

# Effect of *Lactobacillus rhamnosus* GG Supplementation on the Growth Performance, Survival rate and Morphometry of Grass Carp (*Ctenopharyngodon idella*)

Misbah Farooq<sup>1</sup>, Muhammad Zubair Anjum<sup>1\*</sup>, Zahir Muhammad<sup>1</sup>, Maqsood Jan<sup>1</sup>, Muhammad Shahbaz Azhar<sup>1</sup>, Shaina Rasool<sup>1</sup> and Muhammad Qayash Khan<sup>2</sup>

<sup>1</sup>Department of Zoology, Wildlife and Fisheries, PMAS-Arid Agriculture University Rawalpindi, 46300, Pakistan.

<sup>2</sup>Department of Zoology, Abdul Wali Khan University, Mardan, Khyber Pakhtunkhwa, Pakistan.

## ABSTRACT

The current study was conducted to assess the impact of market available probiotic containing *Lactobacillus rhamnosus* GG bacteria on the growth performance and morphometric characters of grass carp for 60 days. A total of 120 fish fingerlings of mean weight  $2.95 \pm 0.27$ g were randomly distributed into four experimental groups having three replicates ( $n=10$ /aquarium) and fed with four experimental diets i.e., D1 (commercial fish feed without probiotics as control), D2, D3 and D4 (commercial fish feed with addition of 2g, 4g and 6g *Lactobacillus rhamnosus* GG/kg respectively) @ 5% of their body weight. Growth performance was determined in terms of final body weight (FBW), weight gain (WG), percent weight gain (%WG), average daily weight gain (AWG), specific growth rate (SGR), feed conversion ratio (FCR) and survival rate (SR) of fingerlings. Morphometric traits were measured including total length (TL), standard length (SL), fork length (FL), head length (HL), eye diameter (ED), dorsal fin length (DFL), pectoral fin length (PFL), pelvic fin length (PvFL), anal fin length (AFL) and caudal fin length (CFL). The highest FBW ( $14.20 \pm 0.08$ g), WG ( $11.25 \pm 0.08$ g), AWG ( $0.18 \pm 0.00$ g), SGR ( $2.61 \pm 0.05\%$  day<sup>-1</sup>), %WG ( $381.340 \pm 14.40$ g), and SR (100%) and lowest FCR ( $1.94 \pm 0.00$ ) were recorded in D4 ( $P < 0.05$ ) followed by D3, D2 and D1. Fish fed with probiotics showed improved ( $P < 0.05$ ) morphometric traits and highest TL ( $15.19 \pm 0.29$ cm), SL ( $13.17 \pm 0.40$ cm), FL ( $14.04 \pm 0.28$ cm), HL ( $1.76 \pm 0.04$ cm), ED ( $0.62 \pm 0.04$ cm), DFL ( $1.82 \pm 0.04$ cm), PFL ( $1.43 \pm 0.04$ cm), PvFL ( $1.36 \pm 0.05$ cm), AFL ( $1.41 \pm 0.09$ cm) and CFL ( $1.82 \pm 0.04$ cm) was observed in D4. The result of this study demonstrated the importance of commercial probiotic as feed additives containing *L. rhamnosus* GG to improve the growth, survival rate and morphometric parameters of grass carp.

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## Authors' Contribution

MZA designed the study and supervised the research work. MF and MJ performed the experiment and compile the data. MSA and SR helped in experimental work. ZM and MQK analyzed the data and guided the manuscript writing. MF processed the data and wrote manuscript.

## Key words

Grass carp, Probiotics, *L. rhamnosus* GG, Growth performance, Morphometry

## INTRODUCTION

Aquaculture industry has become one of the rapidly developing food production sectors (FAO, 2020), contributing to 47 percent of global fish production (FAO, 2018). In recent years, fish production from aquaculture sector increased to 70 million tons (FAO, 2018) that supply

high quality animal protein and provide revenue and jobs around the world. Asia significantly contributes to global aquaculture production of farmed aquatic animals (FAO, 2020) and Pakistan export 10 percent of whole capture (Ullah *et al.*, 2018). Grass carp is natural inhabitant of rivers and lakes in eastern Asia and introduced into Pakistan in 1964 (FAO, 1970). It is widely cultivated fresh water fish species in the world due to its rich nutritional value, high rate of growth and affordable price. In 2018, 5704 thousand tons of grass carp was produced through aquaculture that accounts for 11% of global fish culture production (FAO, 2018).

