Effect of Probiotics *(Bacillus subtilis)* on the Growth and Survival of Fingerlings of Grass Carp, *Ctenopharyngodon idella*

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**Authors’ contributions**

This work was carried out in collaboration between all authors. Author SS, carried out the trial in the laboratory, carried out the field works and wrote the first draft of the manuscript. Author AC and MR designed the study, wrote the protocol and authors MH and AA managed the analyses of the study and IY and SJ managed the literature searches. All authors read and approved the final manuscript.

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**ABSTRACT**

In the modern high intensity aquaculture, probiotics offer an encouraging substitute to chemicals and antibiotics, one such important application of probiotics is their use as growth promoters, in addition to health and water quality management. On the same background, the study was carried to evaluate the effect of dietary incorporation of probiotic - *Bacillus subtilis* on the growth performance of grass carp (*Ctenopharyngodon idella*). The probiotic - *Bacillus subtilis* was mixed with the basal diet (Protein 32%) in three different concentrations (0.5, 1.0 and 1.5 % designated as T₁, T₂, and T₃). The basal feed with no probiotic was used as control (T₀). The impact was recorded for a period of 60 days. Feeding was done twice a day at the rate of 5% of their body weight. Growth performance was evaluated through estimation of weight gain, feed Conversion Ratio (FCR), protein Efficiency Ratio (PER), specific growth rate (SGR) and feed efficiency ratio (FER). It was observed that the probiotic *Bacillus subtilis* fed at 1.5% significantly improved the growth performance of the fish *Ctenopharyngodon idella* showing the highest growth rate followed by 1% and 0.5% probiotic fed diets. Lowest growth rate was recorded in control group.

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1. INTRODUCTION

Grass carp (Ctenopharyngodon idella), also known as the white Amur, is one of the most important cultured freshwater fish species. However, grass carp is also susceptible to various kinds of diseases and this makes it difficult to culture particularly in fingerling stage. Survival of as low as 5% has been recorded in grass carp [1].

With the growing commercialization and amplification of aquaculture production, diseases and deterioration of environmental conditions are major problems in fish farming and face enormous economic losses [2]. An indiscriminate use of chemical additives and veterinary medicines as protective and remedial measures for diseases has resulted in antimicrobial resistance among pathogenic bacteria and tarnished environmental conditions [3].

The Food and Agriculture Organizations of the United Nations defined the development of affordable yet efficient vaccines, the use of immune stimulants, non-specific immune enhancers, and the use of probiotics for the improvement of aquatic environmental quality as major areas for further research in disease control and management in aquaculture.

The research on its use for aquatic animals is growing due to the requirement for environment friendly aquaculture [4]. Appropriate Probiotics application were shown to improve both the growth and the stability of the host’s normal microbiota and prevent colonization by pathogens. Probiotics also influence the mucosal barrier by their trophic effect on intestinal epithelium and stimulate both specific and non-specific components of the immune system [5][6]. They also contribute to higher growth and feed efficiency, avoid intestinal ailments and pre-digestion of anti-nutritional factors existing in the ingredients thus improving nutrients utilization. Probiotics may also detoxify the possibly harmful compounds in feeds, by denaturing the potentially indigestible components in the diet by hydrolytic enzymes such as amylase and protease.

Among many probiotic species discovered Bacillus has proved to hold better probiotic attributes such as their ability to produce non-pathogenic and non-toxic antimicrobial substance that are active against many microbes, together with their sporulation capacity (i.e. which extend their period of effectiveness), gives them dual benefit in terms of survival (heat-tolerance and longer shelf-life) in diverse environments compared to other species such as Lactobacillus spp. [7]. Bacillus species are known to enhance the digestive enzyme activity, antioxidant enzyme activity, expression of immune related genes as well as stress related genes and improve the ability of fish to be resistant against pathogenic microbes [7]. Thus this study was devised to evaluate the effect of probiotic (Bacillus subtilis) on the growth performance of Grass carp (Ctenopharyngodon idella).

