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Research Article

Growth performance and serum immune responses of the common carp (*Cyprinus carpio*) using *Lactococcus lactis* and *Weissella cibaria* as potential dietary probiotics

Bakhshzad Mahmoudi A.[1]; Farokhrouz Lashidani M.[1*]; Zamini A.[1]; Shenavar Masuleh A.[2]; Tehranifard A.[3]

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Abstract

The present study aimed to investigate the effect of *Lactococcus lactis* and *Weissella cibaria* as potential probiotics on growth performance, some blood and immune parameters, digestive and liver enzyme activity, and intestinal bacterial flora, in common carp (*Cyprinus carpio*) juvenile. Fish (17.00±1.3 g) were divided into 10 treatments. The experimental diets of treatments 1, 2, and 3 were supplemented with *Lactococcus lactis* in doses of 1.5×10^7, 3×10^7, and 4.5×10^7 CFU kg^{-1}, the diets of treatments 4, 5, and 6 were supplemented with *Weissella cibaria* in doses of 1.5×10^7, 3×10^7 and 4.5×10^7 CFU kg^{-1}, these two potential probiotics were equally mixed for preparation the diets of treatments 7,8 and 9 which has been added in doses of 1.5×10^7, 3×10^7, and 4.5×10^7 CFU kg^{-1}. A basal diet (19 $mJkg^{-1}$ of energy and 38% protein) without probiotic was fed to the fish in the control group. Fish were randomly divided into 30 tanks and reared in the water with an average water temperature of 24.5°C. They were fed two times a day at 3% of body weight for 8 weeks. Results showed a significant increase in body weight (about 4 g), specific growth rate, and average daily growth in the most of the probiotic supplemented treatments ($p<0.05$) sespecially in treatments 8 and 5. Also, the highest amount of white blood cells, neutrophil, monocytes, Immunoglobulin M, alternative complement pathway activity (ACH50), lysozyme activity, digestive enzymes, and the lowest amount of liver enzymes (Aspartate aminotransferase and Alanine transaminase) were observed in the groups treated with potential probiotics. According to the results, adding 3 to 4.5×10^7 CFU kg^{-1} of the potential probiotics mixture, or 3×10^7 CFU kg^{-1} *W. cibaria*, could improve the growth performance and health status in common carp.

Keywords: *Cyprinus carpio*, Digestive enzymes, Immune parameters, *Lactococcus lactis*, Probiotics, *Weissella cibaria*

1-Department of fisheries, Faculty of Natural resources, Islamic Azad University of Lahijan, Lahijan, Iran.
2-Caspian Sea Sturgeon International Research Center, Agricultural Research, Education, and promotion Organization, Rasht, Iran.
3-Department of Marine Biology, Faculty of Basic Sciences, Islamic Azad University of Lahijan, Lahijan, Iran.
*Corresponding author's Email: dr.farokhrouz@gmail.com

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Introduction

Nowadays, supplementing aquafeeds with probiotics is a new strategy from the nutritional aspect and an alternative remedial agent to overcome antibiotic's adverse influences (Pérez *et al*., 2019, Yeganeh Rastekenari *et al*., 2021, Kahyani *et al*., 2021). Many studies reported the positive effects of probiotics on farmed aquatic species (Irianto and Austin, 2002; Mohapetra *et al*., 2012; Beck *et al*., 2015; Adel *et a*l., 2016; Sayes *et al*., 2018; Mohammadian *et al*., 2019), However, there are still gaps to increase their efficiency for fish culture, which requires continuous research. Probiotic bacteria are live microbial feed supplements that play a beneficial role in the host by alteration in the gut microbial flora (Sayes *et al*., 2018). Probiotics, especially lactic acid bacteria are the major probiotics used in aquaculture (Irianto and Austin, 2002; Mohapetra *et al*., 2012; Hoseinifar *et al*., 2014; Mohammadian *et al*., 2017; Sayes *et al*., 2018) and their positive effects on improving fish immune and growth performance have been proven (Gatesope, 2008; Beck *et al*., 2015; Adel *et al*., 2016; Mohammadian *et al*., 2017; Won *et al*., 2020).