With increasing commercialization and aquaculture yield, disease outbreaks and environmental degradation are major barriers to sustainable fish production and cause huge economic losses (Bondad-Reantaso *et al.*, 2005). Fish sensitivity to numerous pathogenic organisms enhanced

\* Corresponding author: [zubair.anjum@uaar.edu.pk](mailto:zubair.anjum@uaar.edu.pk)  
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by increasing intensity of fish farming, which creates environmental stress that can substantially limit growth and cause infectious illness in farmed fish (Fan *et al.*, 2018). The use of antibiotics, antimicrobials and chemotherapy in aquaculture disease control and growth enhancement results in the formation of resistant pathogenic bacterial strains which has decreased their effectiveness for animals (Pandey *et al.*, 2022; Lee *et al.*, 2016). However, residual antibiotics in aquaculture products may show some side effects on human health and aquatic environment (Cabello, 2006; WHO, 2006).

Probiotics are considered to be safe alternative to antibiotics which are environment friendly and successfully implemented as feed additives in fish farming. Probiotics are the live microbiota used as a feed additive which when ingested by the host, increase the stability of intestinal microbes and therefore improves the growth and resistance against pathogens of the host organism (Dimitroglou *et al.*, 2011). Probiotics enhance digestion process by releasing enzymes, better nutritional values, inhibit pathogenic activity, antitumor process and act as immune modulators for host (Lara-Flores *et al.*, 2003; Wang *et al.*, 2007). According to WHO and FAO, living microbes that impart beneficial effects on the host health when taken in sufficient quantity are probiotics. Currently all types of probiotics belonging to genera *Bacillus*, *Lactobacillus*, *Enterococcus*, *Carnobacterium*, and yeast has extensively applied in aquaculture system (Van Doan *et al.*, 2019). Probiotics provided to the host either by addition to supplementary feed or in culture water (Carnevali *et al.*, 2014).

Lactic acid bacteria are beneficial flora prevalent in the gut, actively involved in formation of antimicrobials substance, enhanced immune response, greater fish resistance to bacterial infections, utilization of some indigestible carbohydrates and increased accessibility of nutrients, (Fuller, 1989; Nikoskelainen *et al.*, 2001). In recent years, it has been commonly used dietary additive to prevent fish from a variety of infectious diseases (Geng *et al.*, 2012) and found to be very efficient in fish culture. Several human probiotics has been successfully implemented for aquatic animals demonstrating positive impact on growth performance and resistance against diseases (Pirarat *et al.*, 2015). *Lactobacillus rhamnosus* strains administered as probiotics in many trials enhance the hosts growth rate and gastrointestinal defense (Klopper *et al.*, 2018; Miyauchi *et al.*, 2009; Sewaka *et al.*, 2019). Proper dose of probiotics as feed additive is very essential to get better results. There is no study reported regarding the effect of *Lactobacillus rhamnosus* GG on the growth rate and morphometry of grass carp. Therefore, current study was conducted to evaluate the impact of different doses

of *L. rhamnosus* GG on the growth and morphometric parameters of grass carp.

## MATERIALS AND METHODS

### Experimental site

The experimental research was conducted at Aquaculture and Fisheries Laboratory, Department of Zoology, Wildlife and Fisheries, Faculty of Sciences, PMAS-Arid Agriculture University Rawalpindi.

### Sample collection

Grass carp fingerlings of initial mean weight of  $2.95 \pm 0.27$ g and mean initial values of morphometric parameters including TL  $6.00 \pm 0.32$ cm, FL  $5.37 \pm 0.24$ cm, SL  $4.8 \pm 0.22$ cm, HL  $1.34 \pm 0.04$ cm, ED  $0.44 \pm 0.04$ cm, DFL  $1.03 \pm 0.07$ cm, PFL  $0.83 \pm 0.06$ cm, PvFL  $0.64 \pm 0.05$ cm, AFL  $0.74 \pm 0.05$ cm, and CFL  $1.24 \pm 0.05$  cm were acquired from commercial fish seed hatchery and conveyed to the aquaculture and fisheries laboratory in polythene bags filled with water and oxygen.

### Preparation of experimental diet

Commercial fish feed pellets (1.5mm) containing 30% crude protein (Marine Grow Fish Feed; Hi-Tech Feeds Private Limited, Pakistan) as a basal diet and market available probiotic bacteria (PREPRO) having *L. rhamnosus* GG was used to prepare four different experimental diets. Basal diet was used as control (D1) without probiotics. Experimental diets D2, D3 and D4 were prepared with addition of 2g, 4g and 6g *L. rhamnosus* GG /kg of basal diet respectively as probiotics.