2. MATERIALS AND METHODS

2.1 Study Period and Site

The experiment was conducted for a period of 60 days from 10th April to 10th June at Fisheries Instructional Research Farm, Shuhama Ganderbal.

2.2 Experimental Setup

Healthy and disease free fingerlings of Ctenopharyngodon idella average weight of 8.30±0.006 gm. were collected from Fisheries Instructional Research Farm, Faculty of Fisheries located at Shuhama campus of SKUAST-K and were acclimatized to laboratory conditions for a week, before the start of experiment. During acclimatization the mixture of soybean and wheat bran (1:1) pellet feed in granular forms were fed to the fish. A completely randomized design was developed. The experiment was carried out in sixteen plastic tubs of eighty litres capacity. Fifteen Ctenopharyngodon idella fishes were stocked in each plastic tub with four replicates for four experimental diets.

The experimental groups were fed with their respective diets @ 5% of body weight twice a day. Faecal matter and left-over feed was removed by siphoning and 30% water was exchanged on alternative days in each tub with fresh water. Water quality parameters in all the tubs was analysed at weekly intervals as per standard method [8].
2.3 Diet Preparation

2.3.1 Procurement of probiotic

The probiotic *Bacillus subtilis* containing 2 Billion CFU of probiotic cells/5ml product was procured from the market manufactured by Riata Life Sciences Pvt. Ltd. Marketed by ARISTO Pharmaceuticals Pvt. Ltd.

2.3.2 Basic composition of diets

Basal diet containing various ingredients was appropriately prepared. In experimental diets, probiotic was added at different concentrations viz., 0.5, 1 and 1.5%.

2.3.3 Feed formulation

The feed ingredients i.e. rice bran, soybean, fish meal, mustard oil cake, wheat flour were finely crushed with an electric grinder and sieved through the standard mesh of 200mm sieve. Feed formulation was done by Pearson’s square method using known values of protein content of ingredients.

2.3.4 Mixing of ingredients and pelletization

The basal diet was prepared by mixing the ingredients in appropriate proportions. Basal diet with no probiotic was designated as control (T₀). Three experimental diets were prepared separately by combining the weighed quantity of chosen feed ingredients followed by addition of three levels of probiotic (0.5, 1, 1.5% /100g). The contents were mixed uniformly with the appropriate quantity of water so as to assure good consistency of the dough. The dough was autoclaved for 10 minutes at 121°C. The diets were designated as T₁, T₂ and T₃. The prepared dough were pelletized using laboratory model of pelletizer having 4mm diameter sieve.

2.4 Water Quality Analysis

The following physico-chemical parameters of water were analyzed fortnightly by standard methods as described by the APHA [8] and Adoni [9].

1. Water Temperature (°C)
2. Hydrogen ion concentration (pH).
3. Dissolved Oxygen (D.O).
4. Free Carbon dioxide (CO₂).
5. Total Alkalinity (mg/l).
6. Total hardness (mg/l).

3. RESULTS AND DISCUSSION

The results of the present study infer that incorporation of *Bacillus subtilis* in the diet of grass carp fingerlings improved the growth performance of the fish in terms of weight gain, specific growth rate (SGR), feed conversion ratio (FCR), feed efficiency ratio (FER) and protein efficiency ratio (PER).

The weight gain recorded was higher in probiotic fed fish than the control. The weight gain estimated in probiotic fed fish of T₁, T₂ and T₃ were 9.76, 10.75 and 12.46 g respectively. The control group (T₀) displayed a lowest weight gain of 9.03 g only. The highest weight gain of 12.46 g was recorded in T₃ group supplemented with 1.5% probiotic/100g of feed.

The highest specific growth rate (SGR) of 1.5 % was recorded in T₃ group followed by T₂ (1.3 %) and T₁ (1.28%) while control group (T₀) without
the supplementation of probiotic showed lowest SGR of 1.20 %.