L.lactis and *W.cibaria* are kinds of lactic acid bacteria as natural flora of different species of aquatic animals, and their gens were registered. The genus *Weissella* is a recently classified member of LAB that is isolated from different sources including soil, food products, plants, animals, humans and fish (Fusco *et al*., 2015). Strains of some *Weissella* species are known as opportunistic pathogen present in humans, animals and fish (Costa *et al*., 2015; Fusco *et al*., 2015) but some of them have also been proposed as potential probiotics (Jesus, 2014; Goh and Philip, 2015; Hashemimofrad *et al*., 2016; Adebayo-Tayo *et al*., 2018; Sharma *et al*., 2018; Dey *et al*., 2019). Effects of *L. lactis* and *W.cibaria* on some fish species have also been studied (Jesus, 2014; Shenavar Masouleh *et al*., 2016; Hashemimonfared *et al*., 2016; Munir *et al*., 2016).

Common carp (*Cyprinus carpio*) is the sixth most cultured species in the world with more than 4 million tons of production per year (FAO, 2018). Recognizing appropriate and new strategies to improve production and breeding conditions, would be helpful in the common carp culture. Therefore, in this study, the effect of diets containing probiotic
bacteria *W.cibaria* and *L.lactis* has been studied in common carp to promote some aspects of production industry of this commercial species.

Material and methods

Fish and diets

Common carp juveniles (17.00±1.3 g) were obtained from a local farm in Gilan province. They were randomly divided into 30 tanks (600L, n=10) after a two-week acclimation period. Fish were divided into 9 treatments with a control, each with 3 replications. Water factors including temperature, dissolved oxygen, pH, ammonium, and hardness were measured routinely during the

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experiment using an alcohol thermometer (China), Oxygen meter (WTW, Germany), pH meter (AZ8584, China), spectrophotometer (HACH IGS, Germany) and titration (complex metric method) respectively. The average water factors were 24.5° C, 6.1 mg L^{-1}, 7.7, 2 mg L^{-1}, and 212.25 mg L^{-1}, respectively. The potential probiotic bacteria used in the present study were *W.cibaria* (10^{10} $CFUg^{-1}$) and *L.lactis* (10^{10} $CFUg^{-1}$). Bacterial probiotics used in the present study are safe and secure (Soltani *et al*., 2013; Soltani *et al*., 2015; Shenavar masuleh *et al*., 2016; Hashemimofrad *et al*., 2016). They were isolated from *Acipenser persicus* intestine (Soltani *et al.*, 2013) in the international sturgeon research institute and recognized by rRNA 16S gene, and registered in NCBI under code 13 (Shenaver masouleh *et al.*, 2016). Potential probiotic Bacteria powder was prepared from Guilan Science and Technology Park. The diets were prepared by spraying a mixture of 50 ml of sterile physiological serum containing 150, 300, and 450 mg of 2 bacterial strains powder per kg of commercial extrude pelleted diets (Faradaneh Co. 35-38% protein, 4-8% fat, 5-11% moisture, 5-11% ash, 4-7% fiber, and 1.0-1.5 phosphorous) based on the recommended dosages by Yeganeh Rastekenari *et al* (2021) and Ghorbani vaghei *et al.* (2021). Then the prepared feed was placed in a dark and cool place to dry. Prepared feed was placed in the refrigerator (4°C) until the feeding trial (Shenavar Masuleh *et al*., 2016). Fish were fed two times a day at 3% of body weight for 60 days (Hosseini *et al*., 2016).

