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Effect of Dietary *Tinospora cordifolia* Supplementation on Growth Performance and Hemato-Biochemical Parameters of the Common Carp (*Cyprinus carpio*)

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ABSTRACT

Medicinal plants contain bioactive compounds that boost fish immunity and offer an effective chemical compound alternative. The present experiment aimed to study the effect of *Tinospora cordifolia* leaf powder on common carp (*Cyprinus carpio*), evaluating growth performance, hemato-biochemical indices, and serum antioxidant-enzymes activity. The fish species were fed at different levels 0, 2, 4, and 6g/ kg of *T. cordifolia* leaf powder for 60 days. The results showed that the diet supplemented with 6g/ kg improved significantly ($P< 0.05$) the growth performance parameters, red blood cell count, hematocrit, hemoglobin, total protein, albumin, and globulin. In addition, the levels of superoxide dismutase, glutathione peroxidase, and lysozyme also rose significantly ($P< 0.05$). On the other hand, glucose levels decreased significantly ($P< 0.05$) with *T. cordifolia* supplementation. This research indicates that *T. cordifolia* leaf powder is a viable feed additive for improving the growth performance and general health of common carp. Consequently, it would assist in enhancing aquaculture's sustainability.

INTRODUCTION

Aquaculture is one of the largest and fastest-growing food production industries. It is crucial in ensuring food security for the global population and sustainable livelihood prospects **(Stentiford *et al.*, 2020)**. The common carp has worldwide cultivation and is the most preferred farm fish species in many regions **(Harikrishnan *et al.*, 2021)**. It accounts for 10% of global aquaculture production **(FAO, 2020)**. Intensive aquaculture rearing causes possible environmental stress in fish, suppressing the immune system and increasing vulnerability to numerous infections **(Yang *et al.*, 2015)**. Herbal immunostimulants are promising in aquaculture since they boost growth performance, fish immunity, and antimicrobial properties, making them viable alternatives to chemical treatments and antibiotics **(Elumalai *et al.*, 2020)**. *Tinospora cordifolia* is a well-known medicinal plant in the Menispermaceae family and is a significant supplier of new medications. It has a wide range of therapeutic applications and phytochemicals

responsible for its pharmacological effects **(Sajith & Farhan, 2022)**. *Tinospora* leaves are 5- 10cm long, heart-shaped, and rich in calcium, phosphorus, and protein **(Sinha *et al*., 2004; Choudhary *et al*., 2014)**. It has been reported that *T. cordifolia* leaf extract-supplemented diets improved growth performance, survival, and haemato-biochemical profiles in catfish *Pangasius sutchi* fingerlings **(Latha *et al*., 2020)**. Similarly, **El Basuini *et al*. (2022)** found that dietary *T. cordifolia* improves growth performance, antioxidative capacity, immune response, and resistance against hypoxia stress in the Nile tilapia. The current study aimed to evaluate the effects of *T. cordifolia* leaf powder on growth performance, hemato-biochemical parameters, and antioxidant-enzyme activities in *C. carpio*, an economically important farmed fish species in Iraq.

MATERIALS AND METHODS

Rearing system and experimental condition

This study was conducted at the University of Basrah, College of Agriculture, Department of Fisheries and Marine Resources, using recirculating aquaculture systems. The system comprises two rearing units, each with six plastic containers with a capacity of 30 liters, oxygen pumps, mechanical and biological filters with a flow rate of 3 liters per minute. Daily monitoring parameters included water temperature, dissolved oxygen, pH, salinity, and weekly ammonia levels measurement. *C. carpio* fingerling was supplied from the Fish Farming Unit of the University of Basrah, then immersed in a salt solution of NaCl at a dosage of 5g/ L for 1.0min to prevent external parasites. Approximately, 120 fish with an average weight of 15.13±0.01g were distributed among twelve tanks, ten fish in each tank, and three replicates for each of the four treatments. Fish were fed twice daily at 2% of their body weight during the two-week acclimatization and 3% of their body weight during the experiment.

