African Journal of Biotechnology Vol. 11(30), pp. 7775-7780, 12 April, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.3448
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

The use of kefir as potential probiotic in Çoruh trout (*Salmo coruhensis*): Effects on growth performance and immunoglobulin (IgM) levels

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Accepted 18 January, 2012

The objective of this study was to evaluate the effects of three different rates of kefir on growth performance and immunoglobulin (IgM) levels of Çoruh trout (*Salmo coruhensis*). The experiment was carried out with the four following treatments: Control group (not supplemented kefir), D1, D2 and D3 (kefir supplemented diet 10, 20, 40 g kg^{-1} fish body mass, respectively). Condition factor (CF), food conversion ratio (FCR), survival rate, and specific growth rate (SGR) were monthly determined and IgM level was measured at the end of the 4 months. Survival ranged from 88.2 to 89.1%, and was independent of dietary treatments (P>0.05). The highest specific growth rate was found for the fish fed D2; although, there was no significant difference in growth parameters between the control, the fish fed D1, D2 and D3 (P>0.05). However, diets contained kefir (D1, D2) increased immunoglobulin level in *S. coruhensis* (P<0.05). It can also be concluded that kefir is crucial for fish production as a potential probiotic.

Key words: *Salmo coruhensis*, kefir, probiotic, growth, immunoglobulin (IgM).

INTRODUCTION

Using of functional foods have become a vital necessity to minimize the use of chemical drugs for treatment of some fish diseases and to reduce their effects on the fish and environment and also decrease the production costs and to obtain more environment-friendly aquaculture productions (Gatesoupe, 1999; Can, 2001; Suzer et al., 2008; Al-Dohail et al., 2009; Merrifield et al., 2010; Ekici et al., 2011).

Kefir is an acidic and mildly alcoholic fermented milk with a complex mixture of bacteria, which are confined to a matrix of discrete kefir grains. The bacteria include various species of lactobacilli, lactococci, leuconostocs and aceterobacteria and yeasts (both lactose-fermenting and nonlactose-fermenting) (Marshall and, Cole, 1985; Koroleva, 1988; Thoreux and Schmucker, 2000). Kefir also exhibits antimicrobial activity *in vitro* against a wide variety of Gram-positive and Gram-negative bacteria and some fungi (Cevikbas et al., 1994; Zacconi et al., 1995). Recently, antibacterial, immunologic and antitumor effects of kefir were studied on human beings (Lin and Change, 2000; Hoolihan, 2001; Liu et al., 2005) and some other animals, rats etc. (Furukawa et al., 1990, 1991; Zacconi et al., 1995; Güven et al., 2003; Cenesiz et al., 2008; Ozcan et al., 2009) although there is lack of information in the literature on growth performances and immunoglobulin level of kefir on the fish species.

IgM, which is an important immunoglobulin class, is important in phylogenetic research being the first immunoglobulin to appear in evolution and commonly the only immunoglobulin class described in fish (Magnadóttir, 1998). A lot of researchers have focused on this immunoglobulin class in their studies on immune system and growth performance of cultured species (Assem and El-Zaeem, 2005; Panigrahia et al., 2005; Salinas et al., 2008; Reyes-Becerril et al., 2008; Al-Dohail et al., 2009; Lim et al., 2010), and, recently, understanding of the

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Abbreviations: IgM, Immunoglobulin**; CF,** condition factor**; FCR,** food conversion ratio**; SGR,** specific growth rate.

structure and function of fish IgM has become all the more important due to the need of the fish farming industry for effective prevention and control of various fish diseases (Magnadóttir, 1998). Kefir have been reported to stimulate the immune system in both *in vitro* and *in vivo* studies (Furukawa et al., 1991; Osada et al., 1994). The immune system was stimulated in rainbow trout by several probiotics (Irianto and Austin, 2002; Raida et al., 2003; Panigrahi et al., 2005; Sharifuzzaman and Austin, 2009). The application of live probiotics may therefore result in elevated health status, improved disease resistance, growth performance, body composition, reduced malformations and improved gut morphology and microbial balance in aquaculture nowadays (Merrifield et al., 2010).

