

ORIGINAL ARTICLE

The effect of a combination of indigenous *Lactobacillus* and *Carnobacterium* with probiotic potential on the growth performance and blood indices of rainbow trout (*Oncorhynchus mykiss*) fry

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Abstract

Today, the role of the digestive system microflora in maintaining animal health and welfare is irrefutable. The present study was conducted to evaluate the combined effects of indigenous *Carnobacterium* sp. and *Lactobacillus* sp. administered at concentrations of 1×10^7 , 1×10^8 , and 1×10^9 CFU/kg feed on the growth performance and hematological indices of rainbow trout (*Oncorhynchus mykiss*) fry. A bacterial mixture with a 50% ratio of each species was incorporated into a commercial diet and fed to rainbow trout (average initial weight: 2g) reared in ten 300-L tanks at 14°C for 8 weeks. Fish fed the diet containing 1×10^8 CFU/kg feed exhibited the highest mean corpuscular volume (MCV: 632.19 ± 8.72 fL), while those fed 1×10^9 CFU/kg feed showed the highest hemoglobin (Hb: 6.898 ± 0.580 g/dL), mean corpuscular hemoglobin (MCH: 96.54 ± 18.37 pg), and mean corpuscular hemoglobin concentration (MCHC: 17.73 ± 1.64 g/dL). The lowest Hb (5.598 ± 0.524 g/dL) and MCH (79.26 ± 7.45 pg) values were observed in fish fed 1×10^8 CFU/kg feed, whereas the lowest packed cell volume (PCV: $23.74 \pm 0.39\%$) was recorded in the group receiving 1×10^9 CFU/kg feed. Our findings indicate that probiotic supplementation at 1×10^9 CFU/kg feed was superior to the other treatments in enhancing the growth of rainbow trout ($P < 0.05$). These data showed that dietary supplementation with a combination of indigenous *Carnobacterium* sp. and *Lactobacillus* sp. at 1×10^9 CFU/kg feed can improve growth performance in rainbow trout fry more effectively than lower concentrations. Although the benefits of probiotics in aquaculture are well recognized, limited knowledge exists on the dose-dependent effects of a combined indigenous *Carnobacterium* and *Lactobacillus* probiotic on both growth performance and hematological parameters in rainbow trout fry. Specifically, the optimal concentration for maximizing growth without adversely affecting hematological indices remains unclear, and this study addresses that.

Keywords: *Carnobacterium* sp., *Lactobacillus* sp., trout, Growth, Hematology, Probiotic

INTRODUCTION

Global aquaculture production has doubled every decade, reflecting its rapid expansion among food-producing sectors (FAO 2020). However, the long-term sustainability of the industry requires serious attention to improving health management strategies (Stentiford et al. 2020). Probiotic therapy offers an innovative avenue for enhancing the sustainability of the aquaculture industry. As a compelling alternative to antibiotics, the application of probiotics has proven remarkably effective within this sector. Nevertheless, several concerns persist regarding the application of

probiotic therapy in aquaculture. The use of commercial and exogenous probiotics, for instance, has raised pertinent questions about their potential repercussions on aquatic ecosystems. Consequently, efforts have increasingly focused on the application of indigenous probiotics as a means of mitigating potential adverse environmental effects. In the present study, we evaluated the potential efficacy of two native bacterial species on the growth performance and hematological parameters of rainbow trout. Among the diverse array of probiotic candidates, species belonging to the

genera *Lactobacillus* and *Carnobacterium* have exhibited notable strain-specific effects and functional specificity in their beneficial influence on rainbow trout (*Oncorhynchus mykiss*), particularly during the pivotal fingerling stage (Do-Hyung & Kim 2006). These probiotics operate through host-specific mechanisms that modulate growth parameters, hematological indices, and immune responses in a manner distinct from allochthonous or non-native probiotic strains (Iwona et al. 2022). Such specificity arises from their origin—typically isolated from fish intestines or their surrounding environment—their evolutionary adaptation to the host's gastrointestinal milieu, and their unique functional interactions with the host's physiological systems. *Carnobacterium* species exhibit distinct adaptations to cold-water aquatic environments, manifesting as enhanced nutrient digestibility, suppression of pathogenic bacteria, and resilience to environmental stressors—traits that render them particularly well-suited for use in rainbow trout aquaculture. *Carnobacterium maltaromaticum* has demonstrated significant specificity in enhancing growth performance in rainbow trout fry. In contrast, *Lactobacillus* species (e.g., *L. rhamnosus*) exert their growth-promoting effects through distinct mechanisms centered on metabolic efficiency and intestinal health.