As obvious from Table A.1, feed conversion ratio (FCR) improved in treatments supplemented with probiotic. All the 3 treatments $T_1$, $T_2$ and $T_3$ incorporated with probiotic showed better results as compared to the control group ($T_0$). Lowest FCR of 1.99 was recorded in $T_3$ group supplemented with 1.5% probiotic/100g of feed, followed by $T_2$ (2.31) having probiotic supplementation of 1% /100g of feed and $T_1$ having probiotic supplementation of 0.5% obtained FCR of 2.54. The control group ($T_0$) displayed highest FCR of 2.76 having no probiotic supplementation. Thus, $T_3$ showed the best result in terms of FCR.

The highest FER of 50.19 was observed in $T_3$ group followed by $T_2$ (43.20) and $T_1$ (39.32) while lowest FER of 36.19 was recorded in control group ($T_0$).

The highest PER of 1.55 was displayed by $T_3$ group followed with $T_2$ (1.34) and $T_1$ (1.22) while the control group ($T_0$) recorded the lowest PER of 1.11.

The analysis of selected water quality parameters like water temperature, pH, dissolved oxygen, free CO$_2$, total alkalinity and total hardness was done on weekly basis in each experimental unit.

The study is in agreement with the findings of Toutou et al. [10] who reported that the incorporation of Bacillus subtilis in the diet of Ctenopharyngodon idella resulted in higher SGR and lowest FCR value of 1.70±0.5. The value of PER also indicated better improvement in protein utilization compared to the control tested group with no probiotic.

Valipour et al. [11] showed that the use of probiotic, Pediococcus acidilactiti @ $3 \times 10^9$ resulted in highest body weight and specific growth rate and also displayed lowest FCR as compared to the control.

Rane and Markad [12] also noted that incorporation of probiotic (Lactic acid bacteria and Yeast) in the feed of zebra fish improved the growth performance in terms of body weight increase (0.42±0.13), survival rate as compared to the control (0.25±0.07) and FCR (4.57) in probiotic fed fish were lower than the control group (8).

![Fig. 2. Growth Performances in terms of Weight gain, Specific growth rate, Feed conversion ratio, Feed efficiency ratio and Protein efficiency ratio](image-url)
Table 1. Mean±S.E of growth performance in terms of weight gain, SGR, FCR, FER and PER of grass carp (*Ctenopharyngodon idella*) fingerlings fed with different experimental diets

| Treatment   | Initial weight (g) | Final weight (g) | Weight gain (g) | Specific growth rate (%) | Feed conversion ratio | Feed efficiency ratio | Protein efficiency ratio |
|-------------|--------------------|------------------|-----------------|--------------------------|-----------------------|-----------------------|-------------------------|
| Control (T\(_0\)) | 8.32±0.02          | 17.35±0.01       | 9.03±0.03       | 120±0                    | 2.76±0.02             | 36.19±0.28             | 1.11±0                  |
| T\(_1\)      | 8.29±0.01          | 18.05±0.22       | 9.76±0.23       | 128±0.02                 | 2.54±0.06             | 39.32±0.96             | 1.22±0.03               |
| T\(_2\)      | 8.31±0.01          | 19.06±0.26       | 10.75±0.25      | 135±0.01                 | 2.31±0.05             | 43.20±0.90             | 1.34±0.02               |
| T\(_3\)      | 8.31±0.02          | 20.77±0.19       | 12.46±0.17      | 151±0.01                 | 1.99±0.02             | 50.19±0.52             | 1.55±0.01               |

T\(_1\) = feed supplemented with 0.5% *B. subtilis*
T\(_2\) = feed supplemented with 1% *B. subtilis*
T\(_3\) = feed supplemented with 1.5% *B. subtilis*
Control (T\(_0\)) = feed with no supplementation