As concerns, Coruh trout (*Salmo coruhensis,* described by Turan et al., 2009) is the new culture species in Black Sea region of Turkey known as *Salmo trutta labrax* (PALLAS, 1811) in previous literature; the knowledge lack information in the literature on the effect of kefir on the growth and immune system. In this framework, the main objective of this study was to examine the effect of dietary kefir on the growth, survival and immunoglobulin (IgM) levels of Çoruh trout (*S. coruhensis*).

MATERIALS AND METHODS

Fish and experimental design

The samples of *S. coruhensis* broodstocks used in the present study were obtained from Çoruh River (Rize) population of *S. coruhensis.* This study was carried out between December 02, 2010 and March 02, 2011 for 4 months at the facility Aquaculture Department Production, Rize, Turkey.

Twelve tanks (50 L) were used and fish were equally allotted to four groups with three replicates for each treatment. Each tank contained 90 fish (9.7±0.2 g). The temperature of the incoming water was 8±2.53°C. Flow rate was 30 L min^{-1}. Oxygen saturation was always higher than 88% (measured by HQ40D multi - Hach Lange). In the present study, fish were exposed to natural photoperiod.

Four diets were prepared to investigate the effects of different levels of kefir on condition factor (CF), food conversion ratio (FCR), survival rate, specific growth rate (SGR) and IgM level in *S. coruhensis.* For this aim, one control and three experimental diets (D1, D2 and D3) were arranged. Kefir was not included to the control group; however, D1, D2 and D3 groups were supplemented with kefir at 10, 20 and 40 g kg^{-1} fish diet mass levels, respectively. The experimental diets were formulated to contain approximately 50% crude protein, 19% crude lipids, crude cellulose 3%, 12% moist and 13% ash. The experiment was carried out with three replicates for each dietary treatment. Daily tank feed was calculated as 3% of the group biomass. All groups were fed the same daily ration of commercial food (Bioaqua, standard extruder). All the fish in each tank received the same feed treatment. The amount fed to each tank was recorded. Each fish was anesthetized (Benzocaine, 50 ppm), and body weight (Wt; to 1 g), and total length (Lt; to 1 mm) were recorded at intervals of 30 days. Condition factor was calculated as (Wt Lt^{-3}) * 100. Food conversion ratio per tank was calculated as (food fed)/(biomass gain). Specific growth rate was calculated as (LnW_t-LnW_0 / t) * 100. Survival rate was calculated as (N_t / N0) * 100 (Duston et al., 2007).

Kefir and feed preparation

Raw milk was obtained from a special milk production farm daily (Rize, Turkey), and heated to 90°C for minimum of 10 min, then cooled to inoculation temperature (25°C) and 5% active kefir grains added. The inoculated milk was incubated at 22°C for 20 h (Marshall and Cole, 1985). At the end of the incubation, the grains were separated from the kefir product by filtration through a plastic sieve, washed and maintained at +4°C in the sterile drinkable water until the next culture passage. Kefir product was maintained at +4°C for 24 h and then used for microbiological and chemical analyses before feeding the fish in treatment groups. Prepared kefir was not used as feed additive if it was stored for more than 3 days (Güven et al., 2003). Prepared feeds were stored under 4°C conditions. After the feed was prepared with kefir, all 4 groups' feed was covered with fish oil at 32 ml to per kilo of feed.

Bacteriological analysis of kefir

Twenty-five milliliters of kefir product was mixed with 225 ml peptone water (Oxoid Ltd., Hampshire, UK). Tenfold serial dilutions from this homogenate were prepared in the same solution and 0.1 ml from these dilution tubes spread-plated onto separate duplicate plates. Lactobacilli were investigated by using MRS agar (Oxoid, CM361) and lactic streptococci were counted by using M17 agar (Oxoid, CM785). Selective enumeration of yeasts was specified via potato dextrose agar (Oxoid, CM 139) (Harrigan and McCance, 1976).

Sampling and measurement of blood serum immunoglobulin M (IgM)

Fish were sampled monthly for growth parameters and at the end of feeding period for enzymatic analyses. At each sampling for enzymatic analyses, three fish from each tank (nine per treatment) were taken at random. All specimens were anaesthetized by immersion in benzocaine solution (50 ppm) before blood drawing. Blood was drawn from the *vena caudalis* using an 18 G×1½ in syringe. Blood serum was obtained by blood centrifugation at 3000 rpm for 15 min.