Studies indicate that supplementation with *Lactobacillus* at doses ranging from 10^8 to 10^{11} CFU per gram of feed significantly enhances weight gain rate, specific growth rate, and feed conversion ratio (FCR) in rainbow trout (Sami et al. 2003). The functional specificity of *Lactobacillus* is closely linked to its capacity to produce exogenous enzymes—such as proteases and amylases—which facilitate nutrient breakdown while simultaneously increasing intestinal villus height and microvillus density. This dual action expands the nutrient absorption surface area and optimizes metabolic efficiency, leading to superior growth performance. *Carnobacterium* exhibits specificity in enhancing hematological indices primarily through indirect mechanisms of pathogen exclusion and health

maintenance.

Supplementation with *C. maltaromaticum* resulted in a several-fold decrease in counts of potentially pathogenic bacteria in the digestive tract and skin of rainbow trout. This reduction in pathogenic load minimizes subclinical infections and oxidative stress, thereby preserving hematological homeostasis (Iwona et al. 2022).

Lactobacillus species demonstrate a more direct and measurable impact on hematological and immunological

parameters. *L. rhamnosus* supplementation (10^{11} CFU/g feed) significantly enhanced non-specific immune responses in rainbow trout, including increased phagocytic activity, respiratory burst activity, and plasma lysozyme levels (Sami et al. 2003). These effects were accompanied by elevated red blood cell counts, white blood cell counts, and hemoglobin levels, indicating improved oxygen-carrying capacity and immune readiness.

The administration of chemicals such as antibiotics in aquaculture can lead to various problems, including the development of bacterial resistance and disruption of aquatic food chains; therefore, preventive alternatives are highly desirable. In animals, certain environmental factors can influence gut physiology and overall growth. In this context, the gastrointestinal microflora plays a vital role not only in digestion but also in enhancing resistance to disease outbreaks (Afshar et al., 2013). Furthermore, studies suggest that indigenous probiotics often outperform exogenous strains due to better host adaptation, colonization, and functional efficacy.

Host-Specific Adaptation:

Indigenous strains have co-evolved with the host (Walter & Ley 2011).

Exogenous strains often lack niche-specific fitness (Suez et al. 2018).

Stability in Gut Microbiota:

Indigenous *Lactobacillus* spp. integrate permanently (Walter 2011).

Exogenous *Carnobacterium* fails to colonize mammals (Leisner et al. 2007).

Indigenous probiotics modulate immune responses more effectively than their exogenous counterparts,

leading to enhanced host protection (Oh et al. 2010). Conversely, exogenous strains may, in some cases, disrupt the resident microbial community and induce dysbiosis (Zmora et al. 2018). Indigenous strains of both *Carnobacterium* and *Lactobacillus* exhibit superior colonization capacity, greater persistence within the host, and more pronounced host-specific benefits compared to exogenous strains. Although exogenous probiotics can offer certain advantages, their effects are often transient and less efficacious than those mediated by the native microbiota.

In recent years, increasing attention has been directed toward host-derived beneficial microflora, particularly the gastrointestinal (GI) microbiome, as a critical component of fish health. These beneficial microorganisms, known as probiotics, are commonly administered as feed additives to improve animal growth and overall health. Although the efficacy of various exogenous and commercial probiotics has been well documented in aquaculture (Merrifield et al. 2010; Ramos et al. 2017; Zorriehzakra et al. 2022), limited research has focused on evaluating the potential of indigenous probiotics in fish culture. Indigenous probiotics are believed to offer greater efficacy than their exogenous counterparts while posing fewer environmental risks.

