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The Effects of Different Dosage of Kefir with Different Durations on Growth Performances and Antioxidant System in the Blood and Liver Tissues of Çoruh Trout (*Salmo coruhensis*)

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Abstract

The objective of the present work was to examine the effects of different doses (0, 10, 20 and 40 g kg^{-1} fish diet mass) of kefir on growth performance and oxidant-antioxidant status in the blood and liver tissues of Coruh trout, *Salmo coruhensis*, in different periods (2 and 3 months). In this study, survival was more than 88.3%, and irrespective of dietary kefir levels at the end of the study. There were no significant differences in SGR (Specific growth rate), FCR (Feed conservation rate), CF (Condition factor) among fish fed diets with 0, 10, 20 and 40 g kg^{-1} kefir, however, these growth values were significantly different in terms of durations between 2-month and 3-month treatment ($P<0.05$). Our results indicated that TAS (Total Antioxidant Status) and TOS (Total Oxidant Status) in blood tissues reduced with kefir treatment at the end of third month ($P<0.05$). Malondialdehyde (MDA) levels in liver decreased in all groups compared with control group and a considerable extent decrease was observed in 40 g doses of kefir treatment at the end of third month. The data obtained from this experiment indicated that the same dose of kefir was more effective at the end of 3-month treatment than 2-month treatment ($P<0.001$). Although there was no statistical difference among groups, an increase in the glutathione peroxidase (GSH-Px) enzyme activity was observed in all groups compared to control groups. While catalase (CAT) activity decreased in all groups compared to control group at the end of second month ($P<0.01$), the decrease was insignificant level at the end of third month. In glutathione (GSH) levels, statistical differences were no observed in all groups compared to the control group with 2-month treatment while there were significant increases with 3-month treatment ($P<0.001$). It was concluded that kefir could play an antioxidant role and its effectiveness depended on dosage and time of application in Coruh trout, *S. coruhensis*.

Keywords: *Salmo coruhensis*, kefir, antioxidant, probiotic, growth.

Farklı Doz ve Sürelerde Uygulanan Kefirin Çoruh Alabalıklarının (*Salmo coruhensis*) Kan ve Karaciğer Dokularındaki Oksidan ve Antioksidan Sistem ile Büyüme Performansı Üzerine Etkileri

Özet

Bu çalışmada balıklara farklı doz (0, 10, 20 ve 40 g kg^{-1} yem) ve sürelerde (2 ay ve 3 ay) kefir uygulanarak kan ve karaciğer dokusundaki oksidan-antioksidan durum ve gelişim performansı incelenmiştir. Çalışmanın sonunda, yaşama oranları % 88.3'ten fazla bulunmuş olup deney ve kontrol grupları arasında fark önemsizdir. SBO (Spesifik büyüme oranı), YDO (Yem değerlendirme oranı), KF (Kondisyon faktörü) bakımından 0, 10, 20 ve 40 g kg^{-1} kefir uygulamaları arasında farklılık önemsizken, bu gelişim değerleri 2. ve 3. aylarda süreç bazında değerlendirildiğinde birbirinden farklı bulunmuştur ($P<0.05$). 3 ay boyunca 20 ile 40 gr kefir uygulamasının balıkların kanındaki TOS'u (Total oksidan durum) ve TAS'ı (Total antioksidan durum) azalttığı gözlenmiştir ($P<0.05$). Karaciğer MDA (Malondialdehyde) düzeylerinde kontrolle karşılaştırıldığı zaman bütün gruplarda düşme görülmüş, en fazla düşüş 40 g kefirin 3 aylık uygulamasında olmuştur. Yapılan çalışmada aynı dozlardaki kefirin 3 aylık uygulamalarının 2 aylık uygulamalarından daha etkili olduğu görülmüştür($P<0.001$). GSH-Px (Glutathione peroxidase) enzim aktivitesinde kontrole göre bütün gruplarda istatistiksel olarak önemli olmayan artış gözlenmiştir. Katalaz (CAT) aktivitesi 2 aylık uygulamaların hepsinde kontrole göre anlamlı azalma gösterirken ($P<0.01$), 3 aylık uygulamalar anlamsız azalmaya sebep olmuştur. GSH (Glutathione) düzeyi 2 aylık gruplarda kontrole göre bir değişikliğe neden olmazken, 3 aylık gruplarda anlamlı artışa yol açmıştır ($P<0.001$). Sonuç olarak kefirin antioksidan özelliğinin olduğu, bunda da, dozun ve sürenin etkili olduğu görülmüştür.

