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Original Article

Effect of Broccoli, *Brassica oleracea* extract on growth performance and some blood parameters of common carp, *Cyprinus carpio*

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Abstract: The herb extract of Broccoli, *Brassica oleracea* was used to feed common carp (19.75±0.057 g) at four incremental levels of 0, 1, 2, and 3% of the diet for 56 days. Fish were fed twice a day at a rate of 3% diets containing 32.7-33.7% crude protein and digestible energy (16.037-16.595Mg/Kg). At the end of the study, the groups fed 2% broccoli extract were significantly better in weight gain (3.36±0.16 g), relative growth rate (17.02±0.77%), specific growth rate (0.30±0.01% day), food conversion ratio (7.74±0.39) and food conversion efficiency (12.93±0.65%). In terms of hematological parameters, albumin showed significant enhancements in 1, 2, and 3% broccoli extract ($P \leq 0.05$). However, globulin levels were not affected by broccoli extract inclusion ($P \geq 0.05$). Total protein and A/G ratio showed significant enhancements in the 2% broccoli extract group ($P \leq 0.05$). According to the findings, using 2% broccoli extract was the best option for growth and blood profile in common carp rearing. Therefore, the present study recommends adding 2% of broccoli extract to improve nutrient efficiency, growth performance, and hematological parameters in *C. carpio* fingerlings.

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Introduction

Aquaculture has grown substantially to meet human demand (Srivastava and Panadey, 2015; Ringø and Song, 2016). Aquaculture is one of the fastest-growing industries, accounting for nearly half of all aquatic products (FAO, 2020). Diseases cause severe losses leading to economic losses in aquaculture. Pathogens such as bacteria, viruses, fungi, and protozoa are major contributors to these losses (Srivastava and Panadey, 2015). Antibiotics and chemotherapy have been widely used to reduce disease risk in fish farms (Abdel-Tawwab et al., 2009; Van Hai, 2015; Tiengtam et al., 2015). In contrast, excessive antibiotic use in aquaculture can harm public health and the water environment (Cabello, 2006; Done et al., 2015). Hence, aquaculture is under pressure to reduce the use of synthetic antibiotics and chemotherapeutics, both of which endanger food safety and hygiene, and disease control in humans, due to the risks posed by chemical residues in foods and antibiotic resistance passed on to human pathogens (Serrano, 2005; Chen et al., 2020). However, many countries have prohibited the use of antibiotics in aquaculture (Vignesh et al., 2011).

Efforts are being made to use plants, plant extracts, and natural plant compounds as potential alternatives to synthetic chemicals to stimulate immune responses and disease resistance (Chakraborty and Hancz, 2011). Fish, unlike vertebrates, lack adaptive immunity and rely entirely on innate immunity (Arala-Chaves et al., 2000; Esteban et al., 2005; Little et al., 2005). The products that can increase host immunity and disease resistance, such as immunostimulants, are being used in disease prevention and have received much attention recently (Srivastava and Panadey, 2015). Plants are the oldest form of healthcare known to

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man due to the widespread presence of various compounds such as alkaloids, flavonoids, phenols, terpenes, steroids, and essential oils. Plants have anti-stress, growth-promoting, appetite-stimulating, immune-stimulating, and anti-microbial properties (Hassan et al., 2018). Because of the properties of their derivatives, they are widely used in fish diets as a growth enhancer and immune stimulator (Citarasu, 2010), in addition to being low-cost, widely available, and easy to prepare (Harikrishnan et al., 2005).

Broccoli, *Brassica oleracea*, is a member of the Brassicaceae family, known as "Cruciferae" due to its distinctive flower confirmation of four petals arranged in a cross-shape. *Brassica* varieties are among the top 10 economic crops in the world (i.e., broccoli, kale, cauliflower, and Chinese cabbage) (Francisco et al., 2017). Since of their high levels of nutrients and health-promoting phytochemicals (such as phenolics, glucosinolates, vitamins, and minerals), these vegetables have been identified as important components of a healthy diet (*Francisco et al., 2017*). Broccoli (*Brassica oleracea* L. var. Italica) has recently gained popularity among wellness consumers. It is a nutritious vegetable with high vitamins and minerals, bioactive phytochemicals (glucosinolates, phenolic compounds, and flavonoids), and antioxidants (Dominguez-Perles et al., 2010). Plant-based foods containing significant amounts of bioactive phytochemicals, such as *B. oleracea* extract, may provide desirable health benefits to common carp, *Cyprinus carpio*, growth performance, and some immune-hematological indicators that are tested as the aim of this study.