Table 2. Mean±S.E of various physico-chemical parameters at 0-day, 1\(^{st}\) week and 2\(^{nd}\) week of the experimental period

| Weekly sampling | Treatments | Mean±S.E | Temperature (°C) | Ph (mg/l) | D.O (mg/l) | Free CO\(_2\) (mg/l) | Total alkalinity (mg/l) | Total hardness (mg/l) | P value |
|-----------------|-----------|----------|-----------------|-----------|-----------|----------------------|------------------------|-----------------------|---------|
| O-day           | Source    |          |                 |           |           |                      |                        |                       |         |
|                 |           |          | 10.5            | 7.7       | 8.7       | 0.3                  | 202.5                  | 156.4                 | >0.05   |
| 1\(^{st}\) week| Control (T\(_0\)) | 10.9±0.129 | 7.7±0.007       | 8.4±0.040 | 0.4±0.040 | 200.8±0.821          | 131.7±1.577            | 135.2±1.962           | >0.05   |
|                 | Treatment 1| 11.2±0.182 | 7.7±0.004       | 8.5±0.168 | 0.4±0.091 | 197.6±1.472          | 132.6±1.962            | 135.2±1.962           | >0.05   |
|                 | Treatment 2| 10.7±0.147 | 7.7±0.013       | 8.3±0.075 | 0.5±0.040 | 196.4±4.070          | 132.0±2.803            | 135.2±1.962           | >0.05   |
|                 | Treatment 3| 11.2±0.244 | 7.7±0.004       | 8.1±0.064 | 0.4±0.081 | 201.2±1.746          | 134.6±2.181            | 135.2±1.962           | >0.05   |
| P value         |           |          |                 |           |           |                      |                        |                       | >0.05   |
| 2\(^{nd}\) week| Control (T\(_0\)) | 12.5±0.645 | 7.7±0.006       | 8.5±0.064 | 0.6±0.040 | 203.6±2.567          | 148.5±4.162            | 148.5±4.162           | >0.05   |
|                 | Treatment 1| 13.0±0.408 | 7.7±0.052       | 8.5±0.129 | 0.6±0.091 | 204.2±3.691          | 146.1±8.02             | 148.5±4.162           | >0.05   |
|                 | Treatment 2| 13.6±0.248 | 7.6±0.019       | 8.4±0.165 | 0.6±0.040 | 201.5±0.989          | 148.5±3.364            | 148.5±4.162           | >0.05   |
|                 | Treatment 3| 12.5±0.645 | 7.7±0.008       | 8.3±0.085 | 0.4±0.119 | 199.8±1.445          | 150.4±2.197            | 148.5±4.162           | >0.05   |
| P value         |           |          |                 |           |           |                      |                        |                       | >0.05   |
Table 3. Mean±S.E of various physico-chemical parameters during 3rd week, 4th week and 5th week of experimental period

| Weekly sampling | Treatments | Mean±S.E | Temperature (°C) | Ph (mg/l) | D.O (mg/l) | Free CO₂ (mg/l) | Total alkalinity (mg/l) | Total hardness (mg/l) |
|-----------------|------------|----------|------------------|-----------|------------|-----------------|------------------------|----------------------|
| 3rd week        | Control (T₀) | 13.8±0.496 | 7.4±0.064          | 8.2±0.064 | 0.5±0.091     | 201.5±1.096      | 140.8±3.208            |
|                 | Treatment 1 | 13.5±0.288 | 7.5±0.091          | 8.2±0.081 | 0.5±0.040     | 201.0±2.050      | 140.3±2.260            |
|                 | Treatment 2 | 14.0±0.408 | 7.4±0.062          | 8.5±0.129 | 0.7±0.091     | 199.6±1.835      | 139.8±1.764            |
|                 | Treatment 3 | 13.8±0.258 | 7.5±0.064          | 8.3±0.119 | 0.6±0.147     | 199.6±0.601      | 139.5±1.874            |
| P value 4th week | Control (T₀) | 14.8±0.336 | 7.6±0.091          | 8.5±0.110 | 0.7±0.006     | 205.8±4.275      | 129.1±1.969            |
|                 | Treatment 1 | 15.0±0.408 | 7.6±0.085          | 8.4±0.108 | 0.6±0.069     | 206.4±1.361      | 133.2±1.654            |
|                 | Treatment 2 | 15.2±0.336 | 7.7±0.057          | 8.3±0.085 | 0.5±0.015     | 203.2±1.293      | 130.6±2.477            |
|                 | Treatment 3 | 14.6±0.408 | 7.5±0.064          | 8.4±0.047 | 0.6±0.033     | 205.5±3.930      | 130.1±1.975            |
| P value 5th week | Control (T₀) | 15.7±0.544 | 7.5±0.064          | 8.5±0.160 | 0.9±0.040     | 209.0±1.688      | 125.4±1.202            |
|                 | Treatment 1 | 15.5±0.204 | 7.6±0.040          | 8.5±0.091 | 0.8±0.040     | 206.6±2.901      | 126.5±2.219            |
|                 | Treatment 2 | 16.5±0.208 | 7.5±0.040          | 8.3±0.108 | 0.9±0.081     | 205.6±1.235      | 129.0±1.270            |
|                 | Treatment 3 | 16.8±0.804 | 7.4±0.047          | 8.4±0.095 | 0.9±0.040     | 209.0±1.745      | 130.3±2.400            |