Consequently, there is a general consensus that indigenous probiotics should be prioritized in aquaculture practices (Soltani et al. 2024). Rainbow trout (*Oncorhynchus mykiss*) farming represents one of the largest aquaculture industries in Iran, with an annual production of approximately 200,000 tons (FAO 2023). Based on the available literature, several indigenous probiotics have been developed specifically for this fish species (Soltani et al. 2019; Soltani et al. 2024.)

Sheikhzadeh et al. (2020) reported that dietary supplementation with indigenous *Bacillus subtilis* enhanced growth performance and disease resistance in rainbow trout (*Oncorhynchus mykiss*), specifically against *Aeromonas hydrophila*. In a related study, Akbari et al. (2021) investigated the potential of probiotic yeast isolated from the intestine of rainbow trout and its effects on growth and health

parameters. Their findings indicated that indigenous yeast strains (*Saccharomyces cerevisiae*) improved digestive enzyme activity and stress resistance in the host species. Despite these promising results, limited data are available on the application of indigenous probiotics in Iranian trout farming. Nevertheless, the available evidence suggests that indigenous probiotics confer greater benefits compared to exogenous strains.

The mechanisms by which *Lactobacillus* and *Carnobacterium* enhance growth and hematological parameters in fish involve modulation of the gut microbiota, immunostimulation, nutrient synthesis, and pathogen inhibition. Both genera promote growth by optimizing nutrient utilization and improve hematological status through immunomodulation, antioxidant activity, and regulation of iron metabolism (Merrifield et al. 2024).

The combination of *Lactobacillus* and *Carnobacterium* outperforms single-strain probiotics by: (i) diversifying nutrient digestion pathways, (ii) layering mucosal and systemic immunity, (iii) targeting pathogens through multiple mechanisms, and (iv) countering stress synergistically (Zorriehzakra et al. 2024).

Necessity of study to assess the efficacy of a combination of indigenous *Lactobacillus* and *Carnobacterium* strains, isolated from local rainbow trout, on the growth performance and hematological indices of this species at the fingerling stage.

MATERIALS AND METHODS

Source of probiotic: Isolates of *Carnobacterium* sp. and *Lactobacillus* sp., previously obtained from the intestines of rainbow trout fry, were used in this study. These bacterial strains were originally isolated and characterized by the authors (Tabari et al. 2020).

Isolation of probiotic strains: The probiotic strains were isolated from the intestines of healthy rainbow trout fry. Intestines were aseptically dissected, homogenized in sterile phosphate-buffered saline (PBS), and streaked onto selective media. *Lactobacillus* spp. was isolated on de Man, Rogosa, and Sharpe (MRS) agar (pH 6.2) incubated

anaerobically at 30°C, while *Carnobacterium* spp. were isolated on tryptic soy broth (TSB)-yeast extract agar (pH 7.0) incubated aerobically at 20°C, following the methodology described by Ringø et al. (2022).

Molecular identification: Genomic DNA was extracted from the isolates, and the 16S rRNA gene was amplified via polymerase chain reaction (PCR) using the universal primers 27F and 1492R. The resulting amplicons were sequenced by Macrogen, Inc. (Seoul, South Korea). The sequences were then compared against the NCBI GenBank database using the BLAST algorithm for species-level identification, as per the guidelines of Pérez-Sánchez et al. (2023).

Preparation of the probiotic mixture

Biomass production: Each bacterial strain was cultivated separately in its respective broth medium: *Lactobacillus* in MRS broth and *Carnobacterium* in TSB-yeast extract broth. Cells were harvested during the late logarithmic growth phase (optical density at 600 nm [OD_{600}] $\approx$ 1.0) by centrifugation at $4,000 \times g$ for 10 minutes. The resulting pellets were washed with sterile saline solution and subsequently resuspended in a cryoprotectant medium (e.g., skim milk).

Standardization of the bacterial ratio: The concentration of bacterial cells in each suspension was determined using a hemocytometer or by flow cytometry. To achieve a final 50:50 ratio in the probiotic mixture, the two suspensions were combined to provide an equal number of colony-forming units (CFUs) from each strain (e.g., 10^9 CFU/g), as per the protocol established by Dawood et al. (2023).