### Study design

The acquired grass carp fingerlings were acclimatized to laboratory conditions using basal diet for 10 days in glass aquaria having the size of 1 x 1x 1.5 feet (length x width x height). After acclimatization, total 120 fish fingerlings were randomly distributed into four experimental groups having three replicates with the stocking density of 10 fish fingerlings per aquarium. Oxygen was supplied to fish using air stone aerators powered by electricity. The fish were fed twice a day at the rate of 5% of their body weight for 60 days. The leftover feed and feces were siphoned out and 50% aquarium water was changed daily for maintenance of water quality.

### Assessment of growth performance, feed utilization and survival rate

Growth performance was assessed in terms of final body weight (FBW), weight gain (WG), specific growth rate (SGR), average daily weight gain (AWG), percent

weight gain (%WG), feed conversion ratio (FCR) and survival rate (SR). Each fish was weighed fortnightly by using electronic balance. Fish weight was used to adjust the feeding rate throughout the experimental time period. Growth parameters were calculated by using equation described by El-Kady *et al.* (2022) and Kanwal and Tayyab (2019).

Weight gain (WG; g) = Final body weight(g)– initial body weight(g)

Average daily weight gain (AWG; g/day) = Final body weight(g) – initial body weight(g) /Number of days

Percent weight gain (WG%) = Final body weight(g)– initial body weight(g)/initial body weight(g)×100

Specific growth rate (SGR; %/day) =  $\ln$  final body weight(g)–  $\ln$  initial body weight(g)/ days×100

Feed conversion ratio (FCR) = Total feed given(g)/ body weight gain(g)

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

#### Measurements of morphometric characters

A total of 10 morphometric characters of fish were measured using procedure described by Khalid and Naeem (2017). The morphometric parameters of fish were measuring by total length (TL), standard length (SL), fork length (FL), head length (HL), eye diameter (ED), dorsal fin length (DFL), pectoral fin length (PFL), pelvic fin length (PvFL), anal fin length (AFL) and caudal fin length (CFL). For this purpose, 5 fish individuals were taken from each aquarium randomly and anesthetized them with clove oil. Each fish was weighed separately by using weighing balance, the morphometric parameters were measured by using measuring scale closest to 0.1cm and released back into their respective aquarium. The TL

was measured from start of snout to the end of caudal fin blade, FL from snout to the bifurcation of caudal fin and SL from the snout tip to start of caudal fin blade. HL measured from snout tip to back end of operculum and ED measured by the space between edges of the eye ball. DFL, PvFL, AFL, PFL and CFL were measured by their longest fin rays from base.

#### Statistical analysis

The growth and morphometric parameters analysis was done by one-way analysis of variance (ANOVA) with Duncan's Multiple Range Test (DMRT) by using SPSS software (version 16.0). A Pearson correlation test was used to identify correlation between final body weight and morphometric traits. The obtained results were presented as means  $\pm$  standard deviation (SD) and at the significance level of  $P < 0.05$ .

## RESULTS

#### Growth performance, feed utilization and survival rate

*L. rhamnosus* GG administered in the diet of grass carp fingerlings improved the growth parameters of fish in terms of FBW, WG, AWG, SGR, %WG, FCR, and SR (Table I). Probiotic treated groups showed improved growth performance ( $P < 0.05$ ) than control group. WG, AWG, %WG and SGR significantly enhanced ( $P < 0.05$ ) in the D4 group treated with 6g of probiotic per kg of basal diet followed by D3, D2 and D1 group. Feed conversion ratio (FCR) improved in dietary groups supplemented with probiotic. Lowest FCR ( $P < 0.05$ ) was observed in D4 than D1, D2 and D3. The survival rate was recorded 100% in all groups.