Growth Performance

Fish weight and length were measured at the beginning and end of the feeding trial to determine the growth performance. Fish were starved for 1 day and anesthetized with clove oil (50 mgL^{-1}) before biometry (Esmaeili *et al*., 2017). At the end of the feeding trial, the percentage of body weight increase (PBWI), condition factor (K), average daily growth (ADG), specific growth rate (SGR) and feed conversion ratio (FCR) were calculated using formulas as below:

$BWI\ (g) = Wt_2 - Wt_1$; $PBWI\ (\%) = [(Wt_2 - Wt_1) / Wt_1] \times 100$; FCR = g dry feed eaten / g live weight gain

$SGR\ (\%\ day^{-1}) = [(LnWt_2 - LnWt_1) / t_2 - t_1] \times 100$ (Merrifield *et al*., 2011); $K = [W / L^3] \times 100$ and $ADG\ (\%) = (Wt_2 - Wt_1) \times 100 / (Wt_1 \times (t_2 - t_1)$ (Bekcan *et al*., 2006)

Where: W= fish weight (g), L=fish length (cm), Ln= natural log, Wt_1= initial weight (g), Wt_2= final weight (g), t_1= first time, t_2 = final time

Blood analysis

At the end of the trial, to measure blood and immune parameters, fish were anesthetized with clove powder (0.5 g L^{-1}) at first. Then blood samples were drawn from the caudal vein and

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transferred to two sets of microtubes, one set containing heparin anti-coagulant and the other non-heparinizes. The first was immediately used for hematological examinations and the second was used for Sera separation by centrifugation at 1500g for 20 min (Binaii *et al.*, 2014). The Blood and immune parameters include red blood cell (RBC), white blood cell (WBC), hematocrit (Hct), hemoglobin (Hb) levels, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and differential leukocyte counts (lymphocytes, neutrophils, and monocytes) (Feldman *et al*., 2000), llysozyme activity (Ellis *et al.*, 1990), immunoglobulin M (Amar *et al*., 2000), alternative complement pathway activity (ACH50) (Ortuno *et al*., 1998), amylase (Ross *et al*., 2000), lipase (Shihabi and Bishop, 1971), protease (Bernfeld, 1955) and liver enzymes (alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate aminotransferase (AST) (Borges *et al*., 2004) were measured (Table 1).

Table 1: Details of amounts of potential probiotics in different treatments.

Bacteria Treatment	*L. lactis* (CFU kg^{-1})	*W. cibaria* (CFU kg^{-1})	Mixture of *W. cibaria* and *L. lactis* (CFU kg^{-1})
1	1.5×10^7	0	0
2	3.0×10^7	0	0
3	4.5×10^7	0	0
4	0	1.5×10^7	0
5	0	3.0×10^7	0
6	0	4.5×10^7	0
7	0	0	1.5×10^7
8	0	0	3×10^7
9	0	0	4.5×10^7
10	0	0	0

Bacteriological examination

At the end of the experiment, 3 fish were randomly sampled from each treatment and the total count of bacteria, as well as LABs count, was examined. Fish were anesthetized at first, then the abdominal surface was sterilized with alcohol (70%). Fish anesthetized with clove oil (50 mgL^{-1}) and humanly sacrificed and the intestine was completely separated. Intestinal contents were collected (it was washed three times using sterile physiological serum) and weighed. The contents of the intestine were diluted using physiological saline and the desired dilutions were prepared, then cultured on Tryptone Soy Agar (Merck, Germany) and MRS agar (Man, Rogosa, and Sharpe) (Difco Detroit, MI, USA) to determine the total count of bacteria and lactic acid bacteria, respectively. Plates were incubated for 48-72 hours at 30-35°C and the number of colonies grown on plates were then counted by colony counter (Ringo and Gatesoupe, 1998; Mahious *et al.*, 2006).

Statistical Analysis

Obtained data were analyzed using SPSS software (Version 20) and graphs

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were drawn using Excel. First, the normality of the data and homogeneity of variance were checked by Kolmogorov-Smirnov and Levene tests, respectively. To compare blood data and growth coefficients, Two-way analysis of variance and Tukey test was used at 95% confidence level.