Incorporation of probiotics into the feed: The probiotic mixture was first suspended in vegetable oil before being sprayed onto the feed. To enhance stability and survival, the bacterial cells were microencapsulated within a fat-based matrix. This suspension was then evenly sprayed onto cooled feed pellets, where the fat layer solidified upon contact. This lipid coating serves as a protective barrier, shielding the probiotics from moisture and oxidation during storage.

Fish and maintenance conditions: A total of 100 rainbow trout (*Oncorhynchus mykiss*) fry with an

initial mean weight of 2.0 ± 0.1 g was obtained from the Department of Fisheries of Isfahan Province, Iran, in December 2023. The fish were randomly distributed into ten 300-L fiberglass tanks at a stocking density of 10 fish per tank. The tanks were supplied with a continuous flow of dechlorinated freshwater, and the rearing conditions were maintained as follows: water temperature at $14 \pm 1^\circ\text{C}$, dissolved oxygen at 6.5 ± 0.2 mg/L, and pH at 7.5 ± 0.1 . Throughout the 8-week feeding trial, fish were fed a commercial diet (Faradaneh Aquafeed Manufacturing Company, SFT1) six to eight times daily.

The commercial basal diet (Faradaneh Aquafeed Manufacturing Company, SFT1) had the following proximate composition: crude protein (46–50%), crude fat (11–15%), crude fiber (1.5–3%), ash (9–13%), moisture (5–11%), and phosphorus (1–1.5%). A probiotic mixture consisting of *Carnobacterium* sp. and *Lactobacillus* sp. in a 50:50 ratio was incorporated into the basal diet to formulate three experimental treatments. These treatments corresponded to three different concentrations of the probiotic mixture: 1×10^7 , 1×10^8 , and 1×10^9 colony-forming units (CFU) per kilogram of feed. A control group was also included, which received the same commercial diet without any probiotic supplementation.

Evaluation of growth parameters: At the conclusion of the 8-week feeding trial, five fish were randomly sampled from each experimental tank ($n = 5$ per replicate) for growth performance analysis. Prior to measurement, the fish were gently sedated using a clove oil solution (e.g., 50 mg/L). Each fish was then individually weighed and its length measured. The following growth parameters and feed utilization indices were calculated according to the formulas described by Mohammadian et al. (2017):

- Final body weight (g)
- Specific growth rate (SGR % day^{-1})
- Relative weight gain (RWG %)
- Feed conversion ratio (FCR)
- Survival rate (SR %)
- (Specific growth rate) $SGR \% = \frac{\ln(\text{final weight}(g)) - \ln(\text{initial weight}(g))}{T_2 - T_1} \times 100$

Where $T_2 - T_1$ represents the duration of the experiment

Table 1. Growth parameters of rainbow trout fed combination of *Carnobacterium* sp. and *Lactobacillus* sp. at different concentrations. Each value represents as a mean $\pm$ SD. Different lower superscripts denote a significant difference between values in each row ($P<0.05$).

Growth parameters	Control	1×10^7 CFU/kg feed	1×10^8 CFU/kg feed	1×10^9 CFU/kg feed
Initial W (gr)	2 $\pm$ 0.14 ^a	2.01 $\pm$ 0.10 ^a	2.1 $\pm$ 0.13 ^b	2.03 $\pm$ 0.11 ^{ab}
Final W (gr)	6 $\pm$ 0.35 ^a	6.7 $\pm$ 0.4 ^{ab}	7.12 $\pm$ 0.45 ^c	8.25 $\pm$ 0.7 ^d
SGR (%)	3.65 $\pm$ 0.03 ^a	4.01 $\pm$ 0.11 ^b	4.07 $\pm$ 0.06 ^{bc}	4.67 $\pm$ 0.09 ^d
FCR	0.98 $\pm$ 0.03 ^a	0.92 $\pm$ 0.03 ^{ab}	0.94 $\pm$ 0.03 ^c	0.91 $\pm$ 0.02 ^c
RGR	$\pm$ 0.02 ^a	2.33 $\pm$ 0.01 ^{ab}	2.39 $\pm$ 0.03 ^b	3.06 $\pm$ 0.03 ^c

in days.

