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Effects of different levels of *Lactobacillus casei* as probiotic on growth performance and digestive enzymes activity of *Barbus gryprus*

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Abstract

The probiotic effects of *Lactobacillus casei* in fish have been documented recently. In this study the effects of different level of *Lactobacillus casei* (PTCC 1608) on the growth performance and digestive enzymes of juvenile *Barbus grypus* were evaluated . Four hundred and eighty juvenile fish (40±4.3 g) were fed with basal diet containing 0 (control), 10^6 (A), 10^7 (B),10^8 (C) CFU g^{-1} *L. casei* for 60 days. Growth performance indices and digestive enzyme activity were examined at 30 and 60 Days of study. Feeding with free probiotic basal diet last for two more weeks too. Growth performance rate evaluated on days 30, 60 and 75 of study. Intestinal samples were taken at the same days of study and digestive enzymes activity including: lipase, **α**-amylase chymotrypsin, trypsin, alkaline phosphates compared among the groups. Results showed that oral administration of *L. casei* for 60 days had significant impact ($P<0.05$) on the specific growth rate (SGR) and feed efficiency rate of *B. grypus*. Dietary administration of *L. casei* (group B) significantly increased the SGR, DWG and RGR compared to the control group at 30 days of study. However group C showed significant increased in SGR, FCR and PER compared to the control group at day 60 ($P<0.05$). Similarly, digestive enzymes activity (chymotrypsin) were significantly elevated in group B at day 30 of study compared to the control group ($P<0.05$). Subsequently, elevated trypsin activity was observed in group C at day 60 of study compared to the control group ($P<0.05$).These results suggest that dietary supplementation of food with *L. casei* at 5×10^7 CFU g^{-1} concentration in day 30 and at 5×10^8 CFU g^{-1} concentration in day 60 are suitable for enhancing the growth and digestive enzymes activity of *B. grypus*.

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Introduction

The quantity of cultured aquatic animals for human consumption was >70 million metric tonnes in 2011 (FAO, 2011). It is expected that the demand for aquatic food will increase, because of the increase in the human population and the perception that aquatic food is healthy. Aquaculture has expanded during the past several decades. The study of new technologies on common rearing species to enhance survival and production potential causes to achieve this goal sooner. New achievements of dietitian science in poultry and aquatic animals to increase economic profits includes symbiotic food supplement use (contains probiotic and prebiotic combination) to improve animal health and growth (Wang *et al*., 2008). Using probiotic is one of the positive achievements in this field and it has been common in aquaculture recently (Makridis *et al*., 2001). The use of probiotic as a food supplement in the culture of aquatic organisms dates back to 1970s. A lot of studies about their effect on fish larva, crustacean and oysters growth and survival have been done up to the present (Ali, 2000). As reported by several authors, probiotics have a good effect on digestive processes in aquatic animals. Moreover, some bacteria may participate in shellfish, shrimp and fish digestive processes and produce outer cellular enzymes such as proteases, lipases and necessary growth factors. Similar observations have been reported for microbial flora Chinese shrimp (*Penaeus chinensis*) that produce complete enzyme for compounds digestion and synthesis *(*Balcazar *et al.,* 2006). Giri *et al.* (Giri *et al*., 2013) examined the effect of *lactobacillus plantarum* in different doses in juvenile fish *Labeo rohita*. Shyne Anand *et al.* (Shyne Anand *et al.,* 2013) studied the effect of microalgae (periphyton) food supplement on growth performance and digestive enzyme activity in *Penaeus monodon* species. Lara-Flores *et al.* (Lara-Flores *et al.,* 2010) studied the effect of bacterial and yeast probiotic on Tilapia digestive enzymes. *Barbus gryprus* is one of the most important freshwater fish in the south part of Iran and has the best meat quality and recently its farming possibility is examining in earthen ponds. It is obvious to identify disease factors for indigenous fish rearing (Jiad *et al*., 1984). According to the lack of information on using Lactobacillus probiotic in Khuzestan province indigenous fish and increase trend to use these bacteria in aquaculture all over the world, the aim of this research were evaluation of different concentrations of *Lactobacillus casei* in *Barbus gryprus* growth factors and digestive system enzymes.

