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Original Article

Effect of Bacflora®F symbiotic mixture on growth and the health of common carp, *Cyrinus carpio*

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Abstract: Adding supplements to fish diets is crucial for sustainable aquaculture. Hence, the present study investigated the effects of graded levels of Bacflora®F (symbiotic mixture) on the growth performance and blood parameters of common carp, *Cyprinus carpio*. The fish were fed a basal diet supplemented with 0.0 (Control), 1.0 g/kg (T1), 2.0 g/kg (T2), and 3.0 g/kg (T3) Bacflora®F for 56 days. Fish were fed twice a day at a rate of 3% diets. The results showed that Bacflora®F supplementation significantly improved the treated fish's growth performance parameters, including FW, WG, DGR, SGR, RGR, and FCR. The findings also revealed improved blood parameters, as evidenced by increased levels of TP, ALB, GLO, RBC, Hct, and Hb, with a significant increase of T3 compared to other treatments. The findings suggest that supplementation of Bacflora®F can help improve the growth and health of common carp, leading to increased production and reduced environmental impact.

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Introduction

The use of antibiotics and chemicals in aquaculture is frequently expensive and unacceptable because it leads to antibiotic and chemical resistance (Bulfon et al., 2017). As a result, immunostimulants such as medical plant extracts or products, probiotics, prebiotics, and synergistic mixtures are used to control fish and shellfish diseases (Kim et al., 1999; Al-Mhanawi et al., 2021a, b). Consumption of food containing probiotic, prebiotic, and synergistic mixtures is led to the positive evenness of beneficial microbes in the intestinal flora; hence, the application of probiotics in aquatic animals has increased for sustainable aquaculture (Burr, 2007). The benefit of using supplements mentioned above improve the values of feed, donating to the digestion of enzymatic, anti-mutagenic and anticarcinogenic activities, inhibiting pathogenic microorganisms, growth-promoting factors, and augmentation of immune response (Burr, 2007; Delbert and Peredo, 2012).

Bacflora-F is a feed additive with a combined probiotic, prebiotic, and acidifying effect. This mixture results in optimum digestion, increased weight gain, improved feed conversion, and better health performance. As a synergy of specific prebiotics plus probiotics in feed, Bacflora-F is a recommended growth promoter and, thus, an alternative to antibiotics. Bacflora-F is useful mixture for establishing and recovering altered intestinal flora after stress conditions, infections, antibiotic treatments, and dehydration. It includes well-chosen specific ingredients to improve gut health (Falowo et al., 2023). A current and evolving modern approach to animal feeding and disease control uses a wide variety of probiotics, such as lactic acid bacteria, *Bacillus* spp., and prebiotics (Falowo, 2022).

This study aims to investigate the effect of Bacflora-F on the growth performance and blood parameters of the common carp, Cyprinus carpio, the most important species in aquaculture. The common carp is farmed worldwide and has significant economic and nutritional value. Understanding the

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Table 1. Ingredient composition of the experimental diets.

Ingredients (%)	Control	T1	T2	T3
Fish meal*	22	22	22	22
Wheat bran	25	24.7	24.5	24.3
Wheat	20	20	20	20
Soybean	30	30	30	30
Vegetable oil	2	2	2	2
Starch	1	1	1	1
Bacflora ® F powder (g/ kg)	0	0.3	0.5	0.7

*Fish meal (55% protein) is locally produced by the Agriculture Collage, Food Industries Department, University of Basrah. All other diet ingredients have been purchased from the local market.

potential benefits of Bacflora-F on the growth and health of this species could have important implications for the aquaculture industry and the wider food supply chain. The results of this study contribute to our understanding of the potential benefits of Bacflora-F in aquaculture.

Materials and Methods

Diet preparation: The basal diet (control diet) comprises fish meal, wheat bran, wheat flour, soybeans, vegetable oil, and starch. To create experimental diets (Table 1), symbiotic mixture (Bacflora®F) powder was added to the basal diet at concentrations of 0.0 (Control), 1.0, 2.0, and 3.0 g/kg. All dry ingredients were mixed gradually using a food mixer, and then oil and water were added. Warm water was added to achieve a consistency suitable for extrusion into small pellets (2 mm diameter). The diets were air-dried in an oven at 45°C for 24 hours and stored at -20°C until use. The proximate composition of the experimental diets was analyzed using the method of AOAC (2000), which includes crude protein, crude lipid, crude ash, and crude carbohydrate (Table 1).

