Mar. Sci. Tech. Bull. (2015) 4(2):21-28
ISSN: 2147-9666

MARINE SCIENCE AND TECHNOLOGY BULLETIN

Effects of different probiotic bacteria on growth, body composition, immune response and hematological parameters of rainbow trout (*Oncorhynchus mykiss*) under sublethal water temperature.

Irfan Selcuk Yazici[1], Olcay Hisar[1]*, Sevdan Yilmaz[2], Murat Yigit[2]

[1]*Department of Basic Sciences, Marine Sciences and Technology Faculty, Canakkale Onsekiz Mart University, Canakkale, Turkey*
[2]*Department of Aquaculture, Marine Sciences and Technology Faculty, Canakkale Onsekiz Mart University, Canakkale, Turkey*

ARTICLE INFO

Article history:

Received: 02.12.2015
Received in revised form: 29.12.2015
Accepted : 30.12.2015
Available online : 26.01.2016

Keywords:

Probiotics
Rainbow trout
Bacillus
Lactobacillus
Growth
Immune response

ABSTRACT

The aim of this study was to evaluate the effects of probiotic bacteria *Lactobacillus plantarum*, *Bacillus subtilis* and their mix additives on growth performance, body composition, immune response and hematological parameters of rainbow trout (*Oncorhynchus mykiss*) under sublethal water temperature. Feeding trial was carried out for 60 days. The first group was fed with commercial feed plus fish oil. The second and third groups were given commercial feed plus *Lactobacillus plantarum* and *Bacillus subtilis* at a rate of 10^7 CFU g^{-1} fish oil, respectively. The fourth group was also fed with commercial feed plus 50% of both probiotic bacteria. After 60 days, there were no differences among the survival rates and body proximate composition of experimental groups. Results also showed that all additives (*L. plantarum*, *B. subtilis* and their mix) to diets had no significant effect on growth parameters of rainbow trout, although the fish fed the diet with *L. plantarum* had statistically better feed conversion ratio (FCR) compared to the other groups ($p<0.05$). However, the additives could improve the immunity and alter hematological parameters of the rainbow trout. In conclusion, it might be suggested that *L. plantarum* can be used as a beneficial dietary probiotic in rainbow trout reared under sublethal water temperature.

Introduction

Trout culture has been started in Turkey since early 1970s, however its major development began during the 1990s with the extension of freshwater farms and deployment of offshore type cages in the Black Sea region. Today, with an annual trout production of 128.059 tons in 2013, Turkey became the top producer of trout in Europe. Out of the total, over 122.873 tons of portion size trout is being produced in fresh water farms, while the smaller amount of 5186 tons comes from the marine cage farms producing larger fish (FAO 2013). With the increasing production by expanding into new directions, intensifying and diversifying (Aras Hisar 2003), the higher disease susceptibility of the cultured organism due to deterioration of water quality and elevation of the other stressful conditions emerge (Subasinghe 2005).

Fish diseases are one of the serious factors limiting the development of the aquaculture industry and antibiotic therapy has been the most common strategy to overcome this problem (Austin and Austin 1999). However the use of antibiotics in fish treatment increases the development of antibiotic resistance in aquatic bacteria, hence it is restricted in many countries (Zilberg et al. 2010). Besides, the residual antimicrobials in aquatic products cause problems for human health (Caruso et al. 2013). Nowadays, several environment-friendly prophylactic and preventive methods such as the use of probiotics and prebiotics are being developed to control disease and maintain a healthy microbial aquatic environment (Verschuere et al. 2000).

Probiotic is defined as any microbial adjunct that has a beneficial effect on the host through an improvement in the use of feed or by enhancing the host response to disease

*Corresponding author
E-mail address: o_hisar@hotmail.com (O. Hisar)
Tel:+: +90 286 218 05 42 fax: +90 286 218 05 43

(Irianto and Austin 2002; Kesarcodi-Watson et al. 2008; Abdel-Tawwab et al. 2008;). Currently, the most common probiotics used in aquaculture belong to *Saccharomyces* sp., *Bifidobacterium* sp., *Vibrio* sp., *Enterococcus* sp., *Bacillus* sp. and *Lactobacillus* sp. (Nayak 2010).

It is well known that the optimal water temperature for the best growth of trout is between 10-17°C (Ineno et al. 2005). However, water temperatures in trout farms where surface water temperature rises to critical levels of 25-26°C (Molony 2001) during the summer period, causes serious problems for the farmers. In those cases, fish farmers have to either harvest the fish prior to the summer period or submerge their fish cages at deeper water layers where a relatively lower water temperature would be available, which then might increase the operating costs at serious levels.

Previous studies were conducted to determine the effects of *Lactobacillus plantarum* and *Bacillus subtilis* in fish species (Nayak 2010; Andani et al. 2012). In these investigations, some parameters such as growth performance and immune response on gut flora were evaluated. However, to our knowledge so far, there is no published information concerning the effects of *L. plantarum* and *B. subtilis* in trout under sublethal water temperatures. Therefore, this study was performed to investigate the effect of probiotic bacteria *L. plantarum*, *B. subtilis* and their mix additives on growth performance, body composition, immune response and hematological parameters of rainbow trout (*Oncorhynchus mykiss*) under sublethal water temperature

Material and Methods

Diet preparation

Oil-free pellet feeds (protein 41-44%) obtained from a commercial fish feed company in Turkey were used in the experiment. Experimental feeds were prepared by adding the different lyophilized probiotic bacteria which were solubilized in fish oil. Four experimental groups consisted of a control group (the group without any probiotics), a *L. plantarum* $1x10^7$ cfu/g group, a *B. subtilis* $1x10^7$ cfu/g group, and a mix additive group of *L. plantarum* $0.5x10^7$ cfu/g + *B. subtilis* $0.5x10^7$ cfu/g group.