Diet preparation

A commercial pallet was used as a basal diet. Its composition was 35.50% protein, 12.20% fat, 24.4% fiber, 16.60% ash, and 10% moisture. The basal diet was ground, 2% of carboxy methyl cellulose (CMC) was added as a binder to increase the stability of the experimental feed in the water; *T. cordifolia* powder was added at different levels of T1(0), T2(2), T3(4), and T4(6) g/kg; each mixture individually was stirred to create a paste. The dough was formed into 2mm thick pellets using a meat grinder. After drying at room temperature, the pellets were stored at 4°C until use.

Growth performance

At the beginning of the feeding trial, the fish's initial body weight (IBW) was determined for each tank. Similarly, the final body weight (FBW) was measured at the end of the trial. Every 15 days during the trial, the fish in each tank were weighed again (WG), and the daily ratio was adjusted based on the updated biomass. The specific

growth rate (SGR), protein efficiency ratio (PER), and food conversion ratio (FCR) were evaluated as described by **Adineh *et al*. (2020)**.

Blood samples

Blood samples were taken from six fish species per treatment at the end of the feeding trial. The fish species were not given food for 24 hours before sampling. Anesthetic lidocaine (lidocaine hydrochloride) was used to sedate the fish at 5mg/ L. According to **Duman *et al*. (2019)**, the tail ablation technique was used to collect the blood samples. Hematocrit tubes were used for blood collection, and two types of capillary tubes were used: heparinized for hematological analysis and un-anticoagulated for serological tests. 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.

Haemato-biochemical assay

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)** and **Witeska *et al.* (2022)**. Biochemical parameters, including total serum protein (TP) and albumin (ALB), were measured by a commercial laboratory kit from Mindray BS-230 and wavelength reaction at 550nm and 630nm, respectively. Globulin levels were calculated by deducting albumin concentration from total protein levels, following the method described by **Wolf and Darlington (1971)**. The fish's blood glucose (GLU) level was assessed using the VivaChek™ device, with a 0.5μL sample size.

Antioxidant-innate immunity enzymes activity assay

Serum antioxidant enzymes, including superoxide dismutase (SOD) and glutathione peroxidase (GPx), were measured using commercial assay kits (BT LAP, China) SH0039, EA0029Fi, at 450nm using the manufacturer's protocol. The lysozyme (LYZ) activity was measured using the turbidimetric technique, implemented by **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 analysis of the data was performed using SPSS version 24 software. One-way ANOVA followed by least significant difference (LSD) post hoc tests were conducted to determine statistically significant differences ($P< 0.05$) between groups. All data are presented as mean ± standard deviation (SD).

RESULTS

Water quality parameters

The water quality parameters were regularly monitored; water temperature, dissolved oxygen, pH, salinity, and ammonia were 23.2 ± 0.7°C, 7.6 ± 0.4mg/ L, 7.5 ± 0.3, 1.4± 0.18ppt, and 0.04 ± 0.01mg/ L, respectively.

Growth performance

The impact of the experimental diets on the growth performance of *C. carpio* are displayed in Table (1). There were significant differences ($P< 0.05$) in the FBW of the fish across the various groups. The highest FW was documented in treatment group T4 (6g/ kg), followed by treatment group T3 (4g/ kg). WG was significantly ($P< 0.05$) increased in all experimental groups compared to the control. The highest WG was recorded in T4 (6g/kg). All the fish species fed with the experimental diets had a lower FCR than the control group. The lowest FCR (1.36± 0.04) was recorded in T4 (6g/ kg) compared to other experimental groups. The experimental groups exhibited an increase ($P< 0.05$) in SGR and PER compared to the control group. Among the experimental groups, fish species in T4 showed the highest SGR and PER values (2.04±0.03, 1.02±0.03), respectively, surpassing the other experimental groups.