Materials and methods

Fish

Four hundred eighty rearing *B. gryprus* (about 40 gr) were purchased from Shushtar city transformed to the lab of Shahid Chamran University. Fish were fed with standard food for two weeks to adapt to the aquarium room condition, then for doing research, they were divided into four groups. They were fed with basal diet (Biomar, France) in the rate of 3% of body weight and three times a day for 60 days and they were also fed with basal food for 15 days to study latency of probiotic effects on digestive enzymes.

Grouping

Aquarium arrangement was in randomized blocks. The fish were divided randomly into four groups and each group was in three repetitions in separate aquarium and each replicate included 30 juvenile fish.

The control group was fed unsupplemented basal diet during the entire trial period. Treatment 1 (T-1), treatment 2 (T-2) and treatment 3 (T-3) were fed with diets containing the different concentration viable probiotic bacteria 5×10^6, 5×10^7 and 5×10^8 CFU g^{-1} *L. casei* respectively. To reach this final concentration, probiotics were slowly applied and mixed in to the diets.

Sampling

Weights of all collected *Barbus grypus* from each aquarium were determined at initial, mid and the end during the 60 days experiment and intestinal samples were taken at 0, 30, 60 and 75 days of study in each treatment.

Growth indices of experimental groups were evaluated based on the following equations: Specific growth ratio ([Ln w2-w1/ daily rearing period ×100]); Food conversion ratio (received food rate (gr) / total weight rate (gr)); Protein efficiency rate: (total received protein rate / wet weight rate (gr)); Condition factors ([w / L^3] ×100): W= fish weight / gr; L= total fish length; daily weight growth (daily weight growth (g/d) test days number / initial weight mean – final weight mean); Survival rate (survived fish number/ initial fish number ×100); Relative growth rate (100×initial weight / initial weight – final weight).

Sampling steps of digestive system

Three fish from each replicate were euthenasied. The fish were dissected out, weighed and separately homogenized with homogeneous buffer solution for assessing pancreas enzymes (trypsin, chymotrypsin, α-amylase and lipase) (Tris-HCL 100 mM , EDTA 0/1 mM, X-100 Triton 0/1 % in 7/8 pH are mixed together) in 1:9 (weight:volume) and for assay alkaline phosphates activity from manitol cold buffer 50 mM , buffer HCL-Tris 2 mM in pH=7 in 1:30 (weight:volume) were used to extract intestine enzyme and it was centrifuged in 10000 rpm for 1 min (Cahu *et al*., 1999). After homogenizing samples in the above buffer, $Cacl_2$ 0.1 M was added to the homogeny and was centrifuged at 9000 rpm for 10 min and the obtained supernatant was used for assessing enzyme. The samples homogenized by an electric homogenizer. (Cahu *et al*., 1999; Rungruangsak *et al*., 2002; Chang *et al*., 2002). All enzymatic assays were conducted within 24 h after extraction.

Digestive enzyme assessment

Total protein content of supernatant was assayed according to Bradford (Bradford, 1976) using bovine albumin as standard. α-amylase activity was measured according to Jiang (Jiang, 1982) and Worthington (Worthington, 1993) using iodine solution to reveal non-hydrolyzed starch. Trypsin activity was measured using N-Banzoeel – L Argenin Eteel Ester (BAEE) as the substrate (Tseng *et al*., 1982). Banzoeel-L-Tirozin Eteel Ester (BTEE) was used as a substrate to determine chymotrypsin enzyme activity (Hummel, 1959). Alkaline phosphatase (AP) activity was measured using p-nitrophenyl phosphate (pNPP) $MgCl_2$ as the substrate (Bessey *et al*., 1946). Lipase activity was determined based on measurement of fatty acids release due to enzymatic hydrolysis of triglycerides in stabilized emulsion of olive oil (Borlongan, 1990; Jin, 1995). Enzyme activities trypsin, chymotrypsin, lipase and α-amylase were expressed as specific activity (Umg^{-1} protein).