Anahtar Kelimeler: *Salmo coruhensis*, kefir, antioksidan, probiyotik, gelişim

Introduction

Most of researchers recommended that feed additives can be used in order to cut down on antibiotics and some chemicals used in aquaculture applications (Gatesoupe, 1999; Can, 2001; Suzer *et al*., 2008; Merrifield, 2010; Ekici *et al*., 2011). Using of functional foods as probiotic has been increasingly become popular. The use of probiotics is an effort to contribute to the sustainable development of aquaculture products and to understand the role played in the antioxidant systems (Ramirez *et al*., 2010).

Free radicals and other reactive oxygen species (ROS) are generated by all aerobic cells (Winston and Di Giulio, 1991). To minimize the negative effects of ROS, fish like other vertebrates possess an antioxidant defense system. Main enzymatic actors in this system are SOD (superoxide dismutase), CAT (catalase), GSH-Px (glutathione peroxidase), GR (glutathione reductase), (glucose-6-phosphate dehydrogenase (G6PD) and GST (glutathione S-transferase) (Halliwell and Gutteridge, 1989; Bayir, 2005). Furthermore, TAS (Total Antioxidant Status) is a quantitative measurement which represents the total contribution from a wide range of antioxidant molecules (Prior and Cao, 1999). Numerous studies have been focused on fish antioxidant enzymes (Aras *et al*., 2009; Reyes-Becerril *et al*., 2008a). Recent studies showed that dietary probiotic stimulates antioxidant responses in juveniles of gilthead sea bream and in leopard grouper *Mycteroperca rosacea* (Reyes-Becerril *et al*., 2008a, 2008b) and in sea bass *Dicentrarchus labrax* (Ramírez *et al*., 2010). Reyes-Becerril *et al*. (2008b) reported that diet supplementation with yeast resulted in lower mortality rates by enhancing humoral and antioxidant immune responses represented by the activity of the SOD enzyme.

Kefir is acidic and mildly alcoholic fermented milk which originated in the Caucasian mountains (Koroleva, 1988). It is the product of fermentation by a mixed group of microflora confined to a matrix of discrete "kefir grains" (Marshall and Cole, 1985). The function of the microorganisms constituting the kefir grains which consist of lactobacilli, lactococci, leuconostoc, acetic acid bacteria and yeasts may include production of lactic acid, antibiotics and bactericides, which inhibit the growth of undesirable and pathogenic microorganisms in kefir milk (Angulo *et al*., 1992**).** The anti-oxidative effect of kefir was studied on human beings (Lin and Change, 2000; Hoolihan, 2001; Liu *et al*., 2005) and some animals, rats etc. (Güven *et al*., 2003; Çenesiz *et al*., 2008; Özcan *et al*., 2009) although there is lack of information in the literature on the growth performance and antioxidant system of kefir related to the aquatic species. In this framework, the main objective of this study was to examine the effect of dietary kefir as a potential probiotic on the growth, survival and the anti-oxidative status of Coruh trout, *Salmo coruhensis*.

Materials and Method

Calculations

The following variables were calculated:

Survival (%) = $100 \times N_t / N_0$

Specific growth rate = $100 \times (\mathrm{Ln}W_t - \mathrm{Ln}W_0) / t$ (SGR: $\%day^{-1}$)

Feed conversation rate (FCR) = $DF/(W_t-W_o)$

Condition factor (CF, %) = $100 \times W_t / L^3$

where N_0 and N_t represent the initial and final numbers of fish in each tank, respectively. W_t is the final body weight (g), W_0 is the initial body weight (g), and t is the experimental duration in day, respectively. DF is the dry diet intake (g). L is the body length of fish (cm).

Experimental Conditions

The juveniles of Coruh trout in the present study were originated from broodstocks that caught from Coruh River (Rize) population of *Salmo coruhensis*. This study was carried out between December 02, 2010 and February 02, 2011 for 3 months at the facility Aquaculture Department Production, Rize, Turkey.