Experimental fish: The experiment was performed at the Fish Nutrition Laboratory, Agriculture College, University of Basrah, Iraq. At the beginning of the trial, batches of 10 fish (10.54±0.15 g) were randomly distributed into 12 rectangular plastic tanks (40 L capacity for each tank) comprising a closed recirculation system. During the trial, fish were fed 3% body weight three times a day for 60 days. Fish were batch weighed on a weekly basis following a 24 h starvation period and reared at 25ºC.

Water quality measurement: A water quality multimeter (Taiwanese origin) was used to measure the temperature, dissolved oxygen (mg/L), pH, and salinity (PSU) of the water in the Recycled Aquaculture System (RAS). The experimental plastic tanks' water was replaced 50% every week to keep a healthy environment with enough oxygen.

Growth performance evaluation: At the end of the feeding period (56 days), fish were weighed and counted to calculate weight gain (WG), relative growth rate (RGR), specific growth rate (SGR), feed conversion ratio (FCR) and Daily Gain Rate (DGR) using the formula described by Jobling and Koskela (1996) as follow:

Weight gain (WG) (g) =Final average weight (g) – initial average weight (g).

Daily Gain Rate (DGR) (g) **=** **(**Weight gain (g)– Initial weight (g))**/** **(**days of the experiment)

Relative growth rate (RGR) (%) = Weight gain (g) / Initial weight (g) × 100

Specific growth rate (SGR) (% per day) = 100 × (ln final weight/ ln initial weight)/ days of the experiment

Feed conversion ratio (FCR) = feed consumed (g) dry weight/ weight gain (g)

Haemato-biochemical assay: After 56 days of the experiment, four fish per treatment were used for blood analysis, and 2.5 ml blood samples were collected by cardiac puncture using 3 ml disposable syringes pre-rinsed with 0.5 M EDTA as an anticoagulant. The blood was kept at -20ºC before analysis. First, total protein (TP) (g/dl), albumin (ALB) (g/dl), and globulin (GLO) (g/dl) were all

Table 2. Growth performance of *Cyprinus carpio* fed synergistic mixture (Bacflora®F) powder-supplemented diets.

Parameters	Control	T1	T2	T3
IW (g)	10.64±0.03[a]	10.32±0.02[a]	10.64±0.03[a]	10.57±0.05[a]
FW (g)	21.68±0.04[d]	22.21±0.08[cd]	23.16±0.02[b]	24.69±0.02[a]
WG (g)	11.04±0.03[b]	11.89±0.02[b]	12.51±0.05[ab]	14.12±0.03[a]
DGR (g)	0.12±0.04[c]	0.13±0.04[bc]	0.14±0.02[b]	0.16±0.35[a]
SGR (% day−1)	0.79±0.08[b]	0.85±0.01[ab]	0.86±0.05[a]	0.94±0.14[a]
RGR (%)	103.88±0.13[d]	115.32±0.27[c]	117.65±0.31[b]	133.42±0.52[a]
FCR	3.48±0.11[c]	3.22±0.04[b]	3.19±0.01[b]	2.85±0.32[a]

Values in each row with different letters significantly differ at $P \leq 0.05$ (mean±SD).

Table 3. Hemato-biochemical parameters in *Cyprinus carpio* fed with the synergistic mixture (Bacflora®F) powder-supplemented diets.

Parameters	Control	T1	T2	T3
TP g/ dL	2.16±0.05[c]	2.41±0.08[b]	2.60±0.08[a]	2.70±0.05[a]
ALB g/dL	1.25±0.05[c]	1.40±0.05[b]	1.62±0.06[a]	1.61±0.08[a]
GLO g/dL	0.87±0.04[b]	1.01±0.07[a]	1.60±0.05[a]	1.07±0.08[a]
RBC ×10^6/µl	1.35±0.04[c]	1.50±0.05[b]	1.53±0.06[ab]	1.53±0.03[a]
Hct %	23.49±1.62[b]	25.51±1.45[b]	31.92±1.12[a]	32.61±1.66[a]
Hb g/dL	9.80±0.73[c]	13.74±0.60[b]	17.33±0.57[a]	17.73±0.55[a]

Values in each row with different letters are significantly different at $P \leq 0.05$ (mean±SD).

measured using a VetScan Chemistry Analyzer. Blood analysis was performed according to the protocol described by Duman et al. (2019).