Materials and Methods

Preparation of aqueous extracts of *Brassica oleracea:* Broccoli was obtained from a local market in Basrah, Iraq, and thoroughly washed under running water to remove dust particles. The soft parts were cut into small pieces and thawed using a home mincer. To make an aqueous extract, 50 g of the minced broccoli was weighed and placed in one liter of Ellen Meyer flasks, 250 ml of deionized water was added, and the mixture was placed in a vibrating incubator at 35 °C for 24 hours. The mixture was filtered through filter paper (Whatman No1) to remove the residue, and the filtrate was stored at -20°C until used.

Diet preparation: Four isonitrogenous and isocaloric experimental rations (diets 1, 2, 3, and 4) were made with varying amounts of *B. oleracea* extract (0, 1, 2, and 3%, respectively). The feed ingredients were ground and milled into pellets. The *B. oleracea* extract was added at the end of the pelleting process. Pellets were packed in polyethylene bags, sealed airtight, labeled after drying and frozen until ready to use. The ingredients and chemical analysis of experimental diets containing varying levels of extracted *B.oleracea* are shown in Table 1.

Rearing system: Inside the laboratory, four recycling aquaculture systems (RAS) units were used. Each unit had three plastic tanks (30×30×60 cm) arranged in two rows on an iron holder, each with a glass tank (30×40×90 cm) for water filtration. Each tank was provided with air pumps for aeration, and heating equipment was used to maintain the temperature of the water. The study lasted for eight weeks (56 days), at the fish labs of the Vertebrates Department (Marine Science Center, University of Basrah). Fingerlings of common carp were obtained from the Fish Culture Unit of Agriculture College, the University of Basrah. Fish were placed in plastic tanks and acclimatized for two weeks before the experiment. Fish had a mean body weight of 19.75±0.057 g. The fish were introduced randomly into four treatments, including diet 1 (0% broccoli extract) as control and diets with broccoli extract at three levels 1, 2, and 3% broccoli extract (diet 2, diet 3, and diet 4, respectively). Each treatment was replicated using three tanks containing 10 fish. The fish were fed twice daily at 09.00 AM and 02.00 PM, at 3% of their body weight. Cleaning of the tanks and water changes were done weekly.

Water quality measurement: A water quality multimeter (Taiwan) was used to measure the

Table 1. Ingredients and chemical analysis of experimental diets containing varying levels of extracted (on a dry matter basis).

Ingredients	Control diet %	*B.oleracea* water extract % in the diets		
	Control (Diet 1)	Diet 2	Diet 3	Diet 4
Fish meal	32	32	32	32
Soya meal	22	22	22	22
Wheat brain	30	29	28	27
Yellow corn	15	15	15	15
Vitamin premix	0.5	0.5	0.5	0.5
Minerals premix	0.5	0.5	0.5	0.5
B.oleracea extract	0	1	2	3
Chemical analysis %				
Moisture	3.2±0.18[a]	3.5±0.21[b]	3.6±0.24[b]	3.7±0.26[b]
Crude Protein	33.98±0.054[a]	33.92±0.057[a]	33.86±0.59[a]	33.80±0.06[a]
Crude Fat	13.8±2.52[a]	13.6±2.87[a]	13.3±3.12[a]	13.1±3.43[a]
Ash	1.7±0.12[a]	1.6±0.15[a]	1.2±0.18[a]	1.5±0.16[a]
NFE	41.2±0.63[a]	40.91±0.71[a]	40.88±0.87[a]	39.1±0.92[b]
Fiber	5±0.32[a]	5.17±0.30[ab]	5.16±0.29[ab]	5.8±0.24[b]
GE (Mg/ Kg) *	16.595±0.032[a]	16.476±0.039[a]	16.361±0.041[a]	16.037±0.042[a]

*According to Smith's equation (1971): Protein × 18.5 + Fat × 33.5 + NFE × 13.8.