Fish were divided into the following groups:

The control group: this group of fish served as the control (not supplemented kefir).

Diet 1 : the group fed with kefir the ration included 10 g kg^{-1} fish diet mass,

Diet 2 : the group fed with kefir the ration included 20 g kg^{-1} fish diet mass,

Diet 3 : the group fed with kefir the ration included 40 g kg^{-1} fish diet mass,

Twelve tanks (50 L) were used and fish were equally allotted to four groups with three replicates for each treatment and fed for 12 weeks. Each tank contained 90 fish (9.7 ±0.2 g). The temperature of the inflow water was 8±2.59°C. Flow rate was 30 l min^{-1}. Oxygen saturation was always higher than 88%. The photoperiod was regional winter natural cycle. All groups were fed the same daily ration of commercial trout food (45% crude protein, 19% crude lipids, 3% crude cellulose, 12% moist and 13% ash). All the fish in each tank received the same feed treatment. The amount fed to each tank was recorded.

Kefir Preparation and Bacteriological Analysis

Raw milk was obtained from a special milk production farm daily (Rize, Turkey), and heated to

90°C for minimum 10 min, then cooled to inoculation temperature (25°C) and 5% active kefir grains were added. The inoculated milk was incubated at 22°C for 20 h (adapted from 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).

For the bacterial analyses of kefir, 25 ml 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, CM139) (Harrigan and McCance, 1976).

Feed Preparation

Daily tank feed was calculated as 3% of the group biomass. Firstly, experimental diets were prepared by adding kefir and then, covered by fish oil at 32 ml to per kilo of feed. The diets were stored at 4°C before feeding.

Sampling

Body weight (W_T; to 1 g), and Total length (TL; to 1 mm) were recorded at intervals of 2 months and 3 months (at least 30 specimens from every group) after each fish was anesthetized. Blood was drawn from the *Vena Caudalis* using an 18 G×1½in Syringe (9 specimens from every group). Blood serum was obtained by blood centrifugation at 3000 rpm for 15 min, and then stored at -80°C until use. Fish were sacrificed by a blow to the head, and livers were dissected (9 specimens from every group), weighed, and immediately frozen in liquid nitrogen and then stored at -80°C until use.

Analysis of Antioxidant Parameters on Liver

To obtain 1:10 (w/v) whole homogenates the liver tissues were homogenized in glass homogenizer with a buffer containing 1.15 % potassium chloride. After homogenate centrifugation (3500 rpm, 15 min, 4°C), the malondialdehyde, glutathione concentrations and the activities of catalase, glutathione peroxidase were determined in the supernatants, immediately. The concentrations of malondialdehyde, reflecting the lipid peroxidation intensity, were measured in the supernatants using the thiobarbituric-acid reaction described by Placer *et al*. (1966) and values were expressed as nmol/g tissue. The concentration of reduced glutathione of the tissue homogenates was determined by the method of Beutler *et al*. (1963) and expressed as $\mu mol \cdot g^{-1}$ tissue. The method is based on the capacity of sulphhydryl groups present in the tissue homogenates to react with 5, 5'-dithiobis-(2-nitrobenzoic acid) (Ellmann's reagent) and form a yellow dye with maximum absorbance at 412 nm. The glutathione peroxidase activity was specified as reported by the method of Lawrence and Burk (1976) and expressed as U/g protein for liver tissue. The liver tissue catalase activity was determined by measuring the decomposition of hydrogen peroxide at 240 nm, according to the method of Aebi (1983) and was expressed as katal/g protein. In parallel, the protein concentration was also measured in the supernatants by using the method of Lowry *et al*. (1975).

Analysis of Antioxidant Parameters on Blood

TAS and TOS were analyzed using Total Antioxidant Status (TAS) Assay Kits and Total Oxidant Status (TOS) Assay Kits (Rel Assay Diagnostics, Turkey) by an automated chemistry analyzer (Roche Cobas MİRA Plus) as reported by the Emecen *et al*. (2010).