Experimental design

The study was conducted at Dardanos Marine Aquaculture Research and Development Center of Canakkale Onsekiz Mart University. Two hundred and forty rainbow trout (mean weight of 12 g) were obtained from a local fish farm and after an acclimatization period of 10 days to the rearing conditions, experimental fish were randomly divided into four groups of triplicate tanks. Water was changed daily at a rate of ~10% of the total volume. Experimental diets were given to fish at 3% of their body weights and feeding was conducted three times a day at 08:00, 12:00, and 16:00 for a total course of 60 days. Feeding rate was recalculated and determined after weighing of fish at 10 days intervals.

Growth performance analyses

The formulas used in determining growth parameters (Bulut et al. 2014);

FCR (feed conversion ratio) = feed intake (g)/ weight gain (g)

SGR (specific growth rate) = 100 [(ln final fish weight (g) - (ln initial fish weight (g)] / experimental days

Whole-body proximate composition

Proximate analyses of the fish were performed using standard methods (AOAC 2000). Moisture was analyzed by drying at 70°C in an oven to a constant weight, crude protein by the Kjeldahl method, and crude ash by incineration at 525°C in a muffle furnace for 12 h. Crude fat was analyzed by methanol/chloroform extraction.

Haematological and blood biochemical analyses

In each experimental period, 3 fish from each tank (9 fish/group in total) were used for blood sampling. The fish were anaesthetized with clove oil which is a natural product. The blood samples were taken without harming the fish by entering from caudal vena with plastic injectors of 5 ml as soon as possible after properly cleaning the back part of anal fin with alcohol in order to prevent mucosa from mixing with blood. The blood samples were put into K3EDTA and serum tubes with jelly. Hematologic, immunologic and biochemical analyses were carried out on blood samples

Haematological analyses were carried out with methods which are routinely used in fish (Blaxhall and Daisley 1973). The total erythrocyte count was calculated by using Thoma slide and by diluting the blood sample with modified Dacie's solution at a rate of 1/200 after taking it into erythrocyte pipette for the count. Microhematocrit method was used in measuring the haematocrit. Haematocrit tubes were filled with blood and were centrifuged for 5 minutes at 10500 g revolution in haematocrit centrifuge and then the readings were measured in haematocrit % value by using a scale. Cyanomethemoglobin method was used for the determination of haemoglobin count. For this purpose, 20 µl blood samples was put into 4 ml Drapkin's solution and then, after an incubation of 10 minutes, the mixture was read at 540 nm and the results were evaluated in g/dl. Differential leukocytes were examined with May-Grunwald-Giemsa stained peripheral blood smears. Each slide was examined under oil-immersion at 100X. For each slide, 100 leukocytes were identified as lymphocytes (LYM), neutrophils (NEU) and monocytes (MON). White blood cells (WBC, 103 mm3) were also counted in blood smears using the indirect method (number of leukocytes in the blood smear 9 erythrocytes quantified in the haemocytometer/7000 erythrocytes in the blood smear) (Yılmaz et al. 2014).

The biochemical parameters glucose (GLU), albumin (ALB), globulin (GLO), total protein (TPROT), triglyceride (TG) and cholesterol (CHO) were determined in the experiment. The analyses were made with spectrophotometer by using kits (Bioanalytic Diagnostic Industry) after the blood serum was separated (Yılmaz et al. 2014).

Immunologic analyses

The respiratory burst (Stasiak and Bauman 1996), lysozyme (Nudo and Catap 2011) and myeloperoxidase (Quade and Roth 1997) activities analyzed by the methods reported in the literature.

Statistical analyses

While Tukey multiple comparison test was used when the data was normally distributed and homogeneous, Kruskal-Wallis Z test was used when the data was not normally distributed and not homogeneous in evaluating the relationships between data obtained from experimental groups. Statistical analyses were carried out using SPSS 17 and NCSS 2007 statistical software.

Results

The growth parameters, feed conversion rate and survival rate of the fish were presented in Table 1. There was no significant effect of diets on survival rate of fish. Although the average final weights of fish in the four experimental groups were statistically similar ($P > 0.05$), the feed conversation rates of fish fed diet containing *L. plantarum* were significantly better than those of the other treatment groups ($P < 0.05$). Analysis of body proximate composition (Table 2) also revealed that moisture, crude protein and ash were unaffected ($P < 0.05$) by the treatments applied in the study.

Although RBC count and Hct, Hgb and LY values were the lowest in fish fed the diet containing *B. subtilis*, WBC count and GR values were the highest in fish fed the diet with *B. subtilis* in our trial. However MO values did not differ among the four experimental groups (Table 3).

From the findings of the present study, it was noted that the serum glucose levels in fish fed diets containing *B. subtilis* were statistically lower than those fed the control diet on day-60, while the cholesterol levels in fish fed diets with *B. subtilis* were statistically lower on day-30 of the experiment ($P < 0.05$). Moreover Albumin ratio in fish fed diets containing *B. subtilis* showed statistically lower values than the other experimental groups only at day-30 ($P < 0.05$). However, approaching the end of the experiment at day-60, it was recorded that the total protein, albumin, globulin ratio and triglyceride amount did not differ among the four experimental groups ($P > 0.05$) (Table 4).

In this study, lysozyme activity was similar in all treatment groups. However, it was found that RBA activity in fish fed diets containing probiotics were statistically higher than that of fish fed the control diet ($P < 0.05$). In addition, myeloperoxidase activity was statistically higher in fish fed diets containing *L. plantarum* and *L. plantarum*+ *B. subtilis* than those fed the control diet ($P < 0.05$) (Figure 1).