The samples were centrifuged at 3000×g for 10 minutes. The collected supernatant serum was stored at -70°C in labeled Eppendorf tubes to analyze biochemical, antioxidant, and immunity enzymes. The hematological parameters, including red blood cell (RBC) count (×10^6/µL), hematocrit (Hct%), and hemoglobin (Hb) concentrations (g/dL) were measured following the method described by Witeska et al. (2017, 2022). Antioxidant-innate immunity enzymes activity assay and serum antioxidant enzymes, including superoxide dismutase (SOD) and glutathione peroxidase (GPx), and Alkaline phosphatase activity (ALP) were measured using commercial assay kits (BT LAP, China) at 450 nm using the manufacturer's protocol. The lysozyme (LYZ) activity was measured using the turbidimetric technique (Siwicki et al., 1994). The LYZ activity was quantified based on the reduction in absorbance, with one unit of activity defined as a decrease of 0.001 per minute (Ellis, 1990).

Statistical analysis: Statistical differences between treatments were evaluated by analysis of variance followed by Least Significant Differences (LSD). The significance level was set at 0.05. All statistical analyses were performed using the SPSS program version 26.

Results

There were significant ($P \leq 0.05$) differences between the experimental treatments for the final weight, weight gain, weight gain daily, qualitative and relative growth rate, and FCR (Table 2). The treatment (T3) was significantly ($P \leq 0.05$) superior to the rest of the treatments in the values of measured parameters (FW, WG, DGR, SGR, RGR, and FCR; 24.69, 14.12, 0.16, 0.94, 133.42, and 2.85, respectively), but there were no significant differences between the treatment T1 and T2.

The results showed that adding the commercial symbiotic (T2 and T3) decreased TP g/dL (2.60, 2.70) in the serum of the experimental fish and significantly increased ALB, GLO, RBC, Hct, and Hb. There were no significant differences between these two treatments (Table 3).

The results also showed that adding the commercially imported symbiotic (T3) to an increase significantly ($P \leq 0.05$) in SOD, GPX, LYZ, and ALP µ/ml (57.55, 37.36, 17.55, and 35.0, respectively), in the serum of the experimental fish. At the same time,

Table 4. Serum antioxidant- innate immunity enzymes activity of *Cyprinus carpio* fed with synergistic mixture (Bacflora®F) powder supplemented

Parameters	Control	T1	T2	T3
SOD µ/ml	38.92±0.69[d]	42.66±1.36[c]	54.31±0.93[b]	57.55±0.93[a]
GPX µ/ml	20.96±0.75[d]	24.43±0.68[c]	35.73±0.99[b]	37.36±1.25[a]
LYZ µ/ml	14.26±0.04[b]	15.45±0.17[b]	17.16±0.04[a]	17.55±0.12[a]
ALP µ/ml	59.0±1.67[c]	47.0±4.15[b]	39.0±3.58[b]	35.0±2.68[a]

Values in each row with different letters are significantly different at $P \leq 0.05$ (mean±SD).

there are no significant differences between T3 and T2 in LYZ. Then, T2 was superior to the T1 and control the remaining parameters (Table 4).

Discussions

Fish face fluctuations in environmental factors throughout their lives, and fish farming relies heavily on controlling environmental factors. Successful and sustainable breeding needs to enhance the quality of the environment so that fish farming can grow and live under ideal control conditions. Some of the major environmental parameters are critical in supporting physiological processes in fish under cultured conditions, and their changes have wide-ranging effects on the general welfare status of fish, survival, and resistance to disease (Cabillon and Lazado, 2019).

The aquaculture sector plays an increasingly important role in improving the sustainability of global fish production and developing this sector. With the advent of new farming practices and technology, some natural nutritional supplements used as feed additives in aquaculture, such as prebiotics and synbiotic mixtures, are considered common dietary supplements. It has the potential to improve the growth performance of fish (Ali and Amal, 2016). A Synbiotic is a dietary supplement that includes a probiotic and a prebiotic in a synbiotic relationship promoting the growth and survival of microflora in the gastrointestinal tract of host species by altering the beneficial bacterial community in the gut (Butt et al., 2021).