Statistical analysis

For data analysis, SPSS®16 edition was used and the effect of probiotic on growth performance and studying digestive enzyme activity between four groups was examined by one-way ANOVA with 95% coefficient assurance and for studying significant means difference Duncan was used in 0/05 significant level. Drawing diagram in Excel software was also done.

Results

The data regarding the growth of *B. gryprus* fed with three different concentrations of probiotic *L. casei* incorporated diets are presented in Table 1. Survival rate was 100% after 60 days of culture for all the groups.

Specific growth ratio (SGR) was influenced by probiotic groups so that on the 30^{th} day of test, probiotic groups (B) had the highest specific growth ratio that had a significant difference with control and (A) groups ($P<0.05$). The results from calculating specific growth ratio on the 60^{th} day showed that group C had a significant difference with other groups ($P<0.05$). condition factor (CF) was influenced by probiotic groups, so that on the 30^{th} day of test, probiotic groups (C) had the highest ratio than other groups and had a significant difference with control group (A, B) ($P<0.05$). The results from calculating condition factor on the 60^{th} day also showed that 5×10^7 group had the highest ratio and a significant difference with other groups ($P<0.05$), but it does not

have a significant difference with control group (P>0.05). Daily weight growth (DWG) influenced by probiotic groups, so that on the 30th day of test, the highest daily weight growth was associated with probiotic group (B) that had a significant difference with control group and group A and C (P<0.05), but it does not have a significant difference with control group (C). The results from calculating daily weight growth on the 60th day showed that group (B) and (C) had the highest daily weight growth and a significant difference with control group (P<0.05). Relative growth rate (RGR) at the 30th day of test, probiotic group (C) does not have a significant difference with control C (P>0.05), but the highest relative growth rate was related to group B and had a significant difference with control (P<0.05). Feed conversion ratio (FCR) that on the 30th day of test, probiotic groups (B) had the best feed conversion ratio and group (A) had the highest ratio and a significant difference with control group (P<0.05), but other groups did not have a significant difference with control group (P>0.05). The results from this calculation of feed conversion ratio on the 60th day showed that group (C) had the best feed conversion ratio and all groups had a significant difference with control group (P<0.05). Protein efficiency ratio (PER) influenced by probiotic groups, so that on the 30th day of test, probiotic groups (C) and (B) did not have a significant difference with control group (P>0.05), however group (A) had a significant difference with other groups (P<0.05). The results from protein efficiency ratio on the 60th day also showed that group (C) had a significant difference with other groups (P<0.05), but group (B) does not have a significant difference with control group (P>0.05).

Table 1. Growth indices of the experimental groups in different sampling steps (the results are given as mean ± SD). Latin lower case letters on standard error shows significant difference in level 0.05 in each column, and Latin capital letters on standard error shows significant difference in level ./.5 in each row). Group A: fish were fed with basal diet containing 5×10^{6} *L. casei* group B: fed with 5×10^{7} *L. casei* bacteria in gr diet, group C: fed with 5×10^{8} *L. casei* bacteria in gr diet.