Discussion

Growth rate, an important factor in aquaculture reflecting the production yield, is influenced by the environmental effects, genetic factors and by the quantity and quality of food (Oduleye 1982). In recent years, there has been great interest in the use of probiotics in aquaculture, and *B. subtilis* and *L. plantarum* probiotics have been shown to improve growth performance and modulate intestinal microbiota in different fish species (Vendrell et al. 2008; Merrifield et al. 2010; Son et al. 2009)

In present study, although different probiotic supplementations to fish diet did not significantly affect survival rate or growth performance, fish fed the diet with *L. plantarum* showed a lower FCR value than those fed diets with *B. subtilis* and the control diet. Similarly to our findings in the present study, Merrifield et al. (2010) recorded that BioPlus 2B® (*B. subtilis* + *B. licheniformis*) which was added to fry feeds had no effect on the growth performance. It was also reported in another study that supplementation of *L. plantarum* to trout feed did not change growth performance (Vendrell et al. 2008). In addition Günther and Jimenez-Montealegre (2004) also found that *B. subtilis* added in the feed does not improve growth of tilapia, in fact an adverse effect was observed. In contrast, however, Giri et al. (2014) reported that growth performance of *Labeo rohita* fish which were fed with diets containing *B. subtilis*, *B. subtilis*+*L. plantarum* and *B. subtilis*+*P. aeruginosa*+*L. plantarum* improved better compared to the control group. Similarly, Son et al. (2009) showed that dietary supplementation of *L. plantarum* improved growth performance of *Epinephelus coioides* fish. These results suggest that *B. subtilis* and/or *L. plantarum* supplementations play a role in enhancing feed intake with a subsequent enhancement of FCR values.

The data from fish body composition analysis showed that there were no significant differences in moisture, ash and crude protein of muscle in fish fed experimental diets. These results agree with the findings of Merrifield et al. (2010), who reported that muscle composition was not affected by dietary yeast as a probiotic. However, in another study conducted on trout, it was determined that supplementation of *B. subtilis* + *B. licheniformis* increased the protein amount and reduced fat and moisture amount which are among body proximate composition values (Bagheri et al. 2008). The differences among studies could be attributed to the fish size differences and the probiotic amounts added to the diets.

Lysozyme activity was similar in all treatment groups in our experiment. However, it was found that RBA activity in fish fed diets containing probiotics were statistically higher than that of fish fed with the control diet. In addition, myeloperoxidase activity was statistically higher in fish fed diets containing *L. plantarum* and *L. plantarum* + *B. subtilis* than those of the control group. Similarly, it was determined that supplementation of BioPlus 2B® (*B. subtilis* + *B. licheniformis*) to trout feeds did not change lysozyme activity (Merrifield et al. 2010). In addition, the serum lysozyme and RBA activity of fish fed the diet containing *L. plantarum* VSG3 were significantly higher than those of fish fed the control diet (Giri et al. 2014). MPO activities were not significantly affected by the dietary *B. subtilis* supplementations in olive flounder (Cha et al. 2013).

The changes in RBC, Hct and Hgb amounts among the hematologic parameters are important indicators each in

Table 1. Growth performance in fish fed experimental diets.

	Diets			
	Control	*L. plantarum*	*B. subtilis*	*L. plantarum+B. subtilis*
Initial weight (gr)	11.6 ± 0.6	11.6 ± 0.2	11.8 ± 1.1	11.7 ± 0.4
Final weight (gr)	36.6 ± 0.3	39.3 ± 0.9	38.3 ± 2.2	39.4 ± 1.5
Feed conversion ratio (FCR)	1.2 ± 0.0^{a}	1.0 ± 0.0^{b}	1.15 ± 0.0^{a}	1.1 ± 0.0^{ab}
Specific growth rate (% day^{-1})	1.9 ± 0.1	2.0 ± 0.0	2.0 ± 0.1	2.0 ± 0.0
Survival	100	100	100	100

Values are provided as mean ± standard error (n=10). Values with different superscripts in horizontal row are significantly different (α=0.05).

Table 2. Proximate composition in rainbow trout fed with experimental diets for 60 days. Values are provided as mean ± standard error (n=10 per treatment).

	Diets			
	Control	*L. plantarum*	*B. subtilis*	*L. plantarum+B. subtilis*
Moisture (g kg^{-1})	71.3 ± 1.1	71.3 ± 1.3	70.9 ± 0.9	70.9 ± 0.8
Protein (g kg^{-1})	17.9 ± 0.8	16.0 ± 0.9	16.7 ± 1.1	16.2 ± 0.6
Fat (g kg^{-1})	7.6 ± 0.68	8.2 ± 0.4	7.9 ± 0.5	8.4 ± 0.6
Ash (g kg^{-1})	2.3 ± 0.3	2.7 ± 0.1	2.8 ± 0.2	3.0 ± 0.3

Table 3. Hematological parameters and white blood cell types for rainbow trout fed diets containing different probiotic supplementations diet for 60 days.

	Control	*L. plantarum*	*B.subtilis*	*L.plantarum+B.subtilis*
RBC (x 10^{6} mm^{-3})	1.7 ± 0.1^{a}	1.7 ± 0.1^{a}	1.3 ± 0.2^{b}	1.6 ± 0.0^{ab}
WBC (x 10^{3} mm^{-3})	44.5 ± 0.3^{b}	44.2 ± 0.4^{b}	55.8 ± 5.6^{a}	45.4 ± 1.3^{b}
Hgb (g dL^{-1})	9.3 ± 0.6^{a}	7.8 ± 0.5^{ab}	6.9 ± 0.3^{b}	7.8 ± 0.4^{ab}
Hct (%)	28.9 ± 0.6^{a}	24.5 ± 1.4^{ab}	21.6 ± 1.1^{b}	24.2 ± 1.2^{ab}
LY (%)	56.4 ± 1.9^{a}	55.0 ± 1.8^{a}	47.6 ± 2.4^{b}	60.5 ± 2.4^{a}
MO (%)	14.8 ± 0.2	14.9 ± 0.1	16.1 ± 1.0	14.6 ± 0.4
GR (%)	28.8 ± 1.7^{b}	30.0 ± 1.7^{ab}	35.4 ± 1.3^{a}	25.0 ± 0.8^{b}

Values are provided as mean ± standard error (n=5). Values with different superscripts in horizontal row are significantly different (α=0.05).

