Pakistan J. Zool., vol. XX(0), pp 000-000, 2026 **DOI**: https://dx.doi.org/10.17582.....

Effect of Dietary *Lactobacillus* Probiotic on the Hematology and Meat Quality of Common Carp (*Cyprinus carpio*)

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Article Information
Received 13 June 2024
Revised 05 January 2026
Accepted 19 January 2026
Available online
(early access)
Published

Authors' Contribution
MZA and SA supervised the research work. MI and MQK provided support with data curation. The experiment was conducted by UH. MF helped with statistical analysis. UH processed the data and wrote manuscript.

Key words
Common carp, Probiotics, *L. rhamnosus* GG and *L. reuteri*, Growth performance, Meat quality, Hematology

ABSTRACT

The present research was performed to determine the influence of commercially obtained probiotic *Lactobacillus rhamnosus* GG and *Lactobacillus reuteri* on the growth factor, meat quality, and hematological parameters of common carp. The feeding trial lasted for 60 days. The fish samples (60) of mean weight 14±2.33 g was divided randomly into four test groups consisting of three replicates (n=5/glass tank).In this experiment four different test feeds i.e., D1 (basal feed as reference diet), D2 (*L. reuteri* 6g/kg as feed additive), D3 (*L. rhamnosus* 6g/kg as feed additive) and D4 (a consortium of *L. rhamnosus* 3g and *L. reuteri* 3g as feed additive in per kg of basal feed) were given to fish @ 5% of their body weight. The effect of probiotics were assessed in terms of determination of growth of fish, its meat quality and changes in haematological parameters. The highest weight gain and specific growth (13.43±1.14), (1.08±0.08), were noted in D3 ($P<0.05$) followed by D4, D2 and D1. The survival rate was observed 100% in all four treatments. The best meat status was noticed in D3 regarding crude protein, crude fat, ash content, and total moisture (55.26±1.00, 21.25±0.94, 14.46±1.03, 57.77±1.57) as compared to the reference group and other experimental groups. Overall, D3 (*L. rhamnosus*) shows better results of hematological parameters. The output of this research showed the significance of feed supplements containing probiotics surely improves the growth, survival rate, meat quality and hematology of common carp.

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INTRODUCTION

Aquaculture is a food industry that is growing at much-accelerated rate as provides major source of protein all over the world (Ahmad *et al*., 2021). Southeast Asia accounts for 33% of total freshwater aquaculture with Indonesia and Vietnam producing the most (2 Million metric tons) per year (Kayansamruaj *et al*., 2020). With an area of 8,563,820 km^2 including water lodging areas, ponds, Rivers, and Lakes, Pakistan has significant freshwater and marine water bodies for aquaculture (Jarwar, 2008). In Pakistan, the estimated perennial production of fish is (0.6 million metric tons) with 37% corresponding to Inland aquaculture and 63% marine aquaculture (Amman *et al*., 2020). The fisheries are not only a good source of protein but also a source of income for 0.9 and 1.7 million Pakistanis (Kobayashi *et al*., 2016).

Cyprinus carpio is a freshwater species native to Central Asia and Eastern region of Europe (Tessema *et al*., 2020). Asia accounts for 90% of Common carp production (Hargreaves, 1998). With the increasing human population at an exponential rate there is always a concern about food security and great demand for seafood is required. Thus aquaculture is the only way to fulfill the requirement of increasing population (FAO, 2016). The inexpensive protein production methods are required to fulfill the increasing population demand; thus the application of probiotics in the aquaculture industry is efficiently meeting the requirements. Probiotics are used widely because they are safe, environmentally friendly, and cost-effective supplemented feed that can be applied to improve the use of farmed industries (Varela *et al*., 2010). Probiotics are live microorganisms that are significant

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0030-9923/2026/0001-0001 $ 9.00/0

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for the health of Humans and Animals (Hill *et al*., 2014). The most common probiotics that are used in aqua culturing are *Saccharomyces, Boulardii, Lactobacillus,* and *Bifidobacterium* (van Baarlen *et al*., 2013). Nowadays the application of probiotics is very effective it is used for treatment against the pathogens, bacterial illness, and syndromes and to boost immune responses (Doron and Gorbach, 2006; Macintyre and Childscymet, 2005). These are efficient in boosting the immunomodulation in organisms by increasing the phagocytic ability against foreign bodies through antibody production (Reid *et al*., 2003).