Table 1. Growth performance in common carp fed with *T. cordifolia* leaf powder-supplemented diets after eight weeks (Mean ± SD)

Parameter	T1	T2	T3	T4
IW (g)	15.13±0.96	15.14±1.77	15.15±0.99	15.12±0.95
FW (g)	37.98±0.96a	45.30±1.77b	47.14±0.99 c	51.46±0.95 c
WG (g)	22.85±0.97a	30.16±1.75b	31.99±0.97b	36.35±0.95 c
FCR	1.64±0.07a	1.5±0.09b	1.47±0.04 bc	1.36±0.04 c
SGR (% day^{-1})	1.53±0.04a	1.83±0.06b	1.89±0.03 b	2.04±0.03 c
PER	0.64±0.03a	0.85±0.05b	0.90±0.03b	1.02±0.03 c

Different letters (a-d) within the same row showed significant differences ($P< 0.05$).

Hematological and biochemical parameter

The hematological-biochemical parameters of common carp subjected to varying levels of *T. cordifolia*; RBC, Hct, and Hb were elevated in the fish-fed experimental diet compared to the control group, as shown in Table (2). Within the experimental groups, the T4 group (6g/ kg) exhibited the highest values for all the aforementioned metrics (1.80 ± 0.07, 35.74 ± 4.37, and 10.95 ± 1.27), respectively. There were significant differences ($P< 0.05$) observed in all treatments of *T. cordifolia* in the biochemical parameters when compared to the control fingerlings. T4 (6g/ kg) fingerlings had the greatest TP, ALB, and GLB values among the experimental groups (4.50 ± 0.19, 1.83 ± 0.04, and 2.67 ± 0.18), respectively. There was a drop in GLU levels after each treatment with *T. cordifolia*, and there was a significant difference of ($P< 0.05$) between treatments, and the lowest value was 54.30±2.43 in T4.

Table 2. Hemato-biochemical parameters in common carp fed with *T. cordifolia* leaf powder supplemented diets after eight weeks (Mean ± SD)

Parameter	T1	T2	T3	T4
RBC $\times 10^6$ / µL	1.26 ± 0.10 a	1.42 ± 0.12 a	1.51 ± 0.25b	1.80 ±0.07c
Hct %	21.25 ±2.03a	25.63 ±2.69 b	26.55 ±3.49b	35.74 ± 4.37c
Hb g/dL	5.17 ± 1.03a	7.77 ±0.81b	8.05 ±1.06b	10.95 ±1.27c
TP g/ dL	2.10± 0.37 a	2.80 ±0.07 b	3.90 ±0.03 c	4.50 ±0.19d
ALB g/dL	1.13 ±0.05 a	1.33 ±0.04 b	1.76±0.11 c	1.83 ±0.04 d
GLB g/dL	0.97± 0.35 a	1.47±0.11 b	2.15± 0.11 c	2.67 ± 0.18d
GLU mg/dL	68.67±2.16 a	64.33± 2.5 b	60.17± 1.72c	54.50 ±2.43 d

Different letters (a-d) within the same row showed significant differences ($P< 0.05$).

Antioxidant–innate immunity parameters

The serum levels of SOD, GPx, and LYZ enzymes were assessed following the feeding trial (Fig. 1). The results indicated an enhancement in SOD and GPx activity, with the highest SOD value observed in T4 (70.02±3.81), followed by T3 (67.22±5.39). The maximum values of GPx activity observed in T4 (18.60 ±3.05) exhibited significant differences ($P< 0.05$) when compared to the other treatments. There was an increase in LYZ activity following each level of *T. cordifolia*. Notably, there was a significant difference ($P< 0.05$) between treatments, with the greatest value (14.08±0.54) in T4.