Based on the results, the treatment of the synbiotic mixture (T3) was significantly superior compared to other treatments, so the use of the synbiotic mixture led to an improvement in the physiological status, which indicates the health status of the fish. Many studies have shown that using a synbiotic mixture has positive effects on fish growth. Ali and Amal (2016) showed that the use of the probiotic diet of common carp cultured in the recirculating aquaculture system significantly increases growth parameters by 0.2%. Qaddoori et al. (2023) demonstrated the positive effect of a synbiotic mixture prepared with the local Iraqi probiotic, the commercial imported probiotic, and the commercial imported synbiotic mixture used as a feed additive in the diets of common carp in growth and health performance, leading numerous beneficial effects for fish such as improved gut microbiota, immunological responses, growth rates, and overall health status.

Several studies have shown an improvement in the criteria for evaluating the diet, which is represented by the ratio of the feed conversion factor ingested when using prebiotics and synbiotic mixtures to improve the nutritional value of the feed intake (Al-Mhanawi et al., 2021a, b; Sutriana et al., 2021; Feng et al., 2022). Hoseinifar et al. (2019) reported using the bacterium *Pediococcus acidilactici* as a probiotic and a prebiotic and mixing them as a synbiotic mixture led to a significant improvement in the final weight, weight gain, and daily growth rate of common carp. The treatment of the synbiotic mixture was higher than using the probiotic and the prebiotic alone. The use of the prebiotic Thepax and the synbiotic mixture Labazyme on young common carp fish had a significant effect on weight gain and specific growth rate. The best results were when adding 1 g/kg of the prebiotic feed Thepax to enhance the growth of fish (Al-Mhanawi et al., 2021a). The study by Sutriana et al. (2021) showed when the prebiotics were added individually or in combination with yeast and galacto oligosaccharide, mannan oligosaccharide, and βglucan had the best results on the growth performance of catfish.

Among the examples of synbiotics that can be utilized in poultry nutrition to improve birds' performance are Bacflora-F feed additives. This feed additive contains probiotics, prebiotics, and an acidifier that can promote optimum digestion, increase weight gain, improve feed conversion, and improve birds' overall performance (Agboola et al., 2021). However, little or no work has been reported on the effect of Bacflora-F as a feed additive on fish performance. The synergy (Bacflora-F) of all ingredients in one formula brings many benefits for animals; organic acids: citric, lactic, and formic acid (associated with protein hydrolysis (digestion), relatively decrease pathogen load in the stomach or gizzard, improved performance parameters, and better growth performance. They possess antimicrobial, antifungal, and antibacterial activities in low-pH environments (Agboola et al., 2021; Falowo, 2022). Inorganic acids: Orthophosphoric acid is a highly bioavailable source of bioavailable phosphorus. It also contains probiotic spores: *Bacillus licheniformis*, *B. subtilis*, *Enterococcus faecium*, and *Lactobacillus acidophilus*, and prebiotic, a high-quality and functional fiber-rich, inactivated yeast extract (*Saccharomyces cerevisiae*), supplying micronutrients and contains nutritional elements for the gut flora; thus the flora does not compete for the energy of the feed with the host, saving costs (Agboola et al., 2021).

The features of Bacflora-F include quick beneficial gut microflora establishment supporting feed consumption, conversion (FCR), and weight gain. It also helps decrease transient pathogens, such as *E. coli*, *Salmonella, Campylobacter*, and *C. perfringens.* It speeds up the recovery of the gut microflora after antibiotic applications and reduces the incidence of wet litter and liquid droppings.

Conclusions

This study concludes that adding Bacflora-F can improve the growth performance and blood parameters of common carp. However, further research is needed to explore its effects on other fish health and welfare aspects. Moreover, the long-term effects of Bacflora-F supplementation and potential side effects remain unknown. While Bacflora-F shows promise in enhancing common carp's growth performance and blood parameters, more research is needed to confirm these results and evaluate the potential risks associated with its use.

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