An ELISA kit (Fish Immunoglobulin M (IgM) ELISA Kit) from Cusabio Biotech (Cat. No. CSB-E12045Fh) were used following the manufacturer's instructions to determine total IgM concentrations in serum. All tests were studied in Bilim Special Veterinary Diagnosis and Analysis Laboratory, Istanbul-Turkey.

Statistical analysis

One-way analysis of variance (ANOVA) was conducted to compare differences among dietary treatments. Overall differences were significant ($P<0.05$), Duncan's multiple range test was used to compare the mean values between individual treatment groups. All tests were performed in SPSS 15.0 software for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Survival and growth parameters

Survival ranged from 88.2 to 89.1%, and was

Table 1. Changes in mean SGR, FCR and CF of *S. coruhensis* in the control and fed D1, D2 and D3.

	Month	Control	Diet 1	Diet 2	Diet 3
SGR	1	1.61±0.15^{a,x}	1.73±0.13^{a,x}	1.82±0.12^{a,x}	1.76±0.08^{a,x}
	2	1.68±0.52^{a,x}	1.74±0.07^{a,x}	1.82±0.09^{a,x}	1.79±0.08^{b,x}
	3	1.14±0.06^{b,x}	1.18±0.08^{b,x}	1.17±0.06^{b,x}	1.16±0.02^{c,x}
	4	1.12±0.21^{b,x}	1.10±0.13^{b,x}	1.21±0.16^{b,x}	1.11±0.08^{a,x}
FCR	1	4.47±0.13^{a,x}	4.83±0.14^{a,x}	4.29±0.10^{a,x}	4.46±0.02^{a,x}
	2	3.14±0.35^{b,x}	3.11±0.16^{b,x}	2.98±0.22^{b,x}	2.71±0.51^{b,x}
	3	2.60±0.18c^{a,x}	2.52±0.20^{c,x}	2.37±0.17^{c,x}	2.12±0.62^{c,x}
	4	2.28±0.13^{c,x}	2.39±0.19^{c,x}	2.32±0.13^{c,x}	2.11±0.73^{c,x}
CF	0	0.90±0.06^{a,x}	0.91±0. 06^{a,x}	0.91±0. 05^{a,x}	0.90±0.05^{a,x}
	1	1.05±0.07^{ab,x}	1.04±0.08^{a,x}	1.03±0.08^{a,x}	1.05±0.07^{ab,x}
	2	1.08±0.07^{b,x}	1.07±0.08^{ab,x}	1.09±0.08^{a,x}	1.06±0.08^{b,x}
	3	1.03±0.07^{ab,x}	1.03±0.08^{b,x}	1.06±0.07^{a,x}	1.03±0.07^{ab,x}
	4	1.03±0.07^{ab,x}	1.04±0.06^{b,x}	1.07±0.08^{a,x}	1.04±0.08^{ab,x}

^{a,b,c} Indicate the differences among the same columns (P<0.05).
^{x,y,z} Indicate the differences among the same rows (P>0.05).

independent of dietary treatments (P>0.05). Fish fed diet with 20 mg kg^{-1} kefir (D2) showed the highest growth rate although there were no significant differences between groups (P>0.05). SGR in the control group was lower compared to the kefir induced groups but these differences were not statistically different (P>0.05) (Table 1). FCR and CF in fish fed kefir supplemented diets and the control group are presented in Table 1. FCR and CF were higher in Diet 2 compared to the other groups but the differences between groups were not statistically significant (P>0.05) (Table 1). Nevertheless, there were also significant differences during the various cycle of the production on SGR, FCR and CF by time (P<0.05).

Kefir analyses

At the end of the microbiological analysis of kefir, lactic acid bacteria, lactic streptococci and yeasts were found to be 1.0 * 10^{8}, 2 * 10^{7} and 3 * 10^{7} CFU/ml, respectively.

Blood serum immunoglobulin M (IgM)

IgM level in fish fed kefir supplemented diets and the control group are presented in Figure 1. The findings of the present study showed that the concentration of 10 and 20 g kg^{-1} kefir addition to diets caused a significant increase in IgM level in *S. coruhensis*.