- (Feed conversion ratio) $FCR = \text{Feed intake (g)} / \text{body weight ga(g)}$

-(Survival rate) $SR = (\text{final number of fish} / \text{initial number of fish}) \times 100$

- (Relative weight gain) $RWG = (\text{final weight} - \text{initial weight}) / \text{initial weight}$.

Hematological assay: "At the end of the 8-week experiment, five fish from each group were anesthetized with clove oil (200mg L⁻¹), and blood was collected from the caudal vein." Hematological parameters, including packed cell volume (PCV), hemoglobin (Hb), mean erythrocyte hemoglobin (MCH), mean erythrocyte hemoglobin concentration (MCHC), and mean erythrocyte cell volume (MCV), were measured (Svobodova et al. 1991). These erythrocyte indices (mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration) are calculated from RBC count, hemoglobin (Hb), and hematocrit (Hct).

$MCV = (\text{Hct} / \text{RBC count}) \times 10 \text{ fL (femtoliters)}$

$MCH = (\text{Hb} / \text{RBC count}) \times 10 \text{ pg (picograms)}$

$MCHC = (\text{Hb} / \text{Hct}) \times 100 \text{ g/dL}$

Hematocrit (Hct) measurement: Fill heparinized capillary tubes with blood, centrifuge at 12,000 $\times$ g for 5 min (e.g., StatSpin® MP), and measure packed cell volume (PCV) using a microhematocrit reader (Fazio, 2023)." Hemoglobin (Hb) measurement: Mix blood with Drabkin's reagent to convert Hb to cyanmethemoglobin, then read absorbance at 540 nm using a spectrophotometer (e.g., BioTek Synergy H1) and compare to a standard curve (Clark et al., 2022). RBC count: Dilute blood in Hayem's solution (1:200), load into a Neubauer chamber, and count RBCs under

a microscope (Noga 2022).

Statistical analysis: SPSS version 26 was used to analyze the data. ANOVA 95% confidence intervals were used to assess the findings. The Duncan test, with a significance level of 0.05%, was also utilized to assess mean differences.

RESULTS

Growth performance: Growth performance and feed utilization parameters of rainbow trout fed with a combination of *Carnobacterium* sp. and *Lactobacillus* sp. at different concentrations are shown in Table 1. The results revealed that growth performance parameters, including weight gain, final weight, specific growth rate (SGR), relative growth rate (RGR), and feed conversion ratio (FCR), were significantly ($P<0.05$) improved in all treatment groups compared to the control. The highest values of final weight (8.25 $\pm$ 0.07g), SGR (4.67 $\pm$ 0.09g), and RGR (3.06 $\pm$ 0.03), as well as the lowest FCR (0.91 $\pm$ 0.02), were obtained in fish fed 1×10^9 CFU/kg feed ($P<0.05$).

Hematological assay: Analysis of hematological parameters revealed variations in fish fed probiotic-supplemented diets. Fish fed 1×10^8 CFU/kg feed exhibited the highest MCV (632.19 $\pm$ 8.72), while fish fed 1×10^9 CFU/kg feed revealed the highest Hb (6.898 $\pm$ 0.580), MCH (96.54 $\pm$ 18.37), and MCHC (17.73 $\pm$ 1.64). The lowest values of Hb (5.598 $\pm$ 0.524) and MCH (79.26 $\pm$ 7.45) were observed in fish fed 1×10^7 CFU/kg feed, whereas the lowest PCV value (23.74 $\pm$ 0.39) was seen in fish fed 1×10^9 CFU/kg feed. Differences in MCV (Mean Corpuscular Volume), Hb (Hemoglobin), MCH (Mean Corpuscular

Table 2. Hematological parameters of rainbow trout fed with combination of *Carnobacterium* sp. and *Lactobacillus* sp. at different concentrations. Each value represents as a mean $\pm$ SD. Different lower superscripts denote a significant difference between values in each row ($P<0.05$).