Table 2. The physical and chemical properties of the water used in experimental tanks of common carp fish fed different levels of *B.oleracea*.

Treatments	Temperature (ºC)	Dissolved xygen (mg/l)	pH	Salinity (PSU)
Diet 1 (0 %)	25.14±0.16	5.22±0.26	7.36±0.04	2.94±0.05
Diet 2 (1%)	25.01±0.10	5.38±0.29	7.38±0.02	2.91±0.04
Diet 3 (2%)	24.81±0.38	5.54±0.15	7.31±0.04	2.93±0.04
Diet 4 (3%)	25.13±0.18	5.03±0.74	7.93±0.04	2.94±0.06

temperature, dissolved oxygen (mg/L), pH, and salinity (PSU) of the water in the Recycled Aquaculture System (RAS). The experimental tanks' water was replaced 50% weekly to maintain a healthy environment.

Growth parameters: 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), Food Conversion Efficiency (FCE), and survival rate as they served as indicators for growth performance, using the formula described by Jobling and Koskela (1996).

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

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

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

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

Feed conversion efficiency (%) = weight gain (g) / feed consumed (g) dry weight × 100

Survival rate (%) = number of fish survived / number of fish stocked × 100

Blood collection: 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 -20°C before analysis. Total protein (g/dl), albumin (g/dl), globulin (g/dl), and albumin/globulin ratio (A/G ratio) are all measured (Velisek et al., 2009).

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

Results

Table 2 shows the mean water quality parameters of the treatments during the experiment. Water quality

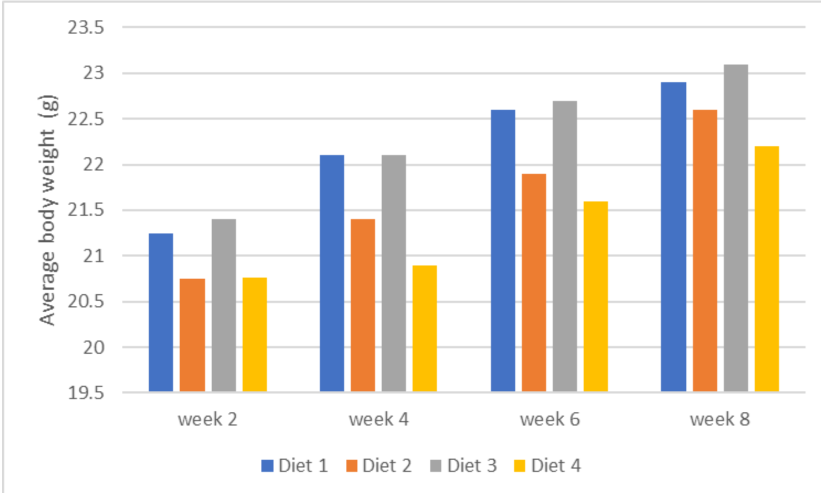

Figure 1. Average body weight of common carp as affected by diets containing different levels of *Brassica oleracea* extract.

Table 3. Growth performance (means ± SD) of common carp fish fed diets containing different levels of *Brassica oleracea* extract.

Items	Control diet %	*B. oleracea* water extract % in the diets		
	Diet 1 (0%)	Diet 2 (1%)	Diet 3 (2%)	Diet 4 (3%)
Initial weight (g)	19.75±0.07[a]	19.74±0.06[a]	19.75±0.07[a]	19.74±0.04[a]
Final weight (g)	22.85±0.12[ab]	22.58±0.12[bc]	23.11±0.23[a]	22.20±0.38[c]
Weight Gain (g)	3.10±0.17[ab]	2.84±0.19[bc]	3.36± 0.16[a]	2.46±0.34[c]
RGR (%)	15.73±0.88[ab]	14.38±1.01[b]	17.02±0.77[a]	12.47±1.71[c]
SGR (% day)	0.28±0.01[ab]	0.26±0.01[b]	0.30±0.01[a]	0.23±0.03[c]
FCR	8.03±0.45[a]	8.76±0.52[a]	7.74±0.39[a]	10.49±1.34[b]
FCE %	12.46±0.70 [a]	11.43±0.70 [a]	12.93±0.65 [a]	9.63±1.32 [b]
Survival rates %	100[a]	100[a]	100[a]	100[a]

The same letter in the same row means it is not significantly different at $P \geq 0.05$.