Parameters	Treatment	30 day	60 day	75 day
SGR	Treatments A	0.06±0.006 ^{aA}	0.07±0.003 ^{aB}	0.10±0.003 ^{aC}
	Treatments B	0.42±0.03 ^{cB}	0.27±0.03 ^{cA}	0.23±0.02 ^{cA}
	Treatments C	0.30±0.01 ^{bA}	0.34±0.003 ^{dB}	0.31±0.003 ^{dA}
	Control	0.22±0.07 ^{bA}	0.175±0.02 ^{bA}	0.14±0.003 ^{bA}
CF	Treatments A	0.39±0.07 ^{bB}	0.24 ±0.10 ^{aAB}	0.08± 0.05 ^{aA}
	Treatments B	34.29±0.01 ^{aA}	0.41 ±0.05 ^{bB}	0.23 ±0.04 ^{bA}
	Treatments C	0.74±0.07 ^{cC}	0.36 ±0.05 ^{abB}	0.08 ±0.05 ^{aA}
	Control	0.51±0.08 ^{bA}	0.37 ±0.06 ^{abA}	0.35± 0.03 ^{cA}
DWG	Treatments A	0.06±0.007^{aA}	0.08±0.03 ^{aB}	0.11±0.003 ^{aC}
	Treatments B	0.54±0.04 ^{cB}	0.35±0.03 ^{cA}	0.32±0.03 ^{cA}
	Treatments C	0.24±0.01 ^{bA}	0.32±0.002 ^{cC}	0.30±0.002 ^{cB}
	Control	0.26±0.08 ^{bA}	0.21±0.02 ^{bA}	0.17±0.002 ^{bA}
RGR	Treatments A	4.37±0.49 ^{Aa}	10.96±0.47 ^{aB}	19.91±0.62 ^{aC}
	Treatments B	34.29±3.17^{cA}	45.58±6.09 ^{cAB}	51.30±7.05 ^{cB}
	Treatments C	23.11±1.09 ^{Ba}	62.00±0.86 ^{dB}	72.10±1.09 ^{dC}
	Control	17.05±6.03 ^{Ba}	27.43±4.05 ^{bB}	28.02±0.82 ^{bB}
FCR	Treatments A	5.39±0.65 ^{bB}	2.96±0.12 ^{cA}	2.20± 0.06 ^{bA}
	Treatments B	0.80±0.06 ^{aA}	1.22± 0.13 ^{bB}	2.20 ±0.24 ^{bC}
	Treatments C	1.09±0.04 ^{aC}	0.67 ±0.005 ^{aA}	0.87± 0.008 ^{aB}
	Control	1.09±0.39 ^{aA}	1.39±0.17 ^{bA}	3.16±0.03 ^{cB}
PER	Treatments A	0.38±0.04 ^{aA}	0.70± 0.02 ^{aB}	0.94± 0.02 ^{bC}
	Treatments B	2.5±0.12 ^{bC}	1.71± 0.17 ^{bB}	0.95±0.10 ^{bA}
	Treatments C	1.91±0.07 ^{bA}	3.06± 0.02 ^{cC}	2.37± 0.02 ^{cB}
	Control	2.07±0.69 ^{bB}	1.50± 0.18 ^{bB}	0.65± 0.07 ^{aA}

The results of this study showed that α-amylase ratio in 0 and 30th day difference between groups was not observed (P>0.05). The results of 60th day also showed that group (A) had the highest amylase ratio than control group, but this difference was not significant statistically (P>0.05).

Lipase activity influenced by probiotic groups, so that on the 30th day of test probiotic, groups (C) had the highest and most lipase ratio and had no significant difference with control group (P>0.05), but had a significant difference with group (A). The results from 60th day showed that lipase activity decreased, but no significant difference observed between groups and control. (P>0.05). On the 75th day, after stopping probiotic feeding, lipase ratio showed a significant increase in group (A) than other groups, but no significant difference observed between group (B) and (C) (P<0.05).

Table 2. Compare digestive enzyme activity between studying groups in different sampling steps. (The results were reported based on mean ± standard error). The Latin lowercase letters on standard error showed a significant difference in level 0.05 in each column, and the Latin capital letters on standard error showed a significant difference in 0.05 in each row). Group A: fish fed with 5×10^6 basal diet *L. casei* gram/food and group (B) fed with 5×10^7 *L. casei* gram/food group (C) fed with 5×10^8 *L. casei* gram/food.