Table 4. Serum biochemical parameters for rainbow trout fed diets containing different probiotic supplementations diet for 30, and 60 days.

		Control	*L. plantarum*	*B.subtilis*	*L.plantarum+B.subtilis*
Glucose (g/dL)	30th day	65.8 ± 1.5	69.5 ± 1.4	66.4 ± 2.0	67.8 ± 3.2
	60th day	98.8 ± 6.2^{a}	85.6 ± 4.4^{ab}	79.5 ± 5.1^{b}	86.0 ± 2.1^{ab}
T.protein (g/dL)	30th day	10.7 ± 0.5	9.9 ± 0.6	8.4 ± 0.6	9.8 ± 0.6
	60th day	9.5 ± 0.7	9.3 ± 0.4	8.7 ± 0.2	10.0 ± 0.5
Albumin (g/dL)	30th day	0.4 ± 0.0^{b}	0.4 ± 0.0^{b}	0.3 ± 0.0^{a}	0.4 ± 0.0^{b}
	60th day	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
Globulin (g/dL)	30th day	10.3 ± 0.5	9.48 ± 0.63	8.08 ± 0.60	9.44 ± 0.57
	60th day	9.1 ± 0.6	8.96 ± 0.36	8.31 ± 0.21	9.56 ± 0.50
TRI (mg/dL)	30th day	119.7 ± 13.7	109.4 ± 6.6	105.3 ± 11.4	87.3 ± 10.0
	60th day	126.0 ± 10.0	107.13 ± 8.2	115.90 ± 7.8	113.60 ± 16.5
CHOL (mg/dL)	30th day	296.6 ± 8.8^{b}	259.7 ± 9.8^{ab}	245.9 ± 12.2^{a}	238.9 ± 10.4^{ab}
	60th day	274.7 ± 16.6	235.0 ± 11.9	287.9 ± 18.2	251.1 ± 26.0

Values are provided as mean ± standard error (n=5). Values with different superscripts in horizontal row are significantly different (α=0.05).

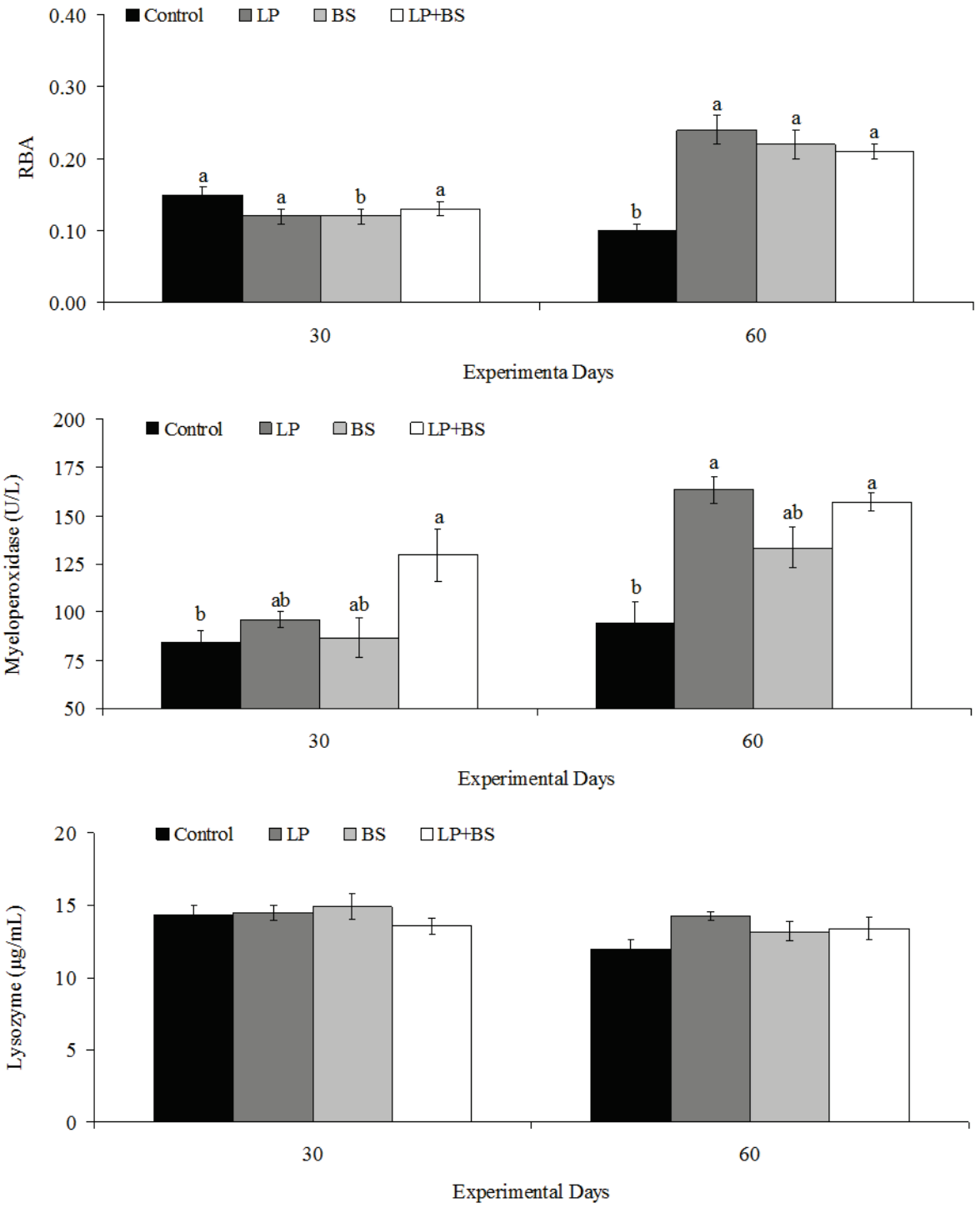

Figure 1. Changes of some immunological parameters in rainbow trout fed with probiotic supplemented diets compared to control diet on days 30, and 60. Asterisks indicate significant differences between treatments ($P < 0.05$).