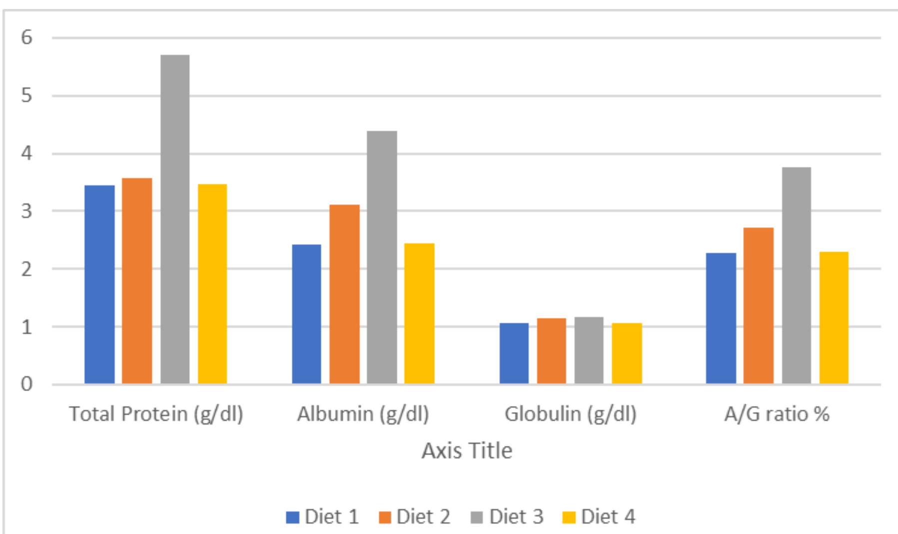

Figure 2. The effect of *Brassica oleracea* extracts on serum total protein (g/dl), albumin (g/dl), globulin (g/dl), and A/G ratio (%) in comparison with the control diet of common carp.

parameters were not different within the recommended ranges for the culture of *C. Carpio* (FAO, 2020), positively reflecting the increased survival rate, which reached 100% for all treatments. Figure 1 illustrates the trend of body weight in fish-fed various levels of broccoli extract during the experiment. The fish fed- diets 1 and 3 have grown faster than those fed diets 2 and 4.

The best growth performance was recorded in the fish-fed diet 3, and the least growth in the fish-fed

Table 4. The effect of *Brassica oleracea* extracts on serum total protein (g/dl), albumin (g/dl), globulin (g/dl), and A/G ratio (%) in comparison with the control diet of common carp.

Treatments (% extract)	Total Proteing/dl	Albuming/dl	Globuling/dl	A/G ratio%
Diet 1 (0%)	3.45±0.32[b]	2.42±0.31[c]	1.06±0.17[a]	2.28±0.24[b]
Diet 2 (1%)	3.58±0.44[b]	3.12±0.55[b]	1.15±0.23[a]	2.71±0.39[b]
Diet 3 (2%)	5.70±0.13[a]	4.38±0.11[a]	1.16±0.26[a]	3.77±0.18[a]
Diet 4 (3%)	3.46± 0.38[b]	2.45± 0.35[bc]	1.07± 0.16[a]	2.29± 0.25[b]

The same letter in the same column means it is not significantly different at $P \geq 0.05$.

diet 4 (Table 3). Dietary supplementation with 2% broccoli extract resulted in significantly higher final weight and weight gain, RGR and SGR. FCR and FCE% in the fish-fed diets with 3% broccoli extract differ ($P \leq 0.05$) from the control one; while a similar value was observed in the fish fed 1 and 2% which did not differ significantly ($P \geq 0.05$) with the control treatment.

Total protein and albumin levels increased significantly ($P \leq 0.5$) with broccoli extract, reaching the highest value at 2% of the diet (5.70±0.13 g/dl and 4.38±0.11, respectively). There were no significant differences between dietary supplementation with 3% broccoli extract and the control one Globulin levels at 1, 2, and 3% of broccoli extracts did not differ significantly from the control. The albumin/globulin (A/G) ratio was highest in diet 2, which differs significantly ($P \leq 0.05$) from other treatments (Fig. 2, Table 4).