Results

Growth performance

The highest amount of body weight gain, percentage of body weight gain, specific growth rate, and average daily growth were observed in treatment 8 and after that in treatment 5, which were significantly different from the control (p<0.05). The data of growth parameters are presented in Table 2.

Table 2: Growth parameters of common carp juveniles fed diets supplemented with different amounts of potential probiotics *L. lactis* and *W. cibaria* after 8 weeks.

Index Treatment	ADG (g)	K	FCR	SGR (%d^{-1})	PBWI (%)	PBW(g)	Final weight (g)
1	0.36 ± 0.01 ^{cd}	0.02 ± 2.58	2.25 ± 0.06 ^{ab}	1.40 ± 0.02 ^{cd}	54.41 ± 0.46 ^{cde}	20.04 ± 0.88 ^{bc}	37 ± 2.12^{c}
2	0.34 ± 0.01 ^{bc}	0.02 ± 2.58	2.16 ± 0.09 ^{a}	1.35 ± 0.02 ^{bc}	53.15 ± 0.36 ^{bc}	18.93 ± 0.56 ^{ab}	36 ± 1.88^{bc}
3	0.33 ± 0.00 ^{ab}	0.01 ± 2.60	2.22 ± 0.05^{ab}	1.30 ± 0.00 ^{ab}	51.70 ± 0.05 ^{ab}	18.53 ± 0.82 ^{ab}	35 ± 1.81^{ab}
4	0.37 ± 0.01 ^{cde}	2.63 ± 0.12	2.26 ± 0.08 ^{ab}	1.43 ± 0.03 ^{cde}	55.00 ± 0.66 ^{cde}	21.00 ± 0.40 ^{cd}	38 ± 1.47^{cd}
5	0.42 ± 0.02 ^{fg}	2.55 ± 0.02	2.13 ± 0.09 ^{a}	1.53 ± 0.04 ^{fg}	57.56 ± 1.02 ^{fg}	23.18 ±0.78 ^{ef}	40 ± 1.74^{ef}
6	0.36 ± 0.02^{cd}	2.57 ± 0.02	2.26 ± 0.05 ^{ab}	1.39 ± 0.05 ^{cd}	54.04 ± 1.16 ^{cd}	20.30 ± 0.96 ^{bcd}	37 ± 1.6^{c}
7	0.40 ± 0.01 ^{ef}	2.61 ± 0.03	2.19 ± 0.03 ^{a}	1.49 ± 0.02 ^{efg}	56.54 ± 0.49 ^{efg}	22.25 ± 0.33 ^{de}	39 ± 1.36^{de}
8	0.43 ± 0.01 ^{g}	2.56 ± 0.05	2.12 ± 0.07 ^{a}	1.57 ± ^{g} 0.02	58.58 ± 0.45 ^{g}	24.30 ± 0.42 ^{f}	41 ± 1.4^{f}
9	0.39 ± 0.02 ^{def}	2.58 ± 0.01	2.14 ± 0.08 ^{a}	1.47 ± 0.04 ^{def}	56.15 ± 0.99 ^{def}	22.07 ± 1.04 ^{de}	39 ± 1.42^{de}
10	0.31 ± 0.02 ^{a}	2.59 ± 0.02	2.43 ± 0.06 ^{b}	1.23 ± 0.04 ^{a}	49.90 ± 1.16 ^{a}	17.06 ± 0.81 ^{a}	34 ± 2.57^{a}
***p*- value**							
W. cibaria	0.001	0.09	0.010	0.010	0.010	0.010	0.010
L.lactis	0.001	0.11	0.060	0.070	0.020	0.010	0.010
L. lactis and *W.cibaria*	0.001	0.10	0.010	0.010	0.010	0.010	0.010

Numbers with different superscripts in the same column are significantly different (p<0.05).