**Table I. Growth Performance, feed utilization and survival rate (Means  $\pm$  SD) of grass carp (*Ctenopharyngodon idella*) fingerlings fed with different experimental diets.**

| Growth parameters | D1                             | D2                             | D3                             | D4                               |
|-------------------|--------------------------------|--------------------------------|--------------------------------|----------------------------------|
| IBW(g)            | 2.93 $\pm$ 0.08                | 2.98 $\pm$ 0.07                | 2.95 $\pm$ 0.07                | 2.95 $\pm$ 0.07                  |
| FBW(g)            | 9.67 $\pm$ 0.08 <sup>d</sup>   | 11.88 $\pm$ 0.07 <sup>c</sup>  | 12.34 $\pm$ 0.10 <sup>b</sup>  | 14.20 $\pm$ 0.08 <sup>a</sup>    |
| WG (g)            | 6.74 $\pm$ 0.07 <sup>d</sup>   | 8.90 $\pm$ 0.05 <sup>c</sup>   | 9.39 $\pm$ 0.12 <sup>b</sup>   | 11.25 $\pm$ 0.08 <sup>a</sup>    |
| AWG (g/day)       | 0.11 $\pm$ 0.00 <sup>d</sup>   | 0.14 $\pm$ 0.00 <sup>c</sup>   | 0.15 $\pm$ 0.00 <sup>b</sup>   | 0.18 $\pm$ 0.00 <sup>a</sup>     |
| WG (%)            | 229.82 $\pm$ 5.44 <sup>d</sup> | 298.89 $\pm$ 3.08 <sup>c</sup> | 318.09 $\pm$ 6.25 <sup>b</sup> | 381.340 $\pm$ 14.40 <sup>a</sup> |
| SGR (%/day)       | 1.98 $\pm$ 0.02 <sup>d</sup>   | 2.30 $\pm$ 0.01 <sup>c</sup>   | 2.37 $\pm$ 0.02 <sup>b</sup>   | 2.61 $\pm$ 0.05 <sup>a</sup>     |
| FCR               | 2.42 $\pm$ 0.02 <sup>a</sup>   | 2.04 $\pm$ 0.00 <sup>b</sup>   | 2.03 $\pm$ 0.04 <sup>b</sup>   | 1.94 $\pm$ 0.00 <sup>c</sup>     |
| SR(%)             | 100                            | 100                            | 100                            | 100                              |

Mean in the same row with different superscripts a, b and c are significantly different ( $P < 0.05$ ). IBM, average initial body weight; FBW, average final body weight; WG, weight gain; AWG, average daily weight gain; WG (%), percent weight gain; SGR, specific growth rate; SR, survival rate. D1, basal diet; D2, basal diet+2g *L. rhamnosus*/kg; D3, basal diet+4g *L. rhamnosus*/kg; D4, basal diet+6g *L. rhamnosus*/kg. Data is presented as mean  $\pm$  standard deviation.

### Fortnightly weight gain

Fortnightly weight gain in D4 was significantly higher than D1, D2 and D3 ( $P < 0.05$ ). All probiotics fed groups showed significant increase in weight gain than control as shown in Figure 1.

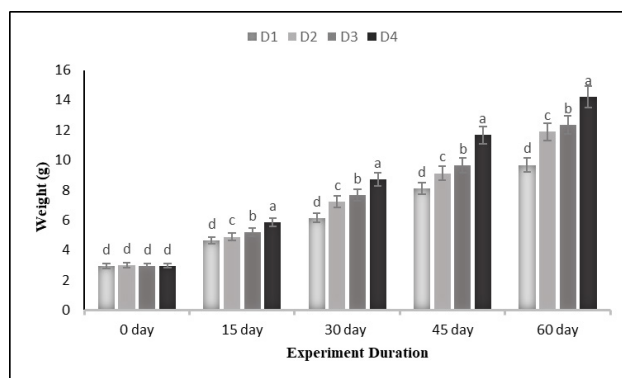


Fig. 1. Effect of different experimental diets on the fortnightly weight gain of grass carp.

The different superscripts on the bars show significant differences among the dietary groups ( $P < 0.05$ ) and same superscripts represent non-significant difference among groups ( $P > 0.05$ ).