Parameters	Treatments	zero day	30 day	day 60	day 75
α-amylase	Treatments A	0.84 ±0.42 ^{aAB}	0.80 ±0.21 ^{abAB}	0.51 ±0.21 ^{bA}	1.20 ±0.61 ^{bB}
	Treatments B	0.61 ± 5.20 ^{aB}	0.61 ±0.18 ^{aB}	0.21 ±0.04 ^{aA}	0.56 ±0.13 ^{aB}
	Treatments C	0.41 ± 0.14 ^{aB}	0.63 ±0.08 ^{aC}	0.20 ±0.08 ^{aA}	0.65 ±0.18 ^{aC}
	Control	0.54 ± 0.19 ^{aA}	0.89 ± 0.25 ^{bB}	0.31 ±0.04 ^{aA}	0.51 ±0.18 ^{aA}
Lipase	Treatments A	1.10 ± 0.51 ^{aA}	1.59 ±0.34 ^{aA}	2.24 ±0.41 ^{aA}	1.20 ±1.50 ^{bB}
	Treatments B	1.23 ± 0.55 ^{abA}	2.45 ±1.83 ^{abA}	1.57 ±0.26 ^{aA}	0.56 ±0.47 ^{aA}
	Treatments C	1.28 ± 0.67 ^{abA}	3.78 ± 1.73 ^{bB}	1.35 ±0.27 ^{aA}	0.65 ±0.33 ^{aA}
	Control	1.79 ± 0.22 ^{bA}	2.76 ± 0.46 ^{abA}	2.10 ±1.45 ^{aAB}	0.51 ±0.24 ^{aA}
Chymotrypsin	Treatments A	0.11 ± 0.05 ^{aB}	0.12 ± 0.03 ^{aB}	0.05 ±0.02 ^{aA}	0.18 ±0.06 ^{bC}
	Treatments B	0.18 ± 0.07 ^{aA}	0.26 ± 0.06 ^{bA}	0.50 ±0.11 ^{bB}	0.18 ±0.06 ^{bA}
	Treatments C	0.13 ± 0.03 ^{aB}	0.13 ± 0.03 ^{aB}	0.06 ±0.02 ^{aA}	0.07 ±0.01 ^{aA}
	Control	0.19 ± 0.03 ^{aAB}	0.15 ±0.03 ^{aA}	0.35 ± 0.28 ^{bB}	0.23 ±0.06 ^{bAB}
Trypsin	Treatments A	1.83 ±0.99 ^{aA}	2.58 ±0.76 ^{bc}	1.39 ± 0.32 ^{aA}	8.88 ± 3.22 ^{bB}
	Treatments B	1.72 ±0.63 ^{aB}	1.51± 0.74 ^{abAB}	0.72 ± 0.20 ^{aA}	3.15 ± 1.26 ^{aC}
	Treatments C	1.74 ±0.62 ^{aA}	3.20 ±1.54 ^{cAB}	3.37 ± 1.81 ^{bB}	3.07 ± 0.87 ^{aAB}
	Control	1.65 ±0.23 ^{aAB}	1.10 ±0.69 ^{aA}	2.43 ± 0.63 ^{bBC}	2.30 ± 0.94 ^{aC}
Alkaline Phosphates	Treatments A	289.03± 24.25 ^{aB}	45 ±21.59 ^{aA}	36.6 ± 7.25 ^{aA}	277.72 ± 137.95 ^{cB}
	Treatments B	217.08± 39.07 ^{aB}	31± 18.61 ^{aA}	27.78 ± 8.79 ^{aA}	59.91 ± 20.44 ^{aA}
	Treatments C	232.49± 147.13 ^{aB}	54 ±17.88 ^{aA}	136.156 ± 45.39 ^{bAB}	62.24 ± 14.54 ^{aA}
	Control	238.98± 126.87 ^{aB}	50 ±16.50 ^{aA}	141.55 ± 83.36 ^{bAB}	172.73 ± 53.74 ^{bAB}

The results of this study showed that chymotrypsin ratio influenced by probiotic groups, so that on the 30th day of test, probiotic groups (B) had the most chymotrypsin ratio and a significant difference with control and other groups (P<0.05). The results on the 60th day showed that group (B) had no significant difference compared to control (P>0.05). On the 75th day after stopping probiotic feeding chymotrypsin ratio decreased in all groups.

The results of this study showed that trypsin ratio influenced by probiotic groups so that, on the 30th day of test the highest trypsin ratio was associated with probiotic group (C) and had a significant difference with control and other groups (P<0.05). The results from the 60th day showed that group (C) had the most ratio and had no significant difference with control (P>0.05) (3.37±1.81). On the 75th day after stopping probiotic feeding, trypsin ratio increased in all groups and the most ratios was associated with group (A).