Lactobacillus is a gram-positive bacterium it is a micro flora that is found in the gastrointestinal tract of most animals and humans. It is effective in enhancing immune protection, combating pathogens, and resisting gastrointestinal environmental changes (van Baarlen *et al*., 2013). The dietary supplementation of *Lactobacillus* spp. improves resistivity against diseases, and innate immune responses and enhances growth in fish species (Son *et al*., 2009). Many different combinations of probiotics have been used to study their impact on fish. Here we use the probiotic *Lactobacillus rhamnosus* and *Lactobacillus reuteri* to assess their influence of variable probiotics dosage *(L. rhamnosus, L. reuteri*) on the growth, meat quality, and hematology of common carp.

MATERIALS AND METHODS

Maintenance of fish

The fingerlings of common carp (14±2.39g) obtained from the Fisheries complex at Rawal town Islamabad were stocked in twelve glass aquaria (1×1×1.5 ft) with 5 fingerlings per aquarium in a triplicate manner.

Experimental plan

Fish were given four different test feeds i.e., D1 (commercially available supplementary reference), D2 (*L. reuteri* 6g/kg as feed supplement), D3 (L. *rhamnosus* 6g/kg as feed supplement) and D4 (a consortium of *L. rhamnosus* 3g and *L. reuteri* 3g as feed supplement in per kg of basic feed). Fish were fed twice a day @ 5% mass weight for two months. The unconsumed feed pellets and faeces were pumped out daily and dirty water was exchanged with clean water after 3 days to maintain water quality. The powdered form of probiotics (*Lactobacillus rhamnosus* GG and *Lactobacillus reuteri*) were purchased from the local drug store.

Assessment of fish growth

Growth performance was determined in terms of weight gain (WG), average daily weight gain (AWG), specific growth rate (SGR), percentage weight gain (%WG), final body weight (FBW), and survival rate (SR). The weighing balance was used to measure the weight of fish fortnightly. The feeding rate was governed in accordance with fish body weight throughout the trial. Growth variables was assessed by using the following formulas stated (Kanwal and Tayyeb, 2019; El-Kady *et al*., 2022).

WG (g)= Final body weight (g) - initial body weight (g)

WG % = Final body weight (g) - initial body weight (g)/ initial body weight (g) ×100

AWG (g/day) = Final body weight (g) - initial body weight gain (g)/Number of days

SGR (%/day) = ln final body weight (g)-ln initial body weight (g)/day×100

SR (%) = Number of fish survived/ total number of fish ×100

Proximate compositionof fish

For determining proximate composition the fish meat was used to determine crude protein, crude fat, crude ash, and total moisture percentage according to the standard protocols of AOAC. Kjeldahl and Soxhlet techniques were used for determining crude protein and crude fat. The fat content was measured using petroleum ether for 8 h, while total ash was done at 550 °C in muffle oven for 12 h. The moisture content was examined by heating the samples in the oven at 60° C in dry dishes for one hour. The moisture content was calculated as follows.

% moisture = Final weight - Initial weight/ initial weight ×100

Hematological analysis

Blood samples were collected through cardiac puncture by using a 2cc syringe heparin-coated EDTA tubes. Red blood cells (RBC) and white blood cells (WBC) were counted by diluting blood with Rees solution and placed under the Neubauer chamber this was practiced according to Svobodova *et al*. (1991). Hemoglobin was measured by cyanmethemoglobin method using test kits. The mean corpuscular hemoglobin concentration (MCHC) was determined using hematological data , MCHC = (Hb/ Ht) × 100 (Blaxhall and Daisley, 1973).

Statistical analysis

The result was examined by one way analysis of variance (ANOVA) with Duncan's Multiple Range Test (DMRT) for recognition of significant differences within the treated groups by using SPSS software (version 16.0). The results were displayed as means ± standard deviation (SD), the significant dissimilarity within the treated group at $P<0.05$.

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RESULTS

Growth performance

Table I shows that overall fish groups fed with supplemented diet with addition of probiotic showed improved growth (P<0.05) than the control group without probiotics in feed. Higher values of WG (13.43±1.14), SGR (1.08±0.08), %WG (92.91±9.52), and AWG (0.22±0.02) observed in D3 group treated with 6g of *L. rhamnosus* per kg of basal diet followed by D4, D2 and D1 group. The survival rate was 100% in all groups.

