25 ± 5.2^{Cc}	100.78 ± 6.6^{Ab}	131.53 ± 8.8^{Cc}
5%	95.62 ± 5.5^{Ab}	114.84 ± 7.5^{Ab}	133.84 ± 8.4^{Bb}	107.89 ± 6.2^{Ab}	114.12 ± 6.8^{Ab}	115.75 ± 6.5^{Ab}
10%	135.21 ± 8.2^{Bc}	–	103.89 ± 5.6^{Aa}	113.60 ± 6.6^{Ab}	123.93 ± 8.2^{Bc}	113.37 ± 5.4^{Ab}
Control	72.84 ± 6.1^{Ba}	88.03 ± 7.2^{Ba}	105.47 ± 6.8^{Ca}	59.66 ± 9.7^{Aa}	58.93 ± 8.6^{Aa}	89.02 ± 8.2^{Ba}

[1]The differences between the averages are shown with different lower case letters in the same column and the different capital letters in the same line were statistically significant ($P < 0.05$).

TABLE 3. *Resistance of rainbow trout juveniles fed with different concentrations of kefir to* Yersinia ruckeri *and* Lactococcus garvieae.

	Groups	Challenge dose (cfu/mL)	Number of fish	Mortality (%)	RPS
Y. ruckeri	%2	4.69×10^7	50	52	–
	%5	4.69×10^7	50	48	7.69
	%10	4.69×10^7	50	48	7.69
	Control	4.69×10^7	50	52	–
L. garvieae	%2	6×10^6	50	30	40.00
	%5	6×10^6	50	36	28.00
	%10	6×10^6	50	24	52.00
	Control	6×10^6	50	50	–

RPS = relative percent survival.

(Table 3). Reduced mortality against *L. garvieae* was noticed in groups fed with 2 and 10% kefir-supplemented feed, whereas the mortality rates were not prominent in groups fed with 5% kefir-supplemented feed. Kefir supplementation did not provide any protection against *Y. ruckeri*, indicating that kefir supplementation may provide protection against Gram-positive bacteria, *L. garviea*.

Discussion

Probiotics have been widely used in aquaculture to enhance the immune system, thereby preventing diseases. Currently, commercial probiotics prepared from various bacterial species such as *Bacillus* sp., *Lactobacillus* sp., *Enterococcus* sp., *Carnobacterium* sp., and yeast are available. Kefir is one such probiotic containing *Bifidobacterium* spp. and *L. acidophilus* (Guzel-Seydim et al. 2011; Ulukoy et al. 2015). Kefir has been proven to stimulate the immune system (Furukawa et al. 1990; Osada et al. 1994). However, the number of studies on the effect of kefir on the immune systems of fish is lacking.

The measurements of nonspecific immune parameters are useful in determining the health status of fish. They are also useful in studying the components of the immune system including different type of cells (in particular leukocytes and macrophages) and their products (myeloperoxidase, superoxides, lysozyme, complement, acute phase proteins, interferons, agglutinins, properdins, and lysins). The results of this study indicated that the hematological parameters, especially total serum protein, total white blood cell counts, and NBT-positive cells were significantly increased in rainbow trout fed with diets containing kefir. Similar results (packed cell volume, hemoglobin, erythrocyte sedimentation rate, red blood cell, white blood cell, and total serum protein) have also been reported in rainbow trout (Faramarzi et al. 2011) and African catfish (Al-Dohail et al. 2009) fed with *L. acidophilus*-supplemented diet.

The lysozyme activity was significantly elevated in rainbow trout fed with diets containing kefir ($P < 0.05$). Although Panigrahi et al. (2004) showed significantly higher serum lysozyme activity in rainbow trout fed with *L. rhamnosus,* Balcázar et al. (2007) observed that the lysozyme activity did not increase in rainbow trout fed with *L. sakei.* Similarly, the lysozyme activity of grouper, *Epinephelus coioides*, fed the *L. plantarum* containing (10^8 and 10^{10} cfu/kg) diets significantly increased compared with other groups (Son et al. 2009).