DISCUSSION

There have been no comparative studies on the effect of kefir on growth and immune system of aquatic species. However, the significance of probiotic in fish production has been confirmed in several studies.

The effect of dietary probiotic on growth and survival rate depended on many factors (Gomez-Gil et al., 2000) such as species composition, application level, frequency of application and environmental conditions. Zhou et al. (2009) and Liu et al. (2010) determined that *Saccharomyce cerevisae* and *Bacillus subtilis* which are two of the microorganisms constituting the kefir grains had beneficial effects on the survival rate. The higher survival rates in the probiotic-treated group could also be attributed to their increased potential to respond to and better tolerate the harmful conditions possibly encountered in the culture tanks, probably due to higher induced HSP70 levels, as reported earlier by Carnevali (2006) in sea bream (*Sparus aurata*). Tovar-Ramírez et al. (2010) found an increase of the final mean weight of sea bass larvae fed a yeast-supplemented diet as reported by Lara et al. (2003) who suggested that yeast (*S. cerevisae*) was an appropriate growth-stimulating additive in tilapia cultivation where higher survival, SGR, PER and FCR values were obtained in probiotic treatments with *Streptococcus faecium* and *Lactobacillus acidophilus*. Similarly, Al-Dohail et al. (2009) reported that significantly better (P<0.05) growth performance was

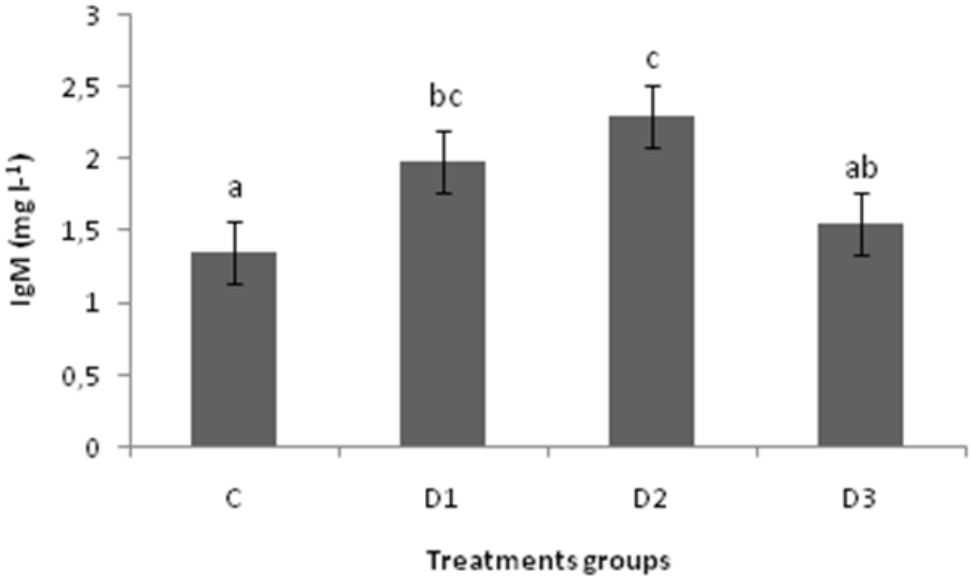

Figure 1. Changes in mean IgM levels of *S. coruhensis* in the control and fed diet D1, D2 and D3.