Hematological parameters

According to the results, no significant differences were observed in the number of RBC, Hct, and Hb between treatments (Table 3, p≥0.05).

Table 3: Amount of blood parameters of common carp juveniles fed diets supplemented with different amounts of potential probiotics *L. lactis* and *W. cibaria* after 8 weeks.

Index Treatment	Lymphocytes (%)	Monocytes (%)	Neutrophil (%)	HCT (%)	HB (g/dL)	WBC (mm^3×1000)	RBC (mm^3×1000)
1	79.00±2.00 ^{ab}	5.33±1.15^{ab}	15.33±1.53^{ab}	37.33±1.15	8.20±0.10	5.60±0.46 ^{bcd}	791.67±25.17
2	76.67±0.58^{a}	6.00±1.00^{b}	17.00±1.00 ^{b}	38.67±1.53	8.67±0.32	6.27±0.61^{cd}	819.67±30.44
3	77.00±3.00^{a}	5.33±1.15^{ab}	16.67±1.53^{b}	40.33±2.08	9.00±0.46	6.20±1.18 ^{cd}	868.33±52.52
4	79.67±1.15^{ab}	4.33±0.58^{ab}	15.00±1.00^{ab}	40.33±1.53	8.90±0.26	4.03±0.38^{ab}	859.33±28.04
5	77.33±1.15^{a}	5.33±0.58^{ab}	17.33±1.53^{b}	39.33±1.53	8.83±0.42	5.50±0.72 ^{bcd}	835.33±28.38
6	77.67±1.53^{a}	5.67±1.15^{ab}	15.33±0.58^{ab}	40.33±0.58	8.97±0.21	4.63±0.42^{abc}	855.00±15.62
7	75.00±2.65^{a}	6.00±1.00^{b}	18.33±1.53^{b}	37.67±1.53	8.33±0.40	7.17±0.55^{d}	802.67±29.91
8	79.00±2.00^{ab}	5.00±1.00^{ab}	15.67±1.53^{ab}	38.00±2.00	8.77±0.45	5.50±0.75^{bcd}	837.00±42.51
9	78.67±0.58^{ab}	5.33±0.58^{ab}	15.33±0.58 ^{ab}	38.00±1.00	8.43±0.21	5.67±0.32^{bcd}	809.00±12.77
10	83.33±1.15^{b}	3.33±1.09^{a}	13.00±1.00^{a}	40.00±1.00	8.20±0.10	3.53±0.32^{a}	853.33±20. 21
***p*-value**							
W. cibaria	0.090	0.010	0.010	0.060	0.070	0.010	0.700
L. lactis	0.010	0.010	0.040	0.91	0.001	0.010	0.090
L. lactis & *W. cibaria*	0.000	0.001	0.001	0.100	0.100	0.010	0.110

Numbers with different superscripts in the same column are significantly different (p<0.05).

Also, the lowest number of white blood cells, neutrophils, and monocytes were observed in the control treatment, which was significantly different from most of

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the treatments (p<0.05). The lowest amount of IgM, ACH50, and Lysozyme activity was observed in the control group ($p<0.05$). In general, the highest amount of immune parameters was observed in treatments 5, 8, and 9 (Table 4).

Table 4: Amount of immune parameters of common carp juveniles fed diets supplemented with different amounts of potential probiotics *L. lactis* and *W. cibaria* after 8 weeks.