Table II. Effect of different experimental diets on the morphometric traits (Means $\pm$ SD) of grass carp fingerlings.

| Morpho-<br>metric<br>param-<br>eters | D1                            | D2                            | D3                            | D4                            |
|--------------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| TL                                   | 10.78 $\pm$ 0.33 <sup>d</sup> | 12.94 $\pm$ 0.38 <sup>c</sup> | 13.44 $\pm$ 0.42 <sup>b</sup> | 15.19 $\pm$ 0.29 <sup>a</sup> |
| FL                                   | 9.72 $\pm$ 0.30 <sup>d</sup>  | 12.00 $\pm$ 0.35 <sup>c</sup> | 12.45 $\pm$ 0.38 <sup>b</sup> | 14.04 $\pm$ 0.28 <sup>a</sup> |
| SL                                   | 9.07 $\pm$ 0.36 <sup>d</sup>  | 11.35 $\pm$ 0.43 <sup>c</sup> | 11.71 $\pm$ 0.37 <sup>b</sup> | 13.17 $\pm$ 0.40 <sup>a</sup> |
| HL                                   | 1.62 $\pm$ 0.04 <sup>c</sup>  | 1.72 $\pm$ 0.05 <sup>b</sup>  | 1.72 $\pm$ 0.07 <sup>ab</sup> | 1.76 $\pm$ 0.04 <sup>a</sup>  |
| ED                                   | 0.52 $\pm$ 0.04 <sup>b</sup>  | 0.53 $\pm$ 0.04 <sup>b</sup>  | 0.55 $\pm$ 0.05 <sup>b</sup>  | 0.62 $\pm$ 0.04 <sup>a</sup>  |
| DFL                                  | 1.61 $\pm$ 0.05 <sup>c</sup>  | 1.71 $\pm$ 0.03 <sup>b</sup>  | 1.73 $\pm$ 0.04 <sup>b</sup>  | 1.82 $\pm$ 0.04 <sup>a</sup>  |
| PFL                                  | 1.30 $\pm$ 0.06 <sup>c</sup>  | 1.34 $\pm$ 0.06 <sup>bc</sup> | 1.37 $\pm$ 0.07 <sup>b</sup>  | 1.43 $\pm$ 0.04 <sup>a</sup>  |
| PvFL                                 | 1.15 $\pm$ 0.05 <sup>c</sup>  | 1.24 $\pm$ 0.05 <sup>b</sup>  | 1.28 $\pm$ 0.05 <sup>b</sup>  | 1.36 $\pm$ 0.05 <sup>a</sup>  |
| AFL                                  | 1.23 $\pm$ 0.04 <sup>c</sup>  | 1.30 $\pm$ 0.07 <sup>b</sup>  | 1.34 $\pm$ 0.05 <sup>b</sup>  | 1.41 $\pm$ 0.09 <sup>a</sup>  |
| CFL                                  | 1.66 $\pm$ 0.04 <sup>c</sup>  | 1.72 $\pm$ 0.05 <sup>b</sup>  | 1.76 $\pm$ 0.05 <sup>b</sup>  | 1.82 $\pm$ 0.04 <sup>a</sup>  |

For detail of dietary groups and statistical analysis, see caption of Table I. TL, total length; FL, fork length; SL, standard length; HL, head length; ED, eye diameter; DFL, dorsal fin length; PFL, pectoral fin length; PvFL, pelvic fin length; AFL, anal fin length; CFL, caudal fin length. All morphometric parameters are measured in centimeters (cm).

### Morphometric parameters

The TL, FL, SL, HL, ED, DFL, PFL, PvFL, AFL and CFL were significantly enhanced in the D4 ( $P < 0.05$ ) than

D2, D3 and D1. DFL, PvFL, AFL and CFL in D2 and D3 did not significantly differ ( $P > 0.05$ ) from each other. There was no significant ( $P > 0.05$ ) difference in eye diameter and PFL of D1, D2 and D3. HL of D2, D3 and D4 differ non-significantly ( $P > 0.05$ ) to each other presented in Table II. Morphometric traits were positive correlated with final body weight in all groups fed with various experimental diets presented in Table III.

Table III. Weight-lengths correlation of grass carp (*Ctenopharyngodon idella*) fed with different experimental diets.