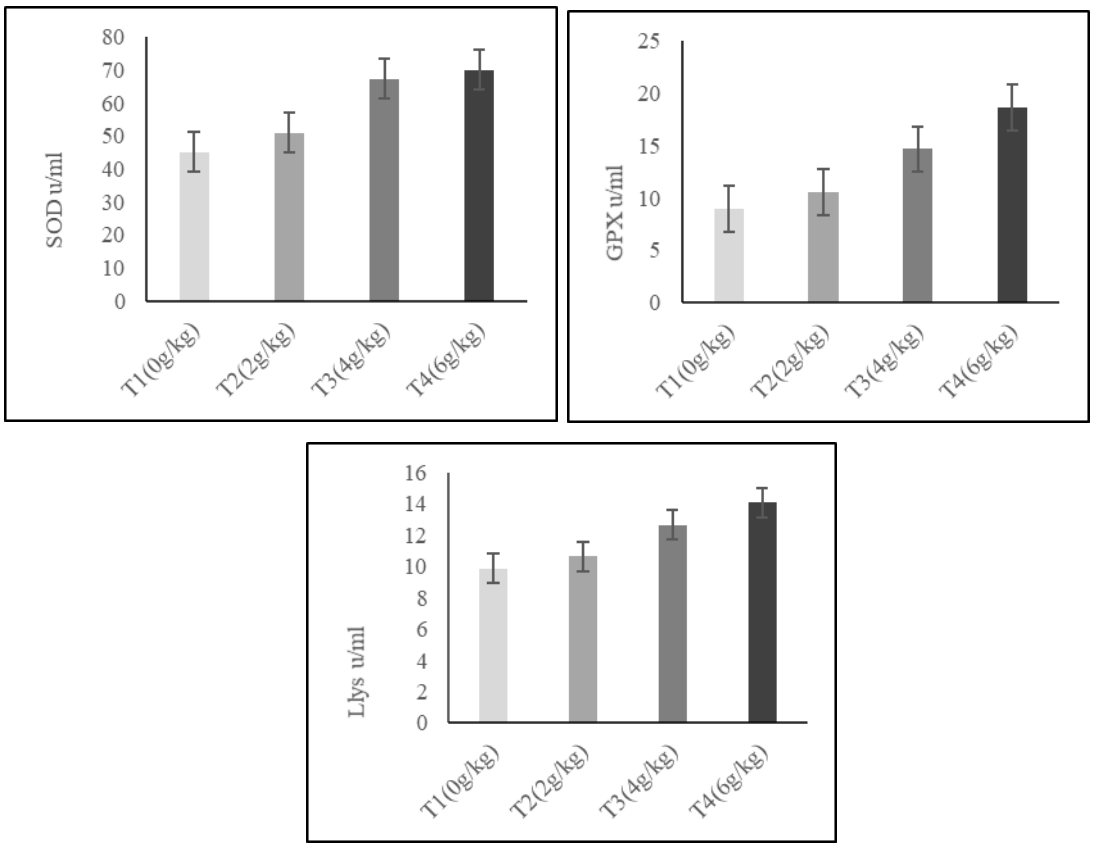

Fig. 1. Serum antioxidant superoxide dismutase (SOD), glutathione peroxidase (GPX), and innate immunity lysozyme (LYZ) activity of common carp fed with *T. cordifolia* leaves powder-supplemented diets after 8 weeks (Mean ± SD)

DISCUSSION

Numerous studies have demonstrated the advantages of medicinal plant-based supplements in aquaculture, improved growth and enhanced immunity **(Yousefi *et al.*, 2021)**. The current study found that *T. cordifolia* had beneficial effects on *C. carpio* growth performance compared to the control group. This might be attributed to *T. cordifolia* being a rich source of polysaccharides and polyphenols that support intestinal immunity and digestive enzyme function **(Saeed *et al.*, 2020)**. Polysaccharides have a crucial role as fermentable "prebiotics" in stimulating the growth and activity of beneficial intestinal bacteria, leading to the secretion of digestive enzymes and improved feed utilization **(Vidhya *et al.*, 2019; Mohammadi *et al.*, 2020)**. In agreement with our results, **Upreti and Chauhan (2018)** recommeneded incorporating up to 1% of *T. cordifolia* leaf powder in *Catla catla* feed to enhance growth and survival up to the fry stage. *Moringa oleifera* leaf powder supplemental diets enhance the immunity and growth performance of rainbow trout *Oncorhynchus mykiss* **(Sanchooli *et al.*, 2023)**.