The results of this study showed that alkaline phosphates influenced by probiotic groups, so that on the 30th day of test, a considerable decrease was observed in alkaline phosphates ratio than 0 day and no significant difference was seen between control

and groups. (P>0.05). The results from the 60th day showed that group (C) had the most ratio but does not have a significant difference with control (P>0.05) (3.37±1.81). On the 75th day after stopping probiotic feeding alkaline phosphates ratio increased in all groups and the most ratio was associated with group (A).

Discussion

Probiotics were defined as living bacteria or planted products. They have useful effects for host by producing prevented materials, competing for chemical materials absorption and adhesion in digestive system (Verschuere *et al.*, 2000; McCracken & Gaskins, 1999). Results of this study showed that growth performance of different levels of probiotic *lactobacillus casei* used with concentration (5×10^7) at 30th day of experiment period and concentration (5×10^8) till 60th day of rearing period caused improvement in *Barbus gryprus*, so that feed conversion ratio, specific growth ratio, relative growth ratio, protein efficiency ratio, daily weight growth were improved significantly than the control group (P<0.05). This growth improvement can cause by positive effect of these bacteria on digestive system flora and increase digestive ratio and absorption used food. Different researchers like this, have done and similar result. They were expressed that adding probiotics to diet of different kinds of fish, increase their growth and this is based on current research result. In the study by Giri *et al.* (Giri *et al.,* 2013) *Lactobacillus plantarum* in three concentrations was added to *Labeo rohita* diet, daily weight growth (WG) and feed conversion ratio (FCR) showed a significant increase and SGR in 10^8, 10^{10} CFU g^{-1} had a significant increase. Similar studies also by Son *et al.* (Son *et al.,* 2009) on *Epinephelus coioides*, Aly *et al.* (Aly *et al.,* 2008) on *Tilapia nilotica* (*Oreochromis niloticus*), Suzer *et al.* (Suzer *et al.,* 2011) on gilthead sea bream (*Sparus aurata*), Al-Dohail *et al.* (Al-Dohail *et al.,* 2009) on *Clarius gariepinus*, Venkat *et al.* (Venkat *et al.,* 2004) on *Macrobrachium rosenbergii* obtained similar results. These results were similar to investigation by Gomez-Gil *et al.* (Gomez-Gil *et al.,* 2000), Nikoskelainen *et al.* (Nikoskelainen *et al.,* 2003) and Taghavi (Taghavi, 2005).

Jafarian *et al.* (Jafarian *et al.,* 2011) reported a significant improvement in growth factor by mixing experimental diets with three mixture of bacterial probiotic including *Lactobacillus*, commercial *bacillus* and isolated *bacillus* from Iranian sturgeon (*Acipencer perscius*) intestine and *Huso Huso* in three levels to trout larval food for 32 days. This finding is in contrast with our results, because growth indices had a direct correlation with probiotic concentration. Lara-flores *et al.* (Lara-flores *et al.,* 2003) reported similar results by using yeast *Saccharomyces cerevisia* ever in Tilapia. Ramos *et al.* (Ramos *et al.,* 2013) studied effect of two commercial probiotics in rainbow trout, for 28 days. They reported no significant effect in growth performance rate, but lasting the treatment for 56 days, induced a significant difference in growth indices and SGR in commercial probiotics. This result was in agreement to current study. Soleimani *et al.* (Soleimani *et al.,* 2012), studies digestive enzyme activity and Caspian roach growth performance in fry step and used FOS (Fructooligosaccharid) a food supplement that is a kind of probiotic in three levels 1%, 2% and 3% for 7 weeks and their study showed that the highest survival was associated with 3% group and higher growth performance and digestive enzyme activity was related to 2% and 3% groups. The results from the effect of using different levels of *lactobacillus casei* probiotic in *B. gryprus* on α-amylase enzymes activity, trypsin, chymotrypsin, alkaline phosphates and lipase showed a significant difference in the above mentioned enzymes, so that chymotrypsin was in (B) concentration on the 30th day. Trypsin was in (C) concentration on the 30th and 60th day in rearing period, treatment group did not show a significant difference than control group in the mid and final rearing period (P>0.05).