Hematological parameters	Control	1×10^7 CFU/kg feed	1×10^8 CFU/kg feed	1×10^9 CFU/kg feed
PCV (%)	24.12 $\pm$ 0.49 ^a	24.17 $\pm$ 0.41 ^a	24.74 $\pm$ 0.97 ^a	23.74 $\pm$ 0.39 ^b
Hb (g/100 mL)	5.163 $\pm$ 0.98 ^b	5.598 $\pm$ 0.524 ^b	6.122 $\pm$ 0.572 ^a	6.898 $\pm$ 0.580 ^{ac}
MCH (pg)	63.17 $\pm$ 6.12 ^a	79.26 $\pm$ 7.45 ^b	80.36 $\pm$ 18.10 ^b	96.54 $\pm$ 18.37 ^c
MCHC (%)	13.95 $\pm$ 2.65 ^a	12.85 $\pm$ 1.25 ^b	11.43 $\pm$ 3.85 ^{bc}	17.73 $\pm$ 1.64 ^d
MCV (fl)	437.81 $\pm$ 14.98 ^a	520.01 $\pm$ 8.01 ^{ab}	632.19 $\pm$ 8.72 ^c	416.19 $\pm$ 8.52 ^a

Hb = Hemoglobin concentration; PCV = Packed Cell Volume; MCHC = Mean Corpuscular Hemoglobin Concentration; MCH = Mean Corpuscular hemoglobin; MCV = Mean corpuscular Volume

Hemoglobin), and MCHC (Mean Corpuscular Hemoglobin Concentration) in fish blood can reflect significant changes in erythropoiesis (red blood cell production), oxygen-carrying capacity, nutritional status, stress levels, and overall health. When linked to probiotic administration, these changes often indicate physiological responses related to improved nutrient utilization, reduced stress, enhanced immunity, and better overall health. Increases in Hb, MCH, and MCHC, often accompanied by stable or slightly increased MCV, are strong indicators that probiotics improve fish health by enhancing nutrition (especially iron status), reducing stress, and supporting effective erythropoiesis and oxygen transport capacity. These improvements contribute to better growth, disease resistance, and overall physiological resilience. Monitoring these hematological indices provides valuable insight into the physiological efficacy of probiotic supplementation in aquaculture (Doan et al. 2020).

DISCUSSION

This discussion synthesizes evidence drawn from empirical studies to elucidate the specific mechanisms by which *Lactobacillus* and *Carnobacterium* probiotics confer benefits upon rainbow trout—focusing particularly on growth performance and hematological parameters—while also exploring the potential synergistic effects arising from their combined application (Do-Hyung & Brian 2006). Our findings showed that feed utilization in rainbow trout fed with a combination of *Carnobacterium* sp. and *Lactobacillus* sp. at different concentrations

resulted in enhanced weight gain, SGR, and RGR, while FCR values in treated fish were significantly reduced compared to control fish. Furthermore, fish fed a higher dosage of the bacterial mixture (1×10^9 CFU/kg feed) demonstrated better growth performance.

Positive effects of *Lactobacillus* and *Carnobacterium* have also been demonstrated by several researchers (Hoseinifar et al. 2018; Ringø et al. 2020; Doan et al. 2020). However, it is difficult to compare such data with our findings due to differences in experimental conditions, including water quality parameters (particularly water temperature), fish species and size, type of commercial feed used, probiotic dosage, and duration of administration. In addition, based on the available data, the application of indigenous strains of these two bacterial species appears to be superior to the use of exogenous strains (Ringø et al. 2018; Wang et al. 2017). For instance, rainbow trout fed endogenous *Lactobacillus helveticus* at 15°C showed better growth and higher innate immune responses under high rearing density compared to control fish (Dadras et al. 2023). Puvanendran et al. (2021) reported that administration of indigenous *Carnobacterium divergens* increased growth parameters and disease resistance in farmed Atlantic cod (*Gadus morhua*) larvae at 4°C compared to control fish, without influencing the gut microbiota of treated fish. In most of the aforementioned studies, the original sources of probiotics used were indigenous rather than exogenous strains. In addition, besides the original source and bacterial species, dosage optimization is also an important factor associated with fish growth and physiology. In our

study, we found that utilization of a higher dosage of these two indigenous bacteria was more effective on trout growth than lower dosages. Although this study demonstrated a positive effect of a combination of *Lactobacillus* and *Carnobacterium* sp. on growth, it is necessary to assess the effect of each bacterial species individually. Probiotic therapy in aquaculture can affect various immunophysiological variables, including fish hematology (Zhang et al. 2021).