Statistical Analysis

One-way analysis of variance (ANOVA) was conducted to compare differences among dietary treatments "except significance of durations on growth parameters (T test was used). When overall differences were significant ($P<0.05$, 0.01, 0.001), 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

Final Balance Sheet of Production

Survival ranged from 88.3% to 89.3%, and was independent of dietary treatments (Table 1). Fish fed the diet with 20 g kg^{-1} kefir showed the highest body weight after 3 months while fish fed the diet with 40 g kg^{-1}kefir had the highest value after 2 months. But, there were no significant differences. However, body weights at the end of second month were significantly different compared to third month ($P<0.05$) (Table 1).

Table 1 Survival and body weights of Coruh trout fed diets containing different concentrations of dietary kefir for 2 and 3 months.

SGR, FCR and CF in fish fed kefir supplemented diets and the control group are presented in Table 2. SGR in fish fed the diet containing 0 g kg^{-1} kefir (control) was lower than the

Table 1. Survival and growth performance of Coruh trout fed diets containing different concentrations of kefir for 2 and 3 months.

Diet no. (Kefir level)	Initial body weight (g) (mean±SE)	2 month-body weight (g) (mean±SE)	3 month-body weight (g) (mean±SE)	Survival (%)
Control (0)	9.68 ±0.08^{a,x}	28.76 ±2.12^{a,y}	39.97±2.44^{a,z}	88.3^{a}
Diet 1 (10)	9.71 ±0.17^{a,x}	30.64 ±1.80^{a,y}	41.88±1.49^{a,z}	88.7^{a}
Diet 2 (20)	9.68 ±0. 25^{a,x}	31.28 ±1.94^{a,y}	43.51±1.08^{a,z}	89.0^{a}
Diet 3 (40)	9.68 ±0.33^{a,x}	31.33 ±2.38^{a,y}	42.83±1.55^{a,z}	89.3^{a}

^{a,b,c} indicate the differences among the same columns (P>0.05, Duncan's test).
^{x,y,z} indicate the differences among the same lines (P<0.05, Duncan's test).

Table 2. SGR, FCR and CF of Coruh trout fed diets containing different concentrations of kefir for 2 and 3 months

Diet no. (Kefir level) [1]	2-month SGR [1]	3-month SGR [1]	2-month FCR[2]	3-month FCR[2]	2-month CF[3] (%)	3-month CF[3] (%)
Control (0)	1.81±0.14 ^{a,x}	1.65±0.21 ^{a,x}	3.22±0.44 ^{a,x}	2.26±0.14 ^{a,y}	1.08±0.07 ^{a,x}	1.03±0.08 ^{a,y}
Diet 1 (10)	1.92±0.18 ^{a,x}	1.70±0.12 ^{a,y}	2.78±0.13 ^{a,x}	2.41±0.21 ^{a,y}	1.07±0.08 ^{a,x}	1.03±0.07 ^{a,y}
Diet 2 (20)	1.95±0.15 ^{a,x}	1.75±0.17 ^{a,y}	2.92±0.12 ^{a,x}	2.34±0.14 ^{a,y}	1.09±0.08 ^{a,x}	1.06±0.07 ^{a,y}
Diet 3 (40)	1.96±0.13 ^{a,x}	1.73±0.09 ^{a,y}	2.75±0.40 ^{a,x}	2.09±0.73 ^{a,y}	1.06±0.08 ^{a,x}	1.03±0.07^{a,y}

^{a,b,c} indicate the differences among the same columns for SGR, FCR and CF, separately, at different dosages (P>0.05).
^{x,y,} indicate the differences among the same lines for SGR, FCR and CF, separately, at different durations (P<0.05).
[1]SGR: specific growth rate, [2]FCR: food conversation rate, [3]CF: condition factor

Diet 1, 2 and 3 treatments while the Diet 2 had the highest SGR after 3 months, but there was no significant difference among groups. The effect of different dosage of kefir on SGR was affected by time (P<0.05) The lowest FCR was in the fish fed the Diet 3 at the end of 2 and 3 months although there were no significant differences. Significant differences were obtained in the treatment groups and control group by time (P<0.05). The highest CF was identified in the Diet 2 at the end of third month. But, condition factor was not significantly affected by time.