Proximate composition

Table II shows proximate composition viz. moisture, crude proteins, total ash, and crude fat of *C. carpio*. The notable higher moisture content was found in D1 (66.33 ±0.57) followed by D2 (63.30±0.99), D4 (60.31±0.88) and D3 (57.77±1.57). Crude protein was found to significantly increase in D3 (55.26 ±1.00), (P< 0.05) than in D4 (52.87±1.52), D2 (50.16±0.95) and D1 (46.83±1.48). The significantly higher percentage of crude fat (32.43 ±0.90), and ash content (22.60 ±1.46) was noted in control group D1 as compared to D2, and D4, and D3, respectively.

Table I. Growth performance and survival rate of common carp (*Cyprinus carpio*) fed with different experimental diets.

Growth parameters	D1	D2	D3	D4
IBW(g)	14.76±0.85	14.73±1.98	14.78±0.14	14.75±0.09
FBW(g)	19.26±1.01^{d}	21.80±1.49^{c}	27.88±1.24^{a}	24.94±1.47^{b}
WG (g)	4.50±0.35^{d}	7.06±0.73^{c}	13.43±1.14^{a}	10.21±0.82^{b}
AWG (g/day)	0.07±0.10^{d}	0.11±0.01^{c}	0.22±0.02^{a}	0.16±0.01^{b}
WG(%)	30.52±2.49^{d}	48.81±10.24^{c}	92.91±9.52^{a}	69.44±5.64^{b}
SGR (%/day)	0.44±0.03^{d}	0.65±0.11^{c}	1.08±0.08^{a}	0.86±0.07^{b}
SR (%)	100	100	100	100

Means in the same row with dissimilar superscripts a, b, c and d is remarkably different (P< 0.05). IBM, average initial body weight; FBW, average final body weight; WG, weight gain; AWG, average daily weight gain; WG (%), percentage weight gain; SGR, specific growth rate; SR, survival rate.D1 basal die; D2, basal diet + 6g *L.* reuteri/kg; D3, basal diet + 6g *L.* rhamnosus/kg; D4, basal diet + 3g *L.* reuteri and 3g *L. rhamnosus* (consortium). Data is showed as mean± standard deviation.

Table II. Effect of commercial probiotics on proximate composition (mean± standard deviation) of common carp.

Treatments	D1	D2	D3	D4
Crude protein	46.83 ±1.48^{d}	50.16 ±0.95^{c}	55.26 ±1.00^{a}	52.87 ±1.52^{b}
Total ash	22.60 ±1.46^{a}	19.87± 1.46^{b}	14.46± 1.03^{d}	17.25± 0.96^{c}
Moisture	66.33± 0.57^{a}	63.30 ±0.99^{b}	57.77 ±1.57^{d}	60.31 ±0.88^{c}
Crude fats	32.43 ±0.90^{a}	24.32± 0.94^{b}	21.25±0.94^{c}	23.32± 0.88^{b}

Means standard deviation in the same row with different superscripts is notably different (P <0.05). D1 basal diet; D2, basal diet + 6g *L.*reuteri/kg; D3, basal diet + 6g *L.* rhamnosus/kg; D4, basal diet + 3g *L. reuteri* and 3g *L. rhamnosus* (consortium).

Table III. Effect of supplemented probiotics on hematological parameters (mean standard deviation) of common carp.

Parameters	D1	D2	D3	D4
RBC×10^{6} (cell/mm^{3})	1.14±0.15^{d}	2.28±0.18^{c}	3.30±0.31^{a}	2.76±0.29^{b}
Hb (g/dL^{-1})	4.74±0.70^{c}	6.15±0.5^{b}	8.86±0.76^{a}	7.86±0.92^{a}
Hct (%)	17.33±2.51^{c}	19.66±0.57^{bc}	24.33±2.08^{a}	23.00±2.64^{ab}
WBC×10^{4} (cell/mm^{3})	2.15±0.13^{d}	2.88±0.30^{c}	4.62±0.39^{a}	3.62±0.52^{b}
MCHC (g/dl)	27.37±0.23^{d}	31.30±0.90^{c}	36.40±0.28^{a}	34.16±0.26^{b}

Red blood cell (RBC); Hemoglobin (Hb); Hematocrit (Hct); White blood cell (WBC); Mean corpuscular hemoglobin concentration (MCHC). Means in the same row with different superscripts a, b, c, and d are significantly different (P< 0.05).

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Hematological parameters

Table III shows hematological parameters at the end of the growth period. The highest values of Hb, MCHC, RBCs and WBC were observed in D3 group as 8.86±0.76, 36.40±0.28, 3.30±0.31, and 4.62±0.39 respectively. The blood parameters were all significantly higher in probiotics fed groups D2, D3, and D4 than control groups (ANOVA: $P < 0.05$).