determining the health conditions of fish and their stress or disease status (Campbell 2004; Başusta 2005). It was found in our study that RBC count was statistically lower in fish fed with feeds containing *B. subtilis* than fish fed the control diet and feeds containing *L. plantarum* and Hct and Hgb counts in fish fed with probiotic supplements were statistically lower than fish fed with control feed. Normally, this change occurs in the presence of a pathogenic disease and it can be explained with the destruction of red blood cells by the leucocytosis activity in an erythrocytic anemia with subsequent erythroblastosis (Haney et al. 1992). This causes changes in serum protein amounts (Harikrishnan et al. 2003), adversely affects the growth (Daniels and Gallagher 2000) and thus causes fish to die. But, it was found in our study that serum protein amounts in fish fed with feeds containing *B. subtilis* were similar with fish consuming other feeds and in addition, no signs of abnormal behavior in fish were observed, no deaths or negatively effect on growth and feed intake of the fish as well. The change in the hematologic parameters could be linked to the water temperature. It was seen that RBC count in rainbow trout was lower during the winter period because it

is easier for the oxygen to circulate in the body in winter than the summer period (Morgan et al. 2008). The fact that RBC, Hct and Hgb parameters were found to be lower in fish fed diets containing *B. subtilis* in our study might be accepted as an indicator of being less affected by water temperature.

It was recorded in our study that LY ratio was statistically lower in fish fed with feeds containing *B. subtilis* than fish fed the other experimental diets ($P < 0.05$) (Table 3). It was found that WBC count was statistically higher in fish fed diets containing *B. subtilis* than those of the control group. Experimental feeds containing *L. plantarum* and diets containing L. plantarum+ B. subtilis showed statistically higher GR ratio compared to the control group. The decrease in the LY ratio and the increase in GR ratio in fish fed diets containing *B. subtilis* and the abundance of WBC in the blood may be an indicator that the innate immunity is getting stronger (Morgan and Iwama 1997).

Especially, it might be concluded as a result of the experiment that serum glucose levels in fish fed diets containing *B. subtilis* were statistically lower than those of the control group. Similarly to our study, it was reported that serum glucose levels in *Clarias gariepinus* fish fed diets containing *L. acidophilus* were statistically lower than those fed the control diet (Al-Dohail et al. 2009).

Increase in total protein, albumin and globulin ratios in fish is an indicator of a strong immune response (Al-Dohail et al. 2009). However, in our study results showed that albumin ratio in fish fed diets containing *B. subtilis* was statistically lower than those of the other experimental groups only at day-30, while at the end of the trial at day-60, it was recorded that total protein, albumin and globulin ratios were statistically similar among all experimental groups. Similar to our findings, it was reported that serum total protein, albumin and globulin ratio in common carps fed with feeds containing *B. subtilis*+ *B. licheniformis* was similar with fish fed the control diets (Eslamloo et al. 2013).

Blood lipids in fish containing phospholipids, fatty acids, cholesterol and their esters and, especially phospholipids and triglycerides are the most common fat classes. Triglycerides are the most common fat sources in fat depots and food and are used in transferring and storing energy, cholesterol on the other hand is an essential component for all cell membranes and leads the biosynthesis of steroid hormones and bile acids (Mayes and Botham, 2003). The triglyceride amounts were found to be similar among experimental groups in our study. It was determined that cholesterol levels in fish fed diets containing *B. subtilis* were statistically lower 30 days after the start of the experiment than fish fed the control diets. The fact that serum cholesterol levels were lower in fish fed diets containing *B. subtilis* might be accepted as an indicator of being healthier than those fed the other test diets (Yılmaz et al. 2014). But, as a result of the experiment, it was seen that the cholesterol levels were similar in all experimental groups.

In conclusion, the present study designates that the supplementation of only *L. plantarum* 10^7 cfu/g to the feed reduces the FCR value and increases the RBA and myeloperoxidase activities among immunologic parameters. Therefore, it could be concluded that *L. plantarum* supplementation can be preferred instead of *B. subtilis* or *L. plantarum*+*B. subtilis* supplementation to trout feeds under sublethal water temperature.

References

Abdel-Tawwab, M., Abdel-Rahman, A.M. and N.E. Ismael. 2008. Evaluation of commercial live bakers' yeast, *Saccharomyces cerevisiae* as a growth and immunity promoter for fry Nile tilapia, *Oreochromis niloticus* (L.) challenged in situ with *Aeromonas hydrophila*. Aquaculture, **280(1):**185-189.

Al-Dohail, M.A., Hashim, R. and M. Aliyu-Paiko. 2009. Effects of the probiotic, *Lactobacillus acidophilus*, on the growth performance, haematology parameters and immunoglobulin concentration in African Catfish (*Clarias gariepinus*, Burchell 1822) fingerling. Aquaculture Research, **40(14)**:1642-1652.