observed in *Clarias gariepinus* fingerling maintained on the diet supplemented with *L. acidophilus*, Similar and Carnevali (2006) who reported that growth in sea bass juvenile was significantly (P<0.05) better in the treated groups than the control when *Lactobacillus delbrueckii* was used as a probiotic via rotifer carriers and Artemia nauplii for 70 days. Wang and Zirong (2006), Noh et al. (1994), Bogut et al. (1998) and Yanbo and Zirong (2006) all reported significantly better growth performance and FCR in common carp when fed diets enriched with probiotics. In contrast, Waché et al. (2006) reported that neither survival nor growth was significantly affected by the probiotic treatment with *Saccharomyces cerevisiae* in another study, in rainbow trout *(Onchoryncus mykiss).* Similarly, on the effectiveness of commercial probiotics in northern white shrimp *Penaeus monodon* ponds Shariff et al. (2001) reported that survival rate did not increase in probiotic-induced groups compared to the control group. In the present study, there was only a rise in growth performance in Coruh trout but not at significant level by feeding dietary kefir, for a period of 4 months. Our results which are similar to those reported by Shariff et al. (2001) and Waché et al. (2006) indicated that dietary kefir did not affect growth and survival of fish. It was not possible to discriminate the contribution of yeast among the effect on growth (Gatesoupe, 2007). The growth performance may be affected by the other environmental conditional, especially by the bacterial bloom on the culture environment (Can et al., 2010). This study was conducted in winter period when the bacterial activation is low. The pathogen effects may be possible to observe due to high activation of pathogenic bacteria if the study is conducted in summer period. Moreover, the findings may change if the study repeated by enhancing microbiological challenge tests.

IgM is the main immunoglobulin present in fish (Watts et al., 2001) and probiotics also modulate various immunological parameters in teleosts (Nayak, 2010). The effects of probiotics have been reported to stimulate the immune system in both *in vitro* and *in vivo* studies (Furukawa et al., 1991; Osada et al., 1994; Irianto and Austin, 2002; Raida et al., 2003; Panigrahi et al., 2005). Assem and El-Zaeem (2005) and Panigrahia et al. (2005) suggested that increased total immunoglobulin concentration could be due to an increased immune response in the probiotic group, induced by the presence of *L. acidophilus*. The authors reported higher immunoglobulin levels in the blood plasma of rainbow trout when lactic acid bacteria *Lactobacillus rhamnosus* JCM 1136 were supplemented in the diet of the fish. Al-Dohail et al. (2009) reported that total immunoglobulin in African catfish *(Clarias gariepinus)* (Burchell, 1822) with two probiotic bacteria additives to fish diet was significantly higher (P<0.05) in fish fed the probiotic supplemented diet than in the control diet over the 12-week culture period. Reyes-Becerril et al. (2008) observed a significantly increase (P = 0.004) on immunoglobulin M level in recovered leopard groupers. In contrast, Balcazar et al. (2007) only found rise in immunoglobulin level in *Salmo trutta* but not at significant level by feeding LAB groups of probiotics, which are *Lactococcus lactis* ssp. *lactis, Lactobacillus sakei* and *Leuconostoc mesenteroides,* supplemented at106 CFU/g feed for a period of 2 weeks. Our results indicate that increased levels of serum IgM levels were detected with kefir supplemented diets (Diet 1 and 2) similarly to those

reported earlier in grouper *(Mycteroperca rosacea)* (Reyes-Becerril et al., 2008), African catfish *(Clarias gariepinus)* (Al-Dohail et al., 2009) and rainbow trout *(Oncorhynchus mykiss)* (Panigrahia et al., 2005), which were fed with basal control and probiotic supplemented diets.

Previous study by Rea et al. (1996) indicated that kefir grains contained (cfu/ml) 10^9 lactococci, 10^8 leuconostocs, 10^6 lactobacilli, 10^5 acetic acid bacteria and 10^6 yeasts. In another study, Güven et al. (2003) reported the averages of the total mesophilic aerobic colony counts, lactic acid bacteria, lactic streptococci, enterococci, and yeasts were found to be 1.04×10^9, 9.87×10^8, 4.38×10^8, 7.80×10^4 and 1.26×10^5 CFU/ml, respectively. Our findings in this study showed a bit difference. The microbial content of kefir grains depends primarily on their source. It has been reported that kefir grains contain lactobacilli, lactococci and yeast, and sometimes acetic acid bacteria, depending on the source or country of origin (Guzel-Seydim et al., 2005).

In conclusion, diets contained different levels of kefir affected the immunoglobulin concentrations in *S. coruhensis* but not growth and survival rate. Therefore, our results indicate kefir has the potential to be a promising probiotic and kefir as a probiotic can be used an integral part of the culture practices for improving growth and disease resistance. Further studies are under way to elucidate kefir effects on growth and IgM activity on aquaculture production..

ACKNOWLEDGEMENT

The authors wish to thank Dr. Ilker Zeki KURTOGLU for his efficient technical contribution towards conducting of the experiments.

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