| Param-<br>eters | D1    |      | D2    |      | D3    |      | D4    |      |
|-----------------|-------|------|-------|------|-------|------|-------|------|
|                 | r     | p    | r     | p    | r     | p    | r     | p    |
| W-TL            | 0.970 | 0.00 | 0.686 | 0.00 | 0.846 | 0.00 | 0.942 | 0.00 |
| W-FL            | 0.937 | 0.00 | 0.598 | 0.01 | 0.848 | 0.00 | 0.898 | 0.00 |
| W-SL            | 0.927 | 0.00 | 0.755 | 0.00 | 0.720 | 0.00 | 0.590 | 0.02 |
| W-HL            | 0.878 | 0.00 | 0.612 | 0.01 | 0.515 | 0.01 | 0.820 | 0.00 |
| W-ED            | 0.753 | 0.00 | 0.814 | 0.00 | 0.851 | 0.00 | 0.590 | 0.02 |
| W-DFL           | 0.756 | 0.00 | 0.628 | 0.01 | 0.656 | 0.00 | 0.756 | 0.00 |
| W-PFL           | 0.740 | 0.00 | 0.746 | 0.00 | 0.911 | 0.00 | 0.791 | 0.00 |
| W-PvFL          | 0.781 | 0.00 | 0.874 | 0.00 | 0.853 | 0.00 | 0.873 | 0.00 |
| W-AFL           | 0.878 | 0.00 | 0.820 | 0.00 | 0.552 | 0.03 | 0.765 | 0.00 |
| W-CFL           | 0.821 | 0.00 | 0.584 | 0.02 | 0.886 | 0.00 | 0.639 | 0.01 |

For abbreviation and other statistical detail, see caption of Table II. r, coefficient correlation; W, weight.

## DISCUSSION

Probiotics use is becoming an essential part of aquaculture in gaining high quality production from aquatic animals world-wide (Nazeer *et al.*, 2016). In aquaculture farming, probiotics significantly improved the growth performance and fish health (Aly *et al.*, 2008; Heo *et al.*, 2013). According to past literature review by author, there is no data reported on the impact of *Lactobacillus rhamnosus* GG on *Ctenopharyngodon idella*. In current study, the dietary supplementation of probiotics to fish fingerlings significantly enhanced the growth rate, feed efficiency and morphometric parameters. Highest weight gain, specific growth rate and lowest FCR was observed in D4 group. Reduced FCR represents reduction in the feed requirement for fish growth (Hardy and Kuashik, 2002). D4 group also showed improved morphometric characters and indicate positive correlation between weight and lengths of fish. The results of this experiment are similar to the outcomes of earlier studies that enhanced the growth parameters of grass carp fed with different probiotics diet (Shah *et al.*, 2021; Xue *et al.*, 2020). Possible reason



of fish growth enhancement and feed utilization upon probiotic treatment could be due to bacterial action in establishment of normal intestinal microbiota, improving nutrition via denaturing indigestible feed components via enzymatic action and by producing vitamins including vitamin B12 and biotin (Balcázar *et al.*, 2006; Planas *et al.*, 2004; Suzer *et al.*, 2008). The current results are supported by the conclusions of the preceding studies in tilapia (Pirarat *et al.*, 2011) and *Cirrhinus molitorella* fed with *L. rhamnosus* GG probiotics diet (Yu *et al.*, 2022). This strain could increase the intestinal surface area for nutrient absorption and assimilation in fish body by enhancing the height and width of villi which corresponds to increased growth rate (Caspary, 1992). *L. rhamnosus* GG cells might bound to the mucosal area of the gut wall (He *et al.*, 2017). Bacterial adhesion in intestine starts the synthesis of short chain fatty acids by utilizing sugar (carbohydrates) (Cani *et al.*, 2019) which triggers the release of gastrointestinal peptides or growth factors that supply energy to host organism and may confers the benefits on intestinal health (Blottiere *et al.*, 2003; Pelicano *et al.*, 2005) thus, enhance the feed utilization and growth of fish. *L. rhamnosus* GG administration in rats modulate the gut microbial community involved in butyrate production by the fermentation of carbohydrates, proteins and fibers (Lin *et al.*, 2020). Butyrate involved in the metabolic process of bile salts and modification of toxic and mutagenic substances leading to the maintenance of intestinal health. Chickens fed with diet supplemented with *L. rhamnosus* might release some antimicrobial compounds including hydrogen peroxide, bacteriocins and organic acids having tendency to maintain beneficial intestinal flora and limit the growth of pathogens (Chen *et al.*, 2016). Likewise, the dietary administration of *L. rhamnosus* GG improved the feed digestion capacity by producing the digestive enzymes or enhancing the activities of host enzymes (Wang *et al.*, 2008) reported in Nile tilapia (Goncalves *et al.*, 2011; Ngamkala *et al.*, 2010).

## CONCLUSION

Grass carp fed with commercial probiotic (PREPRO) containing *L. rhamnosus* GG at the rate of 6g per kg of basal diet showed improved growth performance, feed utilization and morphometric parameters. Hence, higher dose is suitable for rearing grass carp.

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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 Agricultural University Rawalpindi.

## Statement of conflict of interest

The authors have declared no conflict of interests.

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