Immunoglobulins are the principal components of the immune response against pathogenic organisms and IgM is a major component of the

fish humoral immune system (Uribe et al. 2011). In this study, the total immunoglobulin levels were significantly increased in rainbow trout fed with diets containing kefir. Similarly, Can et al. (2012) reported increased immunoglobulin levels in *Salmo coruhensis* fed with diets containing kefir (10 and 20 g kefir/kg fish). Consistent with our results, Al-Dohail et al. (2009) also noted that the total immunoglobulin levels were significantly better in a study conducted with African catfish fed with the *L. acidophilus*-supplemented diet. Higher plasma total Ig levels observed in rainbow trout fed with diets containing *L. rhamnosus* compared with control group also supported our findings (Panigrahi et al. 2004).

In recent years, LAB as a dietary supplement have been widely used to protect fish from various infectious diseases (Geng et al. 2012). A significant resistance against *Vibrio anguillarum* was observed in Atlantic cod, *Gadus morhua*, given feed supplemented with LAB (Gildberg et al. 1997). Similarly, Faramarzi et al. (2011) noted that survival rates against *P. aeruoginosa* were significantly increased in rainbow trout fed with the *L. acidophilus*-supplemented diet. Son et al. (2009) reported that grouper, *Epinephelus coioides*, fed with a diet containing *L. plantarum* at 10^6 and 10^8 cfu/kg had significantly higher survival rates than the control group after a challenge with *Streptococcus* sp. In addition, *L. plantarum* administration significantly decreased mortality of rainbow trout (Vendrell et al. 2008), sea bream (Carnevali et al. 2004), and Nile tilapia (Abumourad et al. 2013). In another study (Pérez-Sánchez et al. 2011) oral administration of LAB, *L. plantarum*, *L. lactis*, and *Leuconostocmes enteroides* to rainbow trout for up to 36 d resulted in significant protection ($P < 0.05$) against *L. garvieae* compared with the control group for fish fed with diet supplemented with *L. plantarum*. Araújo et al. (2015) similarly reported the effectiveness of *L. cremoris* WA2-67 to protect rainbow trout against *L. garvieae*. Our findings are consistent with these studies. Oral administration of probiotics and improved protection against pathogens might be explained by the fact that the gastrointestinal tract is a possible entrance for *L. garvieae* (Vendrell et al. 2006) and antagonistic effects of LAB may be beneficial for the control of pathogens in the gastrointestinal tract (Brunt and Austin 2005; Vendrell et al. 2008).

Nikoskelainen et al. (2001) showed that the probiotic bacterium *L. rhamnosus* (ATCC 53103) could reduce mortality of fish challenged with a virulent strain of *Aeromonas salmonicida*. Similarly, in this study, rainbow trout fed with kefir-supplemented feed were challenged (at day 35) with *L. garvieae* and *Y. ruckeri* and the fish group that was fed with 10% kefir-supplemented feed showed a better survival rate against *L. garvieae* than *Y. ruckeri*. Kefir supplementation reduced fish mortality significantly.

The increase in antibiotic resistance coupled with the negative impact of antibiotic use on the environment and the fish microflora prompted many to explore alternative means to combat bacterial fish pathogens.

In conclusion, rainbow trout fed with kefir-supplemented feed, especially the fish fed with feed containing 10% kefir had an increase in the nonspecific immune parameters. These immune parameters included the serum total protein, total white blood cell counts, and NBT-positive cells. The rainbow trout fed with 10% kefir also exhibited better protection against lactococcosis compared with yersiniosis. Therefore, kefir supplementation of feed at a rate of 10% can be suggested in rainbow trout culture to enhance the nonspecific immune system to control lactococcosis.

Further research is needed to determine the precise interactions of kefir contents and the gut flora of fish. Based on our results, kefir reduces mortality rates against lactococcosis and one line of research should look at whether kefir supplementation provides effective protection against other pathogens. Additional research is also needed to determine the economic value of using kefir as a probiotic in aquaculture as well as integration of probiotic additives to fish feed at commercial scale.

Acknowledgments

This study was financially supported by The Scientific and Technological Research Council

of Turkey (TUBITAK; grant number: 111O326). We would also like to thank Dr Huseyin Kucuktas for his valuable suggestions and critical reading of the manuscript.

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