Fish health may be determined using blood hematological and biochemical markers **(Fazio, 2019)**. The current study found an increase in RBC, Hct, and Hb levels in *C. carpio* that were fed with diets enriched with *T. cordifolia*. This might be related to the polyphenol chemicals in *T. cordifolia* functioning as natural antioxidants, possibly preventing hemolysis by free radicals and prolonging the lifespan of RBCs. In line with our findings, the use of mango (*Mangifera indica*), guava (*Psidium guajava*), and noni leaves (*Morinda citrifolia*) in common carp diets increased survival rate and improved the hematological parameters **(Effendi *et al*., 2023)**. The present study revealed that, the increased biochemical parameters in the fingerlings administered diets enriched with *T. cordifolia* increases in total serum protein, albumin, and globulin in fish species may be associated with an active innate immune response **(Yang *et al*., 2015)**. These results are in agreement with the enrichment of goldfish *Carassius auratus* diet with 1.5% chaste tree fruit extract positively impacting growth performance, serum biochemical, and hematological parameters **(Rashmeei *et al*., 2022)**. The growth performance, hematological indices, and biochemical parameters of *Cirrhinus mrigala* fish were shown to exhibit a significant increase ($P< 0.05$) when fed with supplemental *Hygrophila auriculata* **(Kumar *et al*., 2022)**. In the current study, supplementation with *T. cordifolia* leaf powder lowered blood glucose levels in common carp compared to the non-supplemented group. Previous research has suggested that saponarin isolated from *T. cordifolia* leaf extracts exhibits hypoglycemic activity **(Sharma *et al*., 2015)**. The mechanism of the hypoglycemic *T. cordifolia* may be attributed to alkaloids like magnoflorine, palmetine, and jatrorrhizine present in *T. cordifolia*, which have an insulin-mimetic effect **(Patel & Mishra, 2011)**. Similar results have been reported by **Hamdani and Antony (2022)** that included growth enhancement, hematological parameters improvement, and increased digestive enzyme activity, while decreased blood glucose levels in the Nile tilapia *Oreochromis niloticus* fed herbal seed powders Celery *Apium graveolens*, Fenugreek *Trigonella foenum-graecum*, and Coriander *Coriandrum sativum*. The antioxidant enzymes serve as indicators of the body's antioxidant system, which signifies the body's capacity to metabolize oxygen free radicals and safeguard fish tissues against oxidative damage **(Vazirzadeh *et al*. 2020; Deivi *et al*. 2021; Morshedi *et al*. 2021; Rashidian *et al*. 2021)**. The polysaccharide component 'arabinogalactan' from *T. cordifolia* protects against free radicals **(Subramanian *et al*., 2002)**. It has been suggested that *T. cordifolia* has the potential to scavenge free radicals due to the presence of alkaloids such as choline, tinosporin, isocolumbin, palmatine, tetrahydropalmatine, and magnoflorine **(Gupta & Sharma, 2011)**. In the same line, *Mentha longifolia* increased the activity of antioxidant enzymes and reduced oxidative stress in *Caspian kutum* fish **(Gholamhosseini *et al*., 2020)**. Improvements in immunity are closely related to fish's antioxidant capacity **(Hoseinifar *et al*., 2020; El Basuini *et al*., 2021)**. Increased antioxidative capabilities protect immune cells from harm because of reduced ROS attack on DNA and lipid peroxidation **(Caxico *et al*., 2018)**. Many compounds found in *T.*

codifolia, such as 11-hydroxymuskatone, N-methyle-2-pyrrolidone, N-formylannonain, cordifolioside A, magnoflorine, tinocordioside, and syringin, are responsible for immunomodulation **(Parmar *et al*., 2011)**. These natural compounds have been shown to increase macrophage phagocytic activity and nitric acid generation **(Upadhyaya *et al*., 2011)**. *T. cordifolia* has an α-