Andani, H.R.R., Tukmechi, A., Meshkini, S. and N. Sheikhzadeh. 2012. Antagonistic activity of two potential probiotic bacteria from fish intestines and investigation of their effects on growth performance and immune response in rainbow trout (*Oncorhynchus mykiss*). Journal of Applied Ichthyology, **28(5)**:728-734.

AOAC International, 2000. Association of Official Analytical Chemists. Official Methods of Analysis (17th ed.).Washington DC. 2200 pp.

Aras-Hisar, S., Yanik, T. and O. Hisar. 2003. Hatchery and growth performance of two trout pure breeds, *Salvelinus alpinus* and *Salmo trutta fario*, and their hybrid. The Israeli Journal of Aquaculture-Bamidgeh, **55**:154-159.

Bagheri, T., Hedayati, S.A., Yavari, V., Alizade, M. and A. Farzanfar. 2008. Growth, survival and gut microbial load of rainbow trout (*Onchorhynchus mykiss*) fry given diet supplemented with probiotic during the two months of first feeding. Turkish Journal of Fisheries and Aquatic Sciences, **8**:43-48.

Barnes, M.E., Durben, D.J., Reeves, S.G. and R. Sanders. 2006. Dietary yeast culture supplementation improves initial rearing of McConaughy strain rainbow trout. Aquaculture Nutrition, **12(5)**:388-394.

Başusta, G.A. 2005. Fish hematology and hematological techniques. In: Karatas M (ed) Research techniques in fish biology (in Turkish), 1st edn. Nobel Publications, Ankara, pp 275-300.

Blaxhall, P.C. and K.W. Daisley. 1973. Routine haematological methods for use with fish blood. Journal of Fish Biology, **5(6)**:771-781.

Bulut, M., Yigit, M., Ergün, S., Kesbic, O.S., Acar, Ü., Gültepe, N., Karga, M. and T.W. Campbell. 2004. Clinical chemistry of fish and amphibians. In: Thrall MA, Baker DC, Campbell TW, DeNicola D, Fettman MJ, Lassen ED, Rebar A, Weiser G (eds) Veterinary hematology and clinical chemistry, 1st edn. Lippincott Williams & Wilkins, Pennsylvania, pp 499–517.

Caruso, D., Lusiastuti, A.M., Slembrouck, J., Komarudin, O. and M. Legendre. 2013. Traditional pharmacopeia in small scale freshwater fish farms in west Java, Indonesia: An ethnoveterinary approach. Aquaculture, **416**:334-345.

Cha, J.H., Rahimnejad, S., Yang, S.Y., Kim, K.W. and K.J. Lee. 2013. Evaluations of *Bacillus* spp. as dietary additives on growth performance, innate immunity and disease resistance of olive flounder (*Paralichthys olivaceus*) against *Streptococcus iniae* and as water additives. Aquaculture, **402**:50-57.

Chiu, C.H., Cheng, C.H., Gua, W.R., Guu, Y.K. and W. Cheng. 2010. Dietary administration of the probiotic, Saccharomyces cerevisiae P13, enhanced the growth, innate immune responses, and disease resistance of the grouper, Epinephelus coioides. Fish & Shellfish Immunology, **29(6)**:1053-1059.

Daniels, H.V. and M.L. Gallagher. 2000. Effect of dietary protein level on growth and blood parameters in summer flounder, *Paralichthys dentatus*. Journal of Applied Aquaculture, **10(1)**:45-52.

De Schrijver, R. and F. Ollevier. 2000. Protein digestion in juvenile turbot (*Scophthalmus maximus*) and effects of dietary administration of *Vibrio proteolyticus*. Aquaculture, **186(1)**:107-116.

Diab, A.S., Abdel-Hadi, Y.M., Ahmed, M.H., Saber, S.F. and M.E. Aboel-Atta. 2006. Out-door study on the use of Echinacea (*Echinacea purpurea*), Marjoram (*Origanum dictamnus*) and yeast (*Saccharomyces cerevisiae*) as feed additives for *Oreochromis niloticus*. Egyptian Journal of Agricultural Research, **84(18)**:537-551.

Eslamloo, K., Akhavan, S.R. and M.A. Henry. 2013. Effects of dietary administration of *Bacillus probiotics* on the non-specific immune responses of tinfoil barb, *Barbonymus schwanenfeldii* (Actinopterygii: Cypriniformes: Cyprinidae). Acta Ichthyologica et Piscatoria, **43(3)**:211-218.

FAO, 2013. Food and Agriculture Organization of the United Nations. Fisheries and Aquaculture Department, Information and Statistics Branch.

Fauconneau, B. 1984. The measurement of whole body protein synthesis in larval and juvenile carp (*Cyprinus carpio*). Comparative Biochemistry and Physiology Part B, **78(4)**:845-850.

Fournier, V., Gouillou-Coustans, M.F., Metailler, R., Vachot, C., Moriceau, J., Le Delliou, H. and S.J. Kaushik. 2002. Nitrogen utilisation and ureogenesis as affected by dietary nucleic acid in rainbow trout (*Oncorhynchus mykiss*) and turbot (*Psetta maxima*). Fish Physiology and Biochemistry, **26(2)**:177-188.

Giri, S.S., Sukumaran, V., Sen, S.S. and P.K. Jena. 2014. Effects of dietary supplementation of potential probiotic *Bacillus subtilis* VSG1 singularly or in combination with *Lactobacillus plantarum* VSG3 or/and *Pseudomonas aeruginosa* VSG2 on the growth, immunity and disease resistance of *Labeo rohita*. Aquaculture Nutrition, **20(2)**:163-171.

Gopalakannan, A. and V. Arul. 2010. Enhancement of the innate immune system and disease – resistant activity in *Cyprinus carpio* by oral administration of β – glucan and whole cell yeast. Aquaculture Research, **41(6)**:884-892.