Healthful macronutrients such as carbohydrates and diet protein were useful in *B. gryprus* digestive enzyme activity. In this study diets formulation with healthful materials and the similar energy rate were

used to compare protein supplement and control group. Shyne Anand *et al.* (Shyne Anand *et al.,* 2013) studied digestive enzyme activity in *Penaeus monodon* shrimp influenced by periphyton food supplement. In the highest periphyton food supplement concentration a significant effect was not seen in digestive enzymes, the highest activity of lipase, trypsin and chymotrypsin was obtained in lower level of periphyton food supplement. Wang *et al.* (Wang *et al.,* 2007) also studied probiotic supplement on *Penaeus vannamei* shrimp that their results showed that food supplement had no effect on digestive enzymes.

It is thought that probiotics influences digestive processes by enhancing the population of beneficial microorganisms, microbial enzyme activity, improving the intestinal microbial balance, consequently improving the digestibility and absorption of food and feed utilization (Suzer *et al.*, 2008). Probiotics effect on digestive enzymes on aquatic animals is seen in studies by different researchers. Thus the mentioned bacteria increase digestive system efficiency by participating in digestive processes and finally improve growth indices. the results of the studies in which used probiotics showed that the mentioned bacteria increase protein, fat and starch in food digestion (Wang and XU, 2006; Soleimani, 2012).

Therefore experimental probiotic bacteria with different concentration (group B and C) can possibly cause increase protein efficiency ratio in diet *B. gryprus* juvenile fish and especially increase chymotrypsin enzyme activity and trypsin that is from protease. Similar cases on produce ability of exogenous enzymes synthesized were reported by bacteria in Chinese shrimp digestive system *P. chinensis* (Wang *et al.,* 2008), Roach *Rutilus rutilus* (Skrodenyte Arbaciauskiene, 2007), *Sparus aurata* (Suzer, 2008), *Epinephelus coioides* (Sun *et al.,* 2011), Indian white shrimp (Ziaei-Nejad *et al.*, 2006), West white leg shrimp (Wang, 2007) and *Artemia urmiana* (Ahmadnia *et al.,* 2011). These confirm the results of the current study in increasing chymotrypsin and trypsin digestive enzymes on the 30th day of test in (B) and (C) concentration in *B. gryprus*. This can be for the presentation of unknown compositions of stimulating growth and digestive enzymes production in diets contain probiotic that cause increase in digestive enzyme production by fish. Probiotic *lactobacillus* in (C) and (B) concentration has more ability in producing protease outer cellular enzyme. Digestive enzymes activity ratio depends on growth steps, food ratio, chemical composition and aquatic feed requirements (Ahmadnia *et al.*, 2011) the lack of significant difference in α-amylase and lipase enzyme activity between treatment and control group is possibly due to food chemical composition, low amount of fat and carbohydrate of consumed food and less stimulation of digestive system to produce lipase and α-amylase enzyme than trypsin and chymotrypsin enzymes. Another reason can be the inability to produce Lipase and α-amylase outer cellular by used bacteria in this test or the lack of stimulation of digestive system to produce these enzymes efficiently. Evaluating digestive enzymes activity level can be used as a suitable indices to compare fish growth ratio, food acceptance and also digestive capacity. According to digestive enzymes production by *B. gryprus* juvenile digestive system and stimulation and outer cellular digestive enzymes production by probiotic bacteria used in this test, enzyme activity arises from bacteria activity and enzyme production by fish juvenile cannot be separated from each other (Suzer *et al.*, 2008). Growth indices improvement in fish fed with *lactobacillus casei* with different levels is possibly associated with vitamins synthesis and enzyme activity increase, which leads to improvement in digestion and weight growth. Decreasing food conversion ratio in this study also expressed efficiently improvement of used food when *lactobacillus casei* on the 30th and 60th days was in 5×10^{7} and 5×10^{8} respectively.

On the 30th day period, concentration (B) had the best growth performance, because it had a significant effect on probiotic enzymes like chymotrypsin and on the 60th day period concentration (C) had the best

growth performance and this can be associated with better digestive enzymes performance.

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