Index Treatment	Lysozyme activity (u/mL/min)	ACH50 (U %)	IgM ($mgdL^{-1}$)
1	27.33±0.88^{a}	128.67±2.76^{b}	43.00±0.58^{a}
2	33.33±1.20^{ab}	130.00±1.15^{b}	48.00±3.06^{b}
3	34.33±1.20^{ab}	133.67±1.73^{bc}	44.33±0.33^{ab}
4	26.33±0.67^{a}	123.33±2.85^{a}	45.33±0.33^{ab}
5	38.67±0.33^{b}	138.33±0.67^{c}	55.67±0.33^{c}
6	35.00±1.00^{ab}	133.67±0.88^{bc}	51.33±2.23^{b}
7	26.67±0.88^{a}	141.00±2.08^{c}	55.33±2.33^{c}
8	36.67±2.60^{ab}	141.33±1.33^{c}	57.67±1.76^{c}
9	35.67±2.33^{ab}	141.00±0.58^{c}	56.00±1.53^{c}
10	24.67±0.88^{a}	123.67±1.86^{a}	41.00±0.58^{a}
***p*-value**			
W. cibaria	0.010	0.001	0.001
L. lactis	0.010	0.001	0.001
L. lactis & *W. cibaria*	0.025	0.000	0.000

Numbers with different superscripts in the same column are significantly different (p<0.05).

The lowest amount of digestive enzymes as well as, the highest amount of liver enzymes was observed in the control treatment, which showed a significant difference from most other treatments (p<0.05). Changes in liver and digestive enzymes are presented in Table 5.

Table 5: Amount of digestive and liver enzymes of common carp juveniles fed diets supplemented with different amounts of potential probiotics *L. lactis* and *W. cibaria* after 8 weeks.

Index Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	Protease (Umg)	Lipase (U/mg)	Amylase (UmgL)
1	271.67±13.84^{bc}	24.00±1.73^{a}	37.33±1.45^{ab}	37.00 ± 1.52^{ab}	17.00 ± 0.57^{ab}	60.33 ± 0.33^{b}
2	255.00±23.17^{b}	22.00±1.53^{a}	34.67±0.88^{a}	40.66 ± 1.45^{ab}	16.33 ± 0.88^{ab}	5.04 ± 58.33^{b}
3	236.00±21.50^{a}	22.33±1.20^{a}	44.67±2.19^{bc}	40.33 ± 2.84^{ab}	17.00 ± 0.57^{ab}	59.66 ± 2.33^{b}
4	211.00±12.08^{a}	23.67±1.19^{a}	38.33±2.60^{ab}	38.00 ± 0.57^{ab}	16.66 ± 0.33^{ab}	54.66 ± 1.20^{b}
5	225.33±12.67^{a}	23.67±0.33^{a}	48.67±0.33^{c}	40.00 ± 0.33^{b}	18.33 ± 0.57^{ab}	59.33 ± 0.66^{b}
6	235.00±11.02^{a}	24.67±0.88^{a}	42.67±1.20^{b}	45.00 ± 0.57^{c}	20.00 ± 1.15^{b}	60.33 ± 0.88^{b}
7	222.67±18.19^{a}	22.33±0.88^{a}	47.33±2.33^{c}	39.00 ± 3.57^{ab}	14.66 ± 1.25^{a}	62.00 ± 0.57^{b}
8	227.67±19.74^{a}	22.18±1.53^{a}	41.33±0.88^{b}	41.33 ± 2.57^{ab}	18.00 ± 0.57^{ab}	63.66 ± 2.18^{b}
9	246.33±14.67^{a}	21.33±0.88^{a}	47.00±2.65^{c}	40.00 ± 3.60^{ab}	19.66 ± 1.254^{b}	60.00 ± 2.51^{b}
10	312.12±14.04^{c}	29.23±0.33^{b}	64.21±2.19^{d}	30.33 ± 2.60^{a}	12.00 ± 0.57^{a}	40.66 ± 4.09^{a}
***p*-value**						
W. cibaria	0.001	0.031	0.030	0.001	0.001	0.070
L. lactis	0.001	0.041	0.040	0.001	0.003	0.060
L. lactis & *W. cibaria*	0.001	0.001	0.001	0.001	0.001	0.001

Numbers with different superscripts in the same column are significantly different (p<0.05).

Bactria flora

The lowest number of lactic acid bacteria grown in the MRS agar medium was observed in the control group, which showed a significant difference with treatments (p<0.05) (Fig. 1).