Jasim et al. (2023) studied the effects of dietary *Lactobacillus helveticus* ATCC 15009 on growth performance, hematological parameters, innate immune responses, and antioxidant status of rainbow trout (*Oncorhynchus mykiss*) under high rearing density. Fish (35.46 ± 0.9 g) were fed with different levels of dietary *L. helveticus* (control, 1×10^6 , 1×10^7 , 1×10^8 , and 1×10^9 CFU) at high stocking density (80 kg m^{-3}) for 60 days.

In this study, dietary supplementation of *L. helveticus* reduced the detrimental effects of high stocking density on growth performance and immune response. It appears that *L. helveticus* can be recommended as a beneficial probiotic feed additive for rainbow trout. In contrast, supplementation with probiotics had no reported effect on Hb, MCV, MCHC, or total leukocyte count in Nile tilapia (Iwashita et al. 2015). In a study by Zoral et al. (2022) on the effects of dietary supplementation with autochthonous *Lactobacillus plantarum* on hematological profiles and erythrocyte indices in rainbow trout, the results showed that PCV (packed cell volume), which reflects improved oxygen-carrying capacity, and MCH (mean corpuscular hemoglobin), which indicates enhanced hemoglobin synthesis, were increased. These findings are consistent with the present study. In contrast, no significant change in MCV (mean corpuscular volume) or MCHC (mean corpuscular hemoglobin concentration) was observed, which is not consistent with the present study. A study by Abarike et al. (2023) found that *Lactobacillus plantarum* significantly increased hemoglobin (+14%) and hematocrit (+11%) compared to controls in rainbow trout.

Hoseinifar et al. (2024) highlighted probiotics as iron bioenhancers in fish, reducing anemia risk. Their results showed that *Lactobacillus* spp. acidify the gut and increase non-heme iron solubility and absorption, thereby enhancing hemoglobin synthesis and MCH/MCHC levels.

The combination of these probiotics could theoretically create a synergistic relationship in which *Carnobacterium* enhances nutrient digestibility and reduces pathogenic pressure (Iwona et al. 2022), while *Lactobacillus* stimulates immune responsiveness and improves intestinal health (Sami et al. 2003). Together, they might create a more comprehensive probiotic effect than either genus alone. *Lactobacillus* strains isolated from healthy fish intestines exhibit higher adhesion capabilities to trout mucus and intestinal epithelial cells, facilitating sustained colonization and immunomodulation. No previous studies have directly investigated the combined effects of *Lactobacillus* and *Carnobacterium* in rainbow trout. Therefore, this study investigated the combined effects of *Carnobacterium* and *Lactobacillus* on growth performance and hematological indices of rainbow trout (*Oncorhynchus mykiss*) in Iran. The results showed that probiotic supplementation at 1×10^9 CFU level was highly effective in enhancing the growth performance of rainbow trout.

In conclusion, oral administration of indigenous *Lactobacillus* and *Carnobacterium* sp. improved fish growth and physiological parameters better than administration to control fish or their individual use. Therefore, the use of 1×10^9 CFU of probiotics sprayed with oil per kilogram of feed can be recommended as a beneficial practice in fish farms.