Günther, J. and R. Jiménez-Montealegre. 2004. Effect of the probiotic *Bacillus subtilis* on the growth and food utilization of tilapia (*Oreochromis niloticus*) and prawn (*Macrobrachium rosenbergii*) under laboratory conditions. *International* Journal of Tropical Biology and Conservation, **1(1)**:937-943.

Haney, D.C., Hursh, D.A., Mix, M.C. and J.R. Winton. 1992. Physiological and hematological changes in chum salmon artificially infected with erythrocytic necrosis virus. Journal of Aquatic Animal Health, **4(1)**:48-57.

Harikrishnan, R., Rani, M.N. and C. Balasundaram. 2003. Hematological and biochemical parameters in common carp, *Cyprinus carpio*, following herbal treatment for Aeromonas hydrophila infection. Aquaculture, **221(1)**:41-50.

Heidarieh, M., Mirvaghefi, A.R., Akbari, M., Farahmand, H., Sheikhzadeh, N., Shahbazfar, A.A. and M. Behgar. 2012. Effect of dietary Ergosan on growth performance, digestive enzymes, intestinal histology, hematological parameters and body composition of rainbow trout (*Oncorhynchus mykiss*). Fish Physiology and Biochemistry, **38(4)**:1169-1174.

Henderson, R.J. 1996. Fatty acid metabolism in freshwater fish with particular reference to polyunsaturated fatty acids. Archives of Animal Nutrition, **49(1):**5-22.

Hoseinifar, S.H., Mirvaghefi, A. and D.L. Merrifield. 2011. The effects of dietary inactive brewer's yeast *Saccharomyces cerevisiae* var. ellipsoideus on the growth, physiological responses and gut microbiota of juvenile beluga (*Huso huso*). Aquaculture, **318(1)**:90-94.

Ineno, T., Tsuchida, S., Kanda, M. and S. Watabe. 2005. Thermal tolerance of a rainbow trout *Oncorhynchus mykiss* strain selected by high – temperature breeding. Fisheries Science, **71(4)**:767-775.

Irianto, A. and B. Austin. 2002. Use of probiotics to control furunculosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Journal of Fish Diseases, **25(6)**:333-342.

Kesarcodi-Watson, A., Kaspar, H., Lategan, M. J. and L. Gibson. 2008. Probiotics in aquaculture: the need, principles and mechanisms of action and screening processes. Aquaculture, **274(1)**:1-14.

Kobeisy, M.A. and S.Y. Hussein. 1995. Influence of dietary live yeast on growth performance and some blood constituents in *Oreochromis niloticus*. In Proc. 5th Science Conference Animal Nutrition, Ismailia, Egypt, (pp. 12-13).

Lara-Flores, M., Olvera-Novoa, M.A., Guzmán-Méndez, B.E. and W. López-Madrid. 2003. Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*, and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia (*Oreochromis niloticus*). Aquaculture, **216(1)**:193-201.

Li, P. and D.M. Gatlin. 2003. Evaluation of brewers yeast (*Saccharomyces cerevisiae*) as a feed supplement for hybrid striped bass (*Morone chrysops×M. saxatilis*). Aquaculture, **219(1)**:681-692.

Li, P., Burr, G.S., Goff, J., Whiteman, K.W., Davis, K.B., Vega, R. R. and D.M. Gatlin. 2005. A preliminary study on the effects of dietary supplementation of brewers yeast and nucleotides, singularly or in combination, on juvenile red drum (*Sciaenops ocellatus*). Aquaculture Research, **36(11)**:1120-1127.

Merrifield, D.L., Dimitroglou, A., Foey, A., Davies, S.J., Baker, R. T., Bøgwald, J. and E. Ringø. 2010. The current status and future focus of probiotic and prebiotic applications for salmonids. Aquaculture, **302(1)**:1-18.

Mayes, P.A. and K.M. Botham. 2003. Lipid transport and storage. Harper's Illustrated Biochemistry. 26th ed. California: Lange Medical Books/McGraw-Hill.

Miller, M.R., Nichols, P.D. and C.G. Carter. 2007. Replacement of dietary fish oil for Atlantic salmon parr (*Salmo salar* L.) with a stearidonic acid containing oil has no effect on omega-3 long-chain polyunsaturated fatty acid concentrations. Comparative Biochemistry and Physiology Part B, **146(2)**:197-206.

Mohanty, S.N., Swain, S. K. and S.D. Tripathi. 1996. Rearing of catla (*Catla catla*) spawn on formulated diets. Journal of Aquaculture in the Tropics, **11**:253-258.

Molony, B. 2001. Environmental requirements and tolerances of rainbow trout *(Oncorhynchus mykiss)* and brown trout *(Salmo trutta)* with special reference to Western Australia: a review. Fisheries Research Division, Report No:**130**.

Morgan, J.D. and G.K. Iwama. 1997. Measurements of stressed states in the field. In: Iwama GK, Pickering AD, Sumpter JP,

Schreck CB (eds) Fish stress and health in aquaculture. Cambridge university Press, Cambridge, pp 247-270.

Morgan, A.L., Thompson, K.D., Auchinachie, N.A. and H. Migaud, 2008. The effect of seasonality on normal haematological and innate immune parameters of rainbow trout *Oncorhynchus mykiss* L. Fish & Shellfish Immunology, **25(6)**:791-799.

Nayak, S.K. 2010. Probiotics and immunity: a fish perspective. Fish & Shellfish Immunology, **29(1)**:2-14.

Nayar, S., Hegde, S., Srinivas Rao, P. and P. Sudha. 1998. Live organisms as feed in aquaculture. Infofish International, 36-48.

Noh, H., Han, K.I., Won T.H. and Y.J. Choi. 1994. Effect of antibiotics, enzymes, yeast culture and probiotics on the growth performance of Israeli carp. Korean Journal of Animal Science, **36**:480-486.