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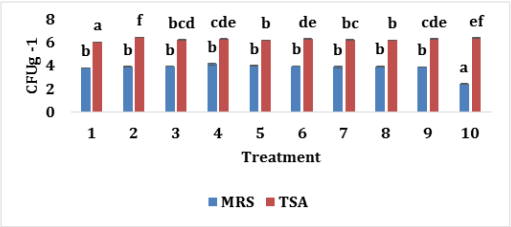


Figure 1: Total count of Bacteria cultured in TSA and MRS agar Interaction p_value (*L. lactis* & *W. cibaria*) for TSA=0.001 and MRS=0.005.

Discussion

In general, the results of the present study indicated the positive effects of two potential probiotics, *W.cibaria* and *L.lactis*, on the growth and immune parameters of the common carp juvenile. The results of growth performance showed that groups fed by the diet supplemented with potential probiotics had better growth performance, especially those in groups 5 and 8.

Enhanced growth performance can be related to an increase in fish appetite due to the stimulation of the digestive system, increase in gastrointestinal efficiency, the population of beneficial microorganisms, and activity of digestive enzymes, also, improvement of intestinal microbial balance leads to better digestion and absorption of feed. Probiotics produce bioactive microbial metabolites such as vitamins, bioactive peptides, organic acids, and fatty acids during fermentation as well as they produce some enzymes (Liu *et al*., 2010) and thus improve the metabolism. Different experiments have shown that probiotics exert their effects through colony formation in the host by secreting growth-promoting nutrients (Bagheri *et al.*, 2008; Mohapatra *et al*., 2012). Irianto and Austin (2002) also stated that adding probiotics to fish feed increase digestive enzyme activity, stimulation of fish appetite, and ultimately increases fish growth. On the other hand, another study reported that biological compounds such as vitamins (especially B vitamins likebiotin and B_{12}), digestive enzymes, proteolytic and peptidolytic enzymes breakdown the indigestible macromolecular compounds by hydrolyzing to peptides and amino acids that could lead to better absorption of nutrients (Abd El-Rhman *et al*., 2009).

Similar to our finding, the positive effects of various probiotics in improving and increasing growth performance have been proven in other studies (Xuxia *et al*., 2010, Beck *et al.*, 2015, Hosseini *et al*., 2016, Hashemi monfared *et al.*, 2016, Nguyen *et al.*, 2017). It should be noted that some bacterial probiotics did not induce desired effects on fish. The reason may be related to type, form, and dose of probiotics, the probiotic carrier, feeding duration, size, and life stage of examined fish (Olsen *et al*., 2001; Mohapatra *et al*., 2012; Yazici *et al.*, 2015).

This is reported that lactic acid bacteria produce compounds such as bacteriocins and thus inhibit the growth of other microorganisms (Vazquez *et al*., 2005) and increase their own population. Lactic acid bacteria can survive effectively in the gastrointestinal tract. They should attach to the intestinal tract to act their probiotic role (Argyri *et al*., 2013; Wang *et al.*, 2014). As a result, according to the positive results obtained after using these potential probiotics, it

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seems that the potential probiotic bacteria used in the present study attached suitably.

In the present study, it was found that the number of lactic acid bacteria, was significantly increased in fish that fed diets supplemented with potential probiotics (p<0.05). A significant increase of lactic acid bacteria was reported in Persian sturgeon (*Acipenser persicus*) (Shenavar masuleh *et al*., 2016) and Nile tilapia (Balcazar and Rojas-Luna, 2007) intestines after consumption of probiotics via their diets. It should be noted that studies about the effect of lactococci probiotics on common carp are limited. Feng *et al.* (2019), reported improvement in growth performance and immunity in common carp. No examination was done about the role of *lactococcus* in the microbial balance of the intestine. According to the results, it can be pointed out that the proper colonization of probiotic bacteria was due to the appropriate conditions of stabilization, colonization, and growth in the intestine of common carp.