Table 4. Mean±S.D of various physico-chemical parameters during 6th week, 7th week and 8th week of experimental period

| Weekly sampling | Treatments | Mean±S.D | Temperature (°C) | Ph (mg/l) | D.O (mg/l) | Free CO₂ (mg/l) | Total alkalinity (mg/l) | Total hardness (mg/l) |
|-----------------|------------|----------|------------------|-----------|------------|-----------------|------------------------|----------------------|
| 6th week        | Control (T₀) | 18.0±0.408 | 7.6±0.069          | 8.1±0.175 | 0.9±0.094     | 207.3±1.717      | 121.3±1.573            |
|                 | Treatment 1 | 18.3±0.387 | 7.7±0.014          | 8.4±0.193 | 0.9±0.110     | 207.3±2.026      | 120.8±1.605            |
|                 | Treatment 2 | 18.2±0.486 | 7.6±0.058          | 8.5±0.081 | 0.9±0.103     | 205.2±1.300      | 124.0±1.718            |
|                 | Treatment 3 | 18.5±0.456 | 7.7±0.020          | 8.3±0.070 | 0.9±0.119     | 206.6±1.080      | 120.1±1.360            |
| P value 7th week | Control (T₀) | 19.3±0.348 | 7.6±0.092          | 7.9±0.246 | 1.1±0.091     | 200.6±2.084      | 122.2±1.598            |
|                 | Treatment 1 | 18.6±0.298 | 7.7±0.006          | 8.0±0.125 | 1.0±0.075     | 198.8±1.179      | 119.8±2.390            |
|                 | Treatment 2 | 19.5±0.408 | 7.7±0.013          | 7.6±0.403 | 1.0±0.129     | 199.2±4.384      | 120.5±2.801            |
|                 | Treatment 3 | 18.2±0.459 | 7.6±0.052          | 7.9±0.217 | 0.9±0.075     | 198.8±1.063      | 124.4±2.499            |
| P value 8th week | Control (T₀) | 21.9±0.193 | 7.8±0.026          | 8.2±0.047 | 1.1±0.135     | 202.1±2.167      | 125.5±2.657            |
|                 | Treatment 1 | 22.0±0.306 | 7.8±0.009          | 8.1±0.155 | 1.1±0.147     | 204.7±1.122      | 123.7±1.601            |
|                 | Treatment 2 | 22.5±0.081 | 7.8±0.045          | 8.2±0.137 | 1.3±0.040     | 201.7±2.554      | 126.3±2.665            |
|                 | Treatment 3 | 22.3±0.182 | 7.7±0.077          | 8.6±0.179 | 1.0±0.040     | 205.4±1.545      | 126.0±2.839            |
| P value          | > 0.05     | > 0.05    | > 0.05            | > 0.05    | > 0.05       | > 0.05          | > 0.05                 |
4. CONCLUSION

In conclusion, probiotic (Bacillus subtilis) may be reflected as a good growth promoter. The study revealed the growth stimulating potential effect of Bacillus subtilis. The results demonstrated that probiotic incorporated diets significantly enhanced the growth of Ctenopharyngodon idella. It can be concluded that the incorporation of Bacillus subtilis in the feed @ 1.5%/100g resulted in better growth performance of the fish in terms increased weight gain, low FCR, high SGR, FER and PER as compared to the control.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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