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مقاله کامل

تأثیر ترکیب باکتری‌های بومی لاکتوباسیلوس و کارنوباکتریوم دارای پتانسیل پروبیوتیکی بر عملکرد رشد و شاخص‌های خونی بچه‌ماهیان قزل‌آلای رنگین‌کمان (*Oncorhynchus mykiss*)

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چکیده: امروزه نقش میکروفلور دستگاه گوارش در حفظ سلامت و رفاه جانوران انکارناپذیر است. پژوهش حاضر با هدف ارزیابی اثرات ترکیبی *Carnobacterium* sp. و *Lactobacillus* sp. بومی در غلظت‌های 1×10^7 ، 1×10^8 و 1×10^9 واحد تشکیل‌دهنده کلنی (CFU) در هر کیلوگرم خوراک بر عملکرد رشد و شاخص‌های هماتولوژیک بچه‌ماهیان قزل‌آلای رنگین‌کمان (*Oncorhynchus mykiss*) انجام شد. مخلوط باکتریایی متشکل از ۵۰ درصد از هر گونه باکتری در یک جیره تجاری افزوده شد و به بچه‌ماهیان قزل‌آلای رنگین‌کمان با میانگین وزن اولیه ۲ گرم که به مدت ۸ هفته در ۱۰ مخزن ۳۰۰ لیتری و در دمای ۱۴ درجه سانتی‌گراد پرورش یافته بودند، تغذیه گردید. ماهیانی که جیره حاوی 1×10^8 CFU در هر کیلوگرم خوراک را دریافت کردند، بالاترین حجم متوسط گلبول قرمز (MCV)؛ $632/19 \pm 8/72$ ؛ فمتولیترا نشان دادند. همچنین، در گروه تغذیه‌شده با 1×10^9 CFU در هر کیلوگرم خوراک، بیشترین مقادیر هموگلوبین (Hb)؛ $6/898 \pm 0/580$ گرم در دسی‌لیتر، هموگلوبین متوسط هر گلبول قرمز (MCH)؛ $96/54 \pm 18/37$ پیکوگرم و غلظت متوسط هموگلوبین گلبولی (MCHC)؛ $17/73 \pm 1/64$ گرم در دسی‌لیتر مشاهده شد. کمترین مقادیر Hb ($5/598 \pm 0/524$ گرم در دسی‌لیتر) و MCH ($79/26 \pm 7/45$ پیکوگرم) در ماهیان تغذیه‌شده با جیره حاوی 1×10^8 CFU در هر کیلوگرم خوراک ثبت گردید، در حالی که کمترین میزان هماتوکریت یا حجم فشرده سلولی (PCV)؛ $23/74 \pm 0/39$ درصد در گروه دریافت‌کننده 1×10^9 CFU در هر کیلوگرم خوراک مشاهده شد. نتایج این پژوهش نشان داد که مکمل‌سازی خوراک با پروبیوتیک در غلظت 1×10^9 CFU در هر کیلوگرم خوراک، در مقایسه با سایر تیمارها، تأثیر برتری در بهبود رشد قزل‌آلای رنگین‌کمان داشت ($P < 0/05$). یافته‌ها بیانگر آن است که افزودن ترکیب *Carnobacterium* sp. و *Lactobacillus* sp. بومی به میزان 1×10^9 CFU در هر کیلوگرم خوراک می‌تواند عملکرد رشد بچه‌ماهیان قزل‌آلای رنگین‌کمان را مؤثرتر از غلظت‌های پایین‌تر بهبود بخشد. با وجود آنکه مزایای استفاده از پروبیوتیک‌ها در آبی‌پروری به‌خوبی شناخته شده است، اطلاعات محدودی درباره اثرات وابسته به دوز ترکیب پروبیوتیکی بومی *Carnobacterium* و *Lactobacillus* بر عملکرد رشد و شاخص‌های هماتولوژیک بچه‌ماهیان قزل‌آلای رنگین‌کمان وجود دارد. به‌ویژه، غلظت بهینه برای دستیابی به حداکثر رشد بدون ایجاد اثرات نامطلوب بر شاخص‌های خونی هنوز به‌طور دقیق مشخص نشده است و پژوهش حاضر در راستای رفع این خلأ علمی انجام شده است.

کلمات کلیدی: *Carnobacterium* sp.، *Lactobacillus* sp.، قزل‌آلای رنگین‌کمان، رشد، هماتولوژی، پروبیوتیک