Discussion

A variety of feed additives are used in aquaculture to promote growth, stimulate immunity, prevent disease, and improve fish antioxidant status (Bilen et al., 2020). Infectious diseases have recently been identified as a major issue in the aquaculture industry, resulting in significant losses for farmers (Erguig et al., 2015; Syahidah et al., 2015; Karga et al., 2020). The use of antibiotics and chemicals in aquaculture is frequently expensive and unacceptable because it leads to antibiotic and chemical resistance (Erguig et al., 2015; Syahidah et al., 2015; Bulfon et al., 2017). Hence, immunostimulants such as medical plant extracts or products have been used to control fish and shellfish diseases and increase phagocytic activity in various fish (Gopalakannan and Arul, 2006; Kim et al., 1999). In aquaculture, many immunostimulants are used to improve fish immune profiles that have the potential to replace antimicrobial agents.

This study showed the effect of dietary plant supplementation of broccoli extract on growth performance and some blood parameters of common carp reared in a recycling aquaculture system. Environmental factors of the systems were within recommended ranges (FAO, 2020), which was contributed by the increased survival rate of fish in all treatments. Fish fed a 2% broccoli extract diet increased their weight gain (WG), relative growth rate (RGR), and specific growth rate (SGR). Positive growth results could be attributed to nutrient protection in the intestine as a result of the *B.oleracea* extract's effectiveness against bacterial pathogens, and increased levels of digestive enzymes, improved cellular respiration, and absorbed nutrients (Citarasu, 2010; Awad and Awaad, 2017; Begnami et al., 2018; Rudiansyah et al., 2022). Food conversion ratio (FCR) and food conversion efficiency (FCE) in fish-fed diets containing 1% and 2% broccoli extract did not differ significantly ($P \geq 0.05$) from the control treatment (diet 1). While the fish-fed diet 4 showed the least growth compared to the control treatment. Oke et al. (2017) proposed that including *B.oleracea* powder in the diet of Nile tilapia at 0.5g/100g would improve feed utilization, survival rate, and non-specific immune infections. Herb active principles have the growth-promoting ability and act as appetizers to trigger the immune system, act as a broad-spectrum antimicrobial, and include anti-stress properties, all of which will play a significant role in fish cultivation (Mahdavi et al., 2013). Many authors have demonstrated that immunostimulants can also promote growth. Influences of dietary medical plants supplementation on growth have been evaluated with several species with different results. *Gynostemma pentaphyllum* is a customary Chinese herbal medicine blended into fish feed, causing a raised weight gain, feed conversion efficiency, and specific growth rate in grass carp, *Ctenopharyngodon idella* (Wu et al., 1998). Dietary supplementation of *Quillaja saponin* increased the growth rate of *C. carpio* (Francis et al., 2002).

The results could indicate that broccoli extract enhances fish growth, feed utilization, and immune function. Dietary broccoli appeared to significantly induce non-specific immune parameters in common carp fish, albumin values showed significant enhancements by 1, 2, and 3% broccoli extract ($P \leq 0.05$). However, broccoli extract inclusion did not affect globulin levels ($P \geq 0.05$). Total protein and A/G ratio showed significant enhancements by 2% broccoli extract compared to other treatments ($P \leq 0.05$). According to the findings of this study, a 2% broccoli extract was the best option for growth and blood profile. Therefore, the present study's data recommends adding 2% of broccoli extract to improve nutrient efficiency, growth performance, and hematological parameters in *C. carpio* fingerlings. Hematological parameter analysis can be used to monitor health status, detect illnesses, and track disease progression and response to therapy (Clauss et al., 2008; Alexander et al., 2010). All the levels of included broccoli in the diet of common carp gave protection in terms of reduced percent mortality which is reflected in the increased relative survival rate values. The results indicate the immunostimulatory and disease resistance properties of broccoli aqueous extract and so its potential to be used as an immunoprophylactic in finfish aquaculture.

As a conclusion, this study found that *B.oleracea* extract has a positive effect on the growth performance of common carp. *Brassica oleracea* extract in the diet can act as a growth promoter, appetite stimulator, and immunostimulant, as well as reduce stress, reduce food losses, and protect fish, resulting in better fish growth.

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