DISCUSSION

Probiotics are now being widely used in the aquaculture industry as it increase the growth and production of cultivated species (Martínez-Cruz *et al*., 2012). In aquaculture, probiotics surely increase the growth performance and health of fish (Anee *et al*., 2021). The dietary supplementation of *Lactobacillus* spp. improves resistivity against diseases, and innate immune responses and enhances growth in fish species (Son *et al*., 2009).The impact of *L. rhamnosus* GG on grass carp was determined on experimental groups and the results showed improved growth and morphometric parameters (Farooq *et al*., 2023). In the current study, the fish fed with addition of probiotics showed improved growth performance, meat quality, and hematological parameters in aliens with the application of probiotic *Lactobacillus plantarum* in carp feed improves growth variables, immunophysiological responses, and disease resistivity in common carp (Soltani *et al*., 2017). The increase in growth parameters like SGR, WG, FBW, and AWG were also observed in *Cyprinus carpio* (Pandey *et al*., 2022). Zhang *et al*. (2019) used *Lactobacillus delbrueckii* as a feed additive in diet resulting the improved growth performance and intestinal enzyme activities in common carp. Probiotics *Lactobacillus acidophilus* and a mix of *Bifidobacterium animalis* sub spp. Lactis with *Bifidobacterium longum* sub spp *used as feed supplement* have been found to have improved effect on the proximate composition of lamb (Santillo *et al*., 2012). Jan *et al*. (2023) performed trial on grass carp and used probiotic *L. rhamnosus* as supplementary diet, the outcomes suggested improved meat quality parameters, similar results were noted in present study. Improved protein content by dietary administration of probiotics in common carp and rainbow trout were also observed previously (Hooshyar *et al*., 2020; Xu *et al*., 2014). Bisht *et al*. (2012) examined the effect of *B. subtilis* (4×10^8 cells 100/g) mixed with rice bran as a diet, the findings showed lower moisture content while higher lipid and protein contents in the experimental diet as compared to reference group. The outcomes of our study were the same as previous studies reported of enhanced crude protein content and other parameters in the experimental feed having probiotics as compared to control diet without addition of probiotics (Asadian *et al*., 2015; Pandey *et al*., 2022). The crude protein parameter showed greater values due to (1) improved digestion, (2) more feed intake, and (3) higher nutritional absorption in the body (Abdel-Tawwab *et al*., 2008). The D3 group (6g/kg *L. rhamnosus*) also showed improved meat quality parameters due to the dietary administration of probiotics. The findings of this trial were same to the outcome of previous work done on common carp which suggested improved growth due to probiotic supplementary diet of different concentrations (Xu *et al*., 2014; Yanbo and Zirong, 2006). Mohammadian *et al*. (2020) conducted research to determine the effect of *Lactobacillus bulgaricus*(10^8 CFU g^{-1}) on hematology and immunological parameters, and the result showed improved blood variables in the probiotic fed diet than control group. The hematological parameters (Ht, PCV, MCV, MCH, MCHC and TLC) significantly increased in the experimental fed diet consist of *L. plantarum* than the control group in common carp (Pandey *et al*., 2022). The greater value of Hct indicates better health conditions in fish (Gallaugher, 1994). The use of a consortium of probiotics (*B. cereus* and *B. subtilis*) in diet improves the value of Hct and Hb in Nile tilapia (Garcia-Marengoni *et al*., 2015). The probiotics-supplemented diet using *L. acidophilus* in Rainbow trout improves RBC and disease resistance in the experimental as compared to the reference group (Faramarzi *et al*., 2011). The probiotics-based diet improve the WBC level, fish respond better to stressors and immune defense is enhanced (Munir *et al*., 2018). The findings of our study explored the improvement in blood variables due to a probiotic supplementary diet, the results were supported by previous studies done on common carp (Dehaghani *et al*., 2015).

CONCLUSION

Common carp fed with commercial probiotic showed better results than control diet. The dietary administration of *L. rhamnosus* added as 6g/kg in supplementary diet showed better growth progress, meat quality, and hematological parameters. Hence further work still needs to be done to evaluate its certain quantity for common carp.

DECLARATIONS

Acknowledgement

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Funding

The study received no external funds.

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IRB approval

The study was approved by the institutional review board of PMAS Arid Agriculture University.

Ethical statement

In our research work, we have adopted all the guidelines authorized by the ethics committee of Pir Mehr Ali Shah Arid Agriculture University Rawalpindi.

Generative AI and AI-assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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