Nudo, L P. and E.S. Catap. 2011. Immunostimulatory effects of Uncaria perrottetii (A. Rich.) Merr.(Rubiaceae) vinebark aqueous extract in Balb/C mice. Journal of Ethnopharmacology, **133(2)**:613-620.

Oduleye, S.O. 1982. Growth and growth regulation in the cichlids. Aquaculture, **27(3)**:301-306.

Oliva-Teles, A. and P. Gonçalves. 2001. Partial replacement of fishmeal by brewers yeast (*Saccaromyces cerevisae*) in diets for sea bass (*Dicentrarchus labrax*) juveniles. Aquaculture, **202(3)**:269-278.

Pooramini, M., Kamali, A., Hajimoradloo, A., Alizadeh, M. and R. Ghorbani. 2009. Effect of using yeast (*Saccharomyces cerevisiae*) as probiotic on growth parameters, survival and carcass quality in rainbow trout *Oncorhynchus mykiss* fry. International Aquatic Research, **1(1)**:39-44.

Quade, M.J. and J.A. Roth. 1997. A rapid, direct assay to measure degranulation of bovine neutrophil primary granules. Veterinary Immunology and Immunopathology, **58(3)**:239-248.

Skalli, A. and J.H. Robin. 2004. Requirement of n-3 long chain polyunsaturated fatty acids for European sea bass (*Dicentrarchus labrax*) juveniles: growth and fatty acid composition. Aquaculture, **240(1)**:399-415.

Smith, M.A.K. 1981. Estimation of growth potential by measurement of tissue protein synthetic rates in feeding and fasting rainbow trout, *Salmo gairdnerii* Richardson. Journal of Fish Biology, **19(2)**:213-220.

Soivio, A., Niemistö, M. and Bäckström. 1989. Fatty acid composition of *Coregonus muksun* Pallas: changes during incubation, hatching, feeding and starvation. Aquaculture, **79(1)**:163-168.

Son, V.M., Chang, C.C., Wu, M.C., Guu, Y.K., Chiu, C.H. and W. Cheng. 2009. Dietary administration of the probiotic, *Lactobacillus plantarum*, enhanced the growth, innate immune responses, and disease resistance of the grouper *Epinephelus coioides*. Fish & Shellfish Immunology, **26(5)**:691-698.

Spisni, E., Tugnoli, M., Ponticelli, A., Mordenti, T. and V. Tomasi, 1998. Hepatic steatosis in artificially fed marine teleosts. Journal of Fish Diseases, **21(3)**:177-184.

Subasinghe, R.P. 2005. Epidemiological approach to aquatic animal health management: opportunities and challenges for developing countries to increase aquatic production through aquaculture. Preventive Veterinary Medicine, **67(2)**:117-124.

Stasiak, S.A. and P.C. Baumann. 1996. Neutrophil activity as a potential bioindicator for contaminant analysis. Fish & Shellfish Immunology, **6(7)**:537-539.

Swain, S.K., Rangacharyulu, P. V., Sarkar, S. and K.M. Das. 1996. Effect of a probiotic supplement on growth, nutrient utilization and carcass composition in mrigal fry. Journal of Aquaculture, **4**:29-35.

Taoka, Y., Maeda, H., Jo, J.Y., Jeon, M.J., Bai, S.C., LEE, W.J., Yuge, K. and S. Koshio. 2006. Growth, stress tolerance and non – specific immune response of Japanese flounder *Paralichthys olivaceus* to probiotics in a closed recirculating system. Fisheries Science, **72(2)**:310-321.

Todorčević, M., Kjær, M.A., Djaković, N., Vegusdal, A., Torstensen, B.E. and B. Ruyter. 2009. N-3 HUFAs affect fat deposition, susceptibility to oxidative stress, and apoptosis in Atlantic salmon visceral adipose tissue. Comparative Biochemistry and Physiology Part B, **152(2)**:135-143.

Tovar, D., Zambonino, J., Cahu, C., Gatesoupe, F. J., Vázquez-Juárez, R. and R. Lésel. 2002. Effect of live yeast incorporation in compound diet on digestive enzyme activity in sea bass (*Dicentrarchus labrax*) larvae. Aquaculture, **204(1)**:113-123.

Vendrell, D., Balcazar, J.L., de Blas, I., Ruiz-Zarzuela, I., Gironés, O. and J.L. Muzquiz. 2008. Protection of rainbow trout (*Oncorhynchus mykiss*) from lactococcosis by probiotic bacteria. Comparative Immunology, **31(4)**:337-345.

Verschuere, L., Rombaut, G., Sorgeloos, P. and W. Verstraete 2000. Probiotic bacteria as biological control agents in aquaculture. Microbiology and Molecular Biology Reviews, **64(4)**:655-671.

Waché, Y., Auffray, F., Gatesoupe, F.J., Zambonino, J., Gayet, V., Labbé, L. and C. Quentel. 2006. Cross effects of the strain of dietary *Saccharomyces cerevisiae* and rearing conditions on the onset of intestinal microbiota and digestive enzymes in rainbow trout, *Onchorhynchus mykiss*, fry. Aquaculture, **258(1)**:470-478.

Yılmaz, S., Ergün, S. and E.Ş. Çelik. 2014. Effect of dietary spice supplementations on welfare status of sea bass, *Dicentrarchus labrax* L. Proceedings of the National Academy of Sciences, **India Section B**:1-9.

Zilberg, D., Tal, A., Froyman, N., Abutbul, S., Dudai, N. and A. Golan – Goldhirsh. 2010. Dried leaves of *Rosmarinus officinalis* as a treatment for streptococcosis in tilapia. Journal of Fish Diseases, **33**:361-369.