Table 2 SGR, FCR and CF of Coruh trout fed diets containing different concentrations of dietary kefir for 2 and 3 months.

Antioxidants and Kefir Analyses

The mean liver MDA, GSH-Px, CAT and GSH values in the control and kefir groups were presented in Table 3. MDA levels on liver tissues in the kefir treatment groups were determined significantly lower compared to the control group and a considerable extent decrease has been seen in 40 g doses of kefir treatment at the end of 3 months. The data from this experiment indicated that this dose of kefir was more effective in 3-months than 2-months treatments (P<0.001). GSH-Px enzyme activity in kefir groups increased compared to the control group although no significant differences were recorded in GSH-Px enzyme activity. CAT activity decreased in kefir groups compared to the control group for 2 months (P<0.01), the decrease was insignificant for 3 months. For GSH levels in kefir treatment groups, statistically differences were not observed compared to the control group at the end of second month but there were significantly differences at the end of third month (P<0.001).

Table 3 Main effects of different level of dietary kefir on activity of antioxidants in Coruh trout fed diets for 2 and 3 months.

TAS and TOS in blood in the control and kefir groups were presented in Table 4. TOS in the Diet 2 and the Diet 3 exhibited a significantly lower levels compared to the control group (P<0.05). TAS in the Diet 3 was also different from the other groups except the Diet 2 group (P<0.05).

Table 4. Main effects of different level of dietary kefir on TOS and TAS activity in Coruh trout fed diets for 3 months

At the end of the microbiological analysis of kefir, lactic acid bacteria, lactic streptococci and yeasts were found as 1.0 x 10^8, 2 x 10^7, and 3 x 10^7 CFU ml^{-1}, respectively.

Discussion

The dietary kefir effects on *Salmo coruhensis* have yet to be examined. Although effect of kefir was studied on human beings and some animals produced in culture conditions, there is lack of information in the literature about the effect of kefir on the growth performance and antioxidant system of Coruh trout and the other aquatic species. Nevertheless, there were a lot of studies about probiotic applications in aquatic species.

The effect of dietary probiotic on growth and survival rate depended on many factors (Gomez-Gil *et al*., 2000) such as species composition, application

Table 3. Main effects of different level of dietary kefir on activity of antioxidants in Coruh trout fed diets for 2 and 3 months

Group	MDA (nmol/g tissue)	GSH-Px (U/g protein)	CAT (k/g protein)	GSH (µmol/g tissue)
Control (2-month)	38.17±8.13^{c}	2.61±0.59^{a}	358.91±50.12^{c}	111.96±33.39^{a}
Diet 1 (2- month)	25.62±3.78^{b}	3.99±1.024^{a}	222.14±38.45^{ab}	114.89±20.96^{a}
Diet 2 (2-month)	25.36±1.37^{b}	5.67±0.47^{a}	216.08±37.87^{ab}	153.56±18.21^{a}
Diet 3 (2-month)	18.15±1.77^{ab}	4.34±0.44^{a}	164.68±34.29^{a}	105.77±21.48^{a}
Control (3-month)	39.79±9.33^{c}	2.57±0.66^{a}	360.60±50.12^{c}	113.20±27.45^{a}
Diet 1 (3-month)	15.42±1.31^{ab}	5.68±0.99^{a}	299.56±32.99^{bc}	219.52±13.49^{b}
Diet 2 (3-month)	12.97±0.95^{ab}	4.70±0.75^{a}	333.23±33.94^{bc}	266.00±13.54^{b}
Diet 3 (3-month)	11.94±0.81^{a}	5.28±0.99^{a}	258.48±35.55^{abc}	226.28±9.19^{b}
P	<0.001	>0.05	<0.01	<0.001

Values in the same column with the same letters are not significantly different (Duncan's test).

Table 4. Main effects of different level of dietary kefir on TOS and TAS activity in Coruh trout fed diets for 3 months.