In the present study, there were no significant changes in RBC number, hematocrit, and hemoglobin levels (p>0.05). In the current research, blood indices did not change as were reported in common carp (Panahi Sahebi *et al*., 2019) and Caspian salmon (*Salmo trutta caspius*) (Hosseini *et al*., 2014). Improper dietary supplements can sometimes cause anemia and reduction of RBC, hemoglobin, and hematocrit, which is usually due to bleeding, hemolysis, or a decrease in RBC production (Hedayati *et al*., 2013), but no negative effect on the hematopoietic process was observed in the present study.

Blood leukocytes such as lymphocytes, neutrophils, and monocytes are parts of the nonspecific cellular immune system. In this study, immune cells were affected by probiotics and the percentage of monocytes and neutrophils in most probiotic-treated fish was significantly more than in the control group. Change in leukocyte number is one of the appropriate indicators that show fish response to various elements like pathogens, etc (Stoskopf, 1993; Nikoskelainen *et al.*, 2003).

It shows that *W. cibaria* and *L. lactis* improved the immune system performance in common carp, especially in combination and the dose of 3×10^7, and 4.5×10^7 CFU kg^{-1}, and 3×10^7 CFU kg^{-1} of *W. cibaria*. Similar to our finding, lactobacilli probiotics increased immune responses and lysozyme activity in Caspian salmon (*Salmo trutta*) (Balcazar and Rojas-Luna, 2007). Besides, an increase in lysozyme activity was reported in rainbow trout fed diet containing *Lactobacillus rhamnous* (Panigrahi *et al*., 2004) and *Pediococcus acidilactici* caused a significant increase in total immunoglobulin and lysozyme activity in *Huso huso* (Ghiasi *et al.*, 2018). The lysozyme levels, especially in serum, reflect the activity of monocytes, neutrophils, and phagocytic cells (Pararat *et al*., 2011). Therefore, in the present study increasing lysozyme along with increasing monocytes and neutrophils can be considered as an

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effective factor in the improvement of the immune system of common carp. On the other hand, an increase in lysozyme activity, ACH50, and immunoglobulin, induced by the metabolic activity of probiotic bacteria are the important mechanisms for promoting the immune system in fish (Pourabbasali *et al.*, 2019).

Liver enzymes are known as indicators for biochemical factors in fish under stress (Newaj-Fyzul *et al.*, 2007). Alanine aminotransferase and AST enzymes are two important enzymes that indicate damage (Pascual *et al.,* 2003; Kumar *et al.,* 2011). Sometimes, ALT and AST secretion in the blood increase following the use of oral additives (Mohapatra *et al*., 2012). Similar to our finding, probiotics *Micrococcus luteus* and *Pseudomonas* spp. in Nile tilapia caused a significant reduction in AST and ALT in probiotic-treated fish in comparison to the control (Abd El-Rhman *et al*., 2009), and a reduction in ALP level was observed in Rainbow trout fed diet supplemented with *Saccharomyces cerevisiae* var. *boularadii* (Wache *et al.,* 2006). In another study, AST and ALT levels in Nile tilapia were not affected by probiotics and no significant difference in AST and ALT levels was observed between probiotic and control treatments (Won *et al.,* 2020).Potential probiotics used in the present study are effective with no side effects on common carp juveniles. Since probiotics improve digestion and absorption of nutrients, increase the absorption of vitamins, and improve immune function, these are effective in reducing stress, improving liver function, and consequently reducing liver enzyme levels.

In conclusion, it could be stated that consumption of potential probiotic bacteria *W. cibaria* and *L.lactis*, especially in combination and at a dose of 3×10^7 CFU kg^{-1}, and after that 3×10^7 CFU kg^{-1} of *W. cibaria* and 4.5×10^7 CFU kg^{-1} combination of these two potential probiotic will improves growth performance, the activity of digestive enzymes, intestinal microbial flora, and immune function in common carp juvenile with no negative effect on the liver.

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