Effects of Protexin Supplementation on Growth, Feed Efficiency and Blood Parameters in Common Carp *Cyprinus carpio* L.

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Abstract

The objective of the current study was to evaluate the impacts of dietary probiotic "Protexin (PROT)" supplements on the growth performance, utilization of the diet, blood biochemical compounds, blood hematology, and liver enzyme activities of the common carp, *Cyprinus carpio*, It was used Fingerlings 120 of common carp Three repetitions for each concentration after 76 days. The dietary treatments of the current study consisted of 0 (control), 0.5, 1, and 2g PROT/kg diet. The results revealed that the fish fed 2g/kg diet (T4) recorded the best values ($P<0.05$) of final body weight (228.94g), daily weight gain (1.493g/day), and specific growth rate (0.890%/day), while the FCR was reduced to 2.773 compared to the control group. Blood biochemical compounds such as total protein (TP), albumin (ALB), and globulin (GLOB), together with hematological compounds such as PCV and hemoglobin (Hb), increased while the albumin globulin ratio (A/G) and liver enzyme activity (GPT, GOT, and ALP) decreased. The current study revealed the positive impacts of dietary probiotics, confirming the effectiveness of PROT as a nutritional strategy to enhance the growth performance of the common carp, *Cyprinus carpio*.

Keywords: Protexin, Common carp, Growth performance, Feed conversion ratio, Aquaculture, Glass tanks.

Introduction

Probiotics are referred to as beneficial microbes that can offer health benefits to the host, equalizing the gut microbiota, improving water quality, helping in food digestion, modulating the host's immune system, enhancing efficiency, and lowering the onset of disease (1).These microbes play a vital role in maintaining fish health, which then increases the host growth rate when supplemented in an appropriate amount through the diet (2)(3).

PROT is a probiotic microbial supplement containing a mixture of lactic acid probiotics, yeast, and fungi. Diversity in microbes in the probiotic enhances its antibacterial activity, adhesion to mucus in the intestine, promoting digestive health, improving the immune system of the fish host species (4). Hossain *et al*. (5) have established that the use of a multi-strain probiotic has more advantages over a single strain of probiotic due to the diversity of the microbes in it.

Peixoto *et al*. (6) stated that the modes through which probiotics act in host health and development include nutrient provision, growth promotion, detoxification, and disease mitigation, among others. Most evidence cited in literature demonstrates that these supplements are imperative for digestion, nutrient absorption, immunity, and disease resistance among fish by competitively excluding pathogenic bacteria. The colonies formed by such probiotics live in the epithelial cells lining the digestive tract.

They block the adhesion of pathogenic microorganisms by forming a physical barrier that hinders attachment sites (7)(8)(9). Although there is an increasing trend in the use of probiotic supplements as feed additives to enhance growth, improve immunity, and reduce antibiotic consumption in fish nutrition, reports on the effect of dietary additions of these supplements, especially PRO —are few in the literature. The study by (10) showed that adding the commercial probiotic Protexin to the diets of common carp fingerlings (*Cyprinus carpio*) improved some hematological parameters and helped reduce the effects of stress resulting from long-distance transport compared to the control group. Therefore, this study focused on assessing growth parameters, feed conversion efficiency, and some hematological and enzymatic parameters of common carp using the probiotic PROT as a dietary supplement.

Materials and methods:

Experiment design

The experiment was divided into four treatments, including the control treatment without any addition, and the second, third, and fourth treatments with the addition of the PROT prebiotic at concentrations of “0.5, 1, and 2 g/kg” of the feed, respectively. Fingerlings 120 of common carp weighing between 11.49 and 11.53 g are used for the study. The fish is sterilized using a saturated saline solution of sodium chloride for approximately “35-40” seconds (11). The fish is then randomly distributed into 12 glass tanks and acclimatized for two weeks in the pre-prepared research tanks with dimensions 30 x 45 x 65 cm, filled with of chlorine-free water from the tap. The temperatures are measured using a mercury thermometer and are on a scale from 0 to 100 °C. The concentrations of the dissolved oxygen are measured using a digital device (Mps556YSI), the pH measured using a HANNA pH meter made in Romania, and the salinity of the water measured using a HANNA electrical conductivity meter multiplied by the factor (0.64).

Feed preparation

The basic diet contains 24% fishmeal, 25% wheat bran, 21% wheat, 30% soybeans, 1% vegetable oil, and 2% starch.

Chemical Analysis of Diets

Analyses were done in the Laboratory for Standardization and Quality Control, Department of Agricultural Research, Ministry of Higher Education and Science, using the general procedures described by AOAC (12), The approximate chemical analysis of feed is given in Table (1).