Group	TOS (mmol/L)	TAS (µmol/L)
Control	25.96±5.53^{a}	0.33±0.02^{a}
Diet 1	27.23±3.49^{a}	0.38±0.03^{a}
Diet 2	15.06±2.25^{b}	0.30±0.02^{ab}
Diet 3	12.61±2.80^{b}	0.24±0.03^{b}
P	<0.05	<0.05

Values in the same row with the same letters are not significantly different (P<0.05, Duncan's test).

level, frequency of application and environmental conditions. In a study relation to effect of probiotic on larvae shrimp *(Penaeus vannamei)* based on water quality, survival rate and digestive enzyme activities He *et al*. (2009) determined that *S. cerevisae*, which is one of the microorganisms constituting the kefir grains, had beneficial effects on the survival rate. In a different study on the effectiveness of commercial probiotics in northern white shrimp *Litopenaeus vannamei* ponds Liu *et al*. (2010) determined that probiotics were able to inhibit the growth of *Vibrio* resulting in an improved shrimp larvae survival rate. On the contrary, Shariff *et al*. (2001) found that treatment of *P. monodon* with a commercial *Bacillus probiotic* did not significantly increase survival. The results obtained in the present study indicated that survival rate was not significantly affected by kefir as reported by Shariff *et al*. (2001). The survival rate can be affected by other environmental conditions, 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 growth performance could be affected by high activation of pathogenic bacteria if the study would be conducted in summer period when the bacterial activation is high. However, it is difficult to directly assess different studies using probiotics, because the efficacy of a probiotic application depended on many factors as reported by Gomez-Gil *et al*. (2000).

Ramírez *et al*. (2010) found an increase of the final mean weight of sea bass larvae fed a yeast-supplemented diet, compared to the control group. Lara Flores *et al*. (2003) suggested that yeast was an appropriate growth-stimulating additive in tilapia cultivation. 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)*. Our results indicated that SGR, FCR and CF in kefir-induced groups, there were no significant differences observed among groups in similar to reported by Waché *et al*. (2006). Gatesoupe (2007) reported that it was not possible to discriminate the contribution of yeast among the effect on growth.

The reactive oxygen species, which are produced under usual oxidative damage, can be eliminated by the body's antioxidant system (Halliwell and Gutteridge, 1989). The antioxidant capacity that TAS expresses includes enzymatic and non-enzymatic antioxidant activities. Wang *et al*. (2006) reported that the higher TAS value, the higher antioxidant capacity it has. However, our results indicated that TAS and TOS in blood tissues reduced with kefir treatment at the end of third month. Decreasing in TAS may be explained by declining activity of antioxidants partially during the detoxification of the oxidants. Diseases can increase free radical damage on fish but no disease symptom was observed during this study. On the other hand, decreased levels of the TOS level in Kefir-induced groups showed that kefir has an antioxidant role in present study. During the oxidative stress, counter-acting antioxidant system plays role for gene expression of protective enzyme and the synthesis of other antioxidant molecules. To defense against the reactive oxygen species the ability of TAS decreases

and maybe according to these levels decline. Use of probiotics may prevent the consumption of cell's antioxidant systems (Castex *et al*., 2010).

In present study, CAT activity, which is one of the antioxidant enzymes, decreased in treatment groups. Indeed, the reduction of antioxidant enzymatic activities were, in some cases, associated with decreased oxidative stress and free radical activities. These were based on the fact that the lower oxidative stress and the fewer antioxidant enzymes were produced (Rahmat *et al*., 2006). In a study on the effect of kefir on damage of carbon tetrachloride in rat, Güven *et al*. (2003) found that GSH-Px enzyme activity increased while MDA levels, which increased with carbon tetrachloride, was decreased by kefir. In a different study related to effect of probiotics on oxidative stress and antioxidant status, Castex *et al*. (2009) determined that CAT activity in digestive gland was decreased by probiotic and GSH-Px enzyme activity increased although GSH level was not affected by probiotic. In this study, MDA levels statistically decreased, but GSH-Px enzyme activity insignificantly increased. The results at the end of 2 months were similar to reported by Castex *et al*. (2009). By contrast in GSH levels, the increase was observed at the end of third month.

It was concluded that kefir could play an antioxidant role and its effectiveness depended on dosage and time of application in Coruh trout, *S. coruhensis*. Therefore, the results showed that kefir had the potential to be a promising probiotic. Further studies are under way to elucidate kefir effects on growth performance and antioxidant system.

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