Table (1). Chemical composition of feed

Moisture (%)	**3.66**
Protein (%)	**33.54**
Fat (%)	**8**
Fiber (%)	**2**
Ash (%)	**5**
Carbohydrates (%)	**47.8**

Growth parameters

Calculations of SGR, FCR, and DWG were performed in accordance with Hepher (13).

Immunological blood tests

Fishes were fasted for 24 hours prior to blood sampling. The blood was withdrawn from different groups of fishes .Special capillary tubes with open ends were used, allowing blood to enter the tube through capillary for, then the tube is closed directly with an artificial clay from the same end from which the blood was drawn, the tubes are placed horizontally in a micro centrifuge for 5 minutes at a speed of 3000 rpm, after which the percentage of the aggregated cell volume is measured using a special ruler, Hematocrit reader [14]

Blood proteins

The levels of serum Alb, and TP were determined with the help of commercially available kits of the German company "Human" and measured at wavelengths of 570 nm and 580 nm. However, the levels of Glob and A/G ratios were calculated using the method of (15), and the following equation is used for the calculation: Glob mg/100ml serum = TP - Alb. Moreover, the A/G ratios were calculated by dividing the albumin levels by the levels of Glob (16).

Liver enzymes U/L

Serum ALT, AST, ALP, and peak regulation values were analysed using dedicated equipment provided by Human GmbH, Germany. Independent measurements were performed using a Philips SP-3000 plus spectrophotometer from Poland, following the system provided by the company, at wavelengths of 400 nm, 340 nm, and 550 nm, with distilled water as a control

Statistical Analysis

The SAS program given by SAS Institute Inc. (17) was used to analysis the data using a completely randomized design (CRD) to find the impact of different treatments on the measured attributes. Duncan’s multiple range test was used for comparisons to find significant ($P < 0.05$) differences (18).

Results

Based on environmental conditions, the water temperature was 26 °C, pH levels ranged between 7.0 and 7.8, and dissolved oxygen levels ranged between 6.0 and 8.2 mg/L. Common carp, abbreviated as *Cyprinus carpio*, is a warm water speciesrequiring water with an optimal temperature of 23-28°C, with 25°C optimal for the growth of the fish (19).

These results shown in Table (2) indicated that PROT supplementations had a significant effect on growth performance of common carp (*Cyprinus carpio*) ($P < 0.05$). There were no significant differences between the initial weight of the treatments, which indicates that all treatments were equal in the initial stage. Final weight, weight gain, and specific growth rate in probiotic treatments were enhanced compared with the control treatment, with the highest value observed in the treatment of 2g PROT /kg diet (T4), then in treatment T3, and then in treatment T2.

The values of FCR in probiotic supplementations were decreased compared with the control treatment, with the lowest value in treatment T4, then in treatment T3, while the lowest growth was observed in the control group with the Highest FCR.

Table (2). Growth Performance Parameters of Common carp (*Cyprinus carpio*) Fed Probiotic – supplemented Diets

Treatment	Initial weight (g)	Final weight (g)	feed conversion ratio	Daily weight gain (g.day-1)	Specific Growth ratio
T1 Control	115.300± 0.472 a	182.433± 1.214 c	4.073± 0.037 a	0.876± 0.012 b	0.600± 0.001 c
T2 PROT (0.5g kg−1)	114.900± 0.458 a	196.450± 3.912 bc	3.453± 0.201 ab	1.070± 0.057 ab	0.706± 0.031 bc
T3 PROT (1g kg−1)	115.100± 0.251 a	220.266± 5.952 ab	2.860± 0.133 b	1.380± 0.080 a	0.843± 0.034 ab
T4 PROT (2g kg−1)	115.266± 0.240 a	228.940± 17.604 a	2.773± 0.323 b	1.493± 0.229 a	0.890± 0.094 a

[abc]Letters indicate significant differences (P < 0.05), [PROT]Protexin

The addition of PRO resulted in a marked increase (P < 0.05) in the serum levels of TP, Alb, and Glob in common carp (*Cyprinus carpio*) compared to the control group (Table 3). Results showed that the highest levels of TP, Alb, and Glob were found in the common carp fed with 2 grams of PRO per kilogram of feed (T4). The albumin/globulin ratio showed a decrease in the probiotic-treated groups, particularly T3 and T4.

Table (3). Influence of Dietary Probiotic Inclusion on Serum (TP, Alb, Glob, and A/G ratio) in Common Carp (*Cyprinus carpio*)

Treatment	TP	Alb	Glob	A/G ratio
T1 Control	± 4.275 0.126 c	± 2.601 0.036 b	± 1.674 0.153 c	± 1.553 0.144 A
T2 PROT (0.5g kg−1)	±4.761 0.324 bc	± 2.439 0.153 b	± 2.322 0.405 b	± 1.050 0.207 B
T3 PROT (1g kg−1)	± 5.598 0.072 b	± 2.412 0.018 b	± 3.186 0.072 a	± 0.757 0.018 C
T4 PROT (2g kg−1)	± 6.300 0.117 a	± 2.763 0.153 a	± 3.537 0.072 a	± 0.781 0.054 C

[abc]Letters indicate significant differences (P < 0.05), [PROT]Protexin

The inclusion of PROT in the feed greatly influenced the activity of liver enzymes, as well as the blood indices in the common carp (*Cyprinus carpio*). The influence was found significant at (P < 0.05). Based on the findings in Table 4, it is evident that the activity of liver enzymes (GPT, GOT, and ALP) was highest in the control group. However, groups treated with probiotics recorded lower activity compared with the control group, with the lowest activity recorded in groups treated with 2 grams of PROT per kilogram of feed (T4). On the other hand, the corrected PCV levels were significantly high in probiotic-treated groups, with the values recorded highest in groups T3 and T4.

Table. (4) Impact of Dietary Probiotic Supplementation on Hepatic Enzyme Activities GPT,GOT,ALP U/L and Hematological Indicators PCV in Common Carp (*Cyprinus carpio*)

Treatment	GPT	GOT	ALP	Pcv
T1 Control	±34.470 0.459 a	± 45.400 3.434 a	± 66.438 0.801 a	22.188± 0.102 d
T2 PROT (0.5g kg−1)	±29.340 0.873 b	± 41.360 2.820 b	± 51.462 2.556 b	±23.127 0.241 c
T3 PROT (1g kg−1)	±30.132 1.008 b	± 41.720 7.650 b	± 51.300 2.295 b	24.024± 0.314 a
T4 PROT (2g kg−1)	± 28.980 0.747 b	± 34.340 1.400 c	± 48.438 1.926 b	26.958± 0.244 a

[abc]Letters indicate significant differences (P < 0.05), [PROT]Protexin

Discussion

The increase in the growth parameters such as final weight, DWG, and SGR of the probiotic-fed fish can be attributed to the improved utilization and assimilation of the diet. This is assumed to be the effect of the improved microbial population in the gut, the enhanced secretion of digestive enzymes, and the improved efficiency of nutrient assimilation (20)(21), while the reduced FCR in the probiotic-supplemented diets can be attributed to the improved feed efficiency. Improved feed efficiency may be the result of improved digestion and the reduced energy loss due to the presence of disease-causing microbes in the diet (22). On the other hand, Aly *et al*(23) and Dawood *et al* (24) also showed similar findings on growth performance and feed conversion ratio when fed probiotic supplements in common carp, as well as other cultured fish species. Moreover, better growth performance at higher levels of probiotic inclusion in T3 and T4 can be explained by a dosage effect, where 2 g PROT/kg diet is the optimum dosage under the present experimental conditions. Increase in serum total protein levels in probiotic-fed fishes indicates better protein metabolism, which can be explained by better nutrient utilization because of a better intestinal microbiotic balance and enzymes (20)(21). The increased levels of Alb indicate enhanced hepatic protein synthesis and nutritional condition, while the increasing concentrations of Glob reveal an enhanced nonspecific immune response in probiotic-treated fish. Increased levels of Alb and Glob consequently lowered the A/G ratio further support the immune stimulation by increased production of Glob, which has been reported in fish studies. These findings confirm the health benefits of the use of dietetic probiotics with respect to the enhancement of the physiological and immunological status of common carp. The marked decrease in the activities of hepatic enzymes GPT, GOT, and ALP in probiotic-fed fish confirms the protective effect of the diet on the hepatic cells and the overall health of the fish. Evidence from studies (27), (28) has shown that marked elevations of these enzymes in the serum are associated with cell stress and tissue damage in the liver. This is attributed to the beneficial effect of the use of probiotics on the balance of the gut microbiota and the reduced production of harmful metabolic products. Moreover, the considerable enhancement of PCV an values for the probiotic-enriched treatment suggests the production of young and efficient red blood cells, which carry oxygen better, an indication of improved physiological health of the fish (24). This concurs with the finding of the study of Nayak (25), The study showed some improvements in blood values in the groups treated with probiotics compared to the untreated groups. Adding probiotics to common carp feed, particularly at a rate of 2 g/kg of feed, may significantly improve the liver health of common carp.

Conclusion

Dietary supplementation with Protexin improved growth performance, feed efficiency, serum protein profile, hematologic, and hepatic function in common carp *Cyprinus carpio*. Maximum responses were noted at the supplementation levels of 2 g/kg diet. It is concluded that this study confirms the approach of using the probiotic effect as a means of enhancing the physiological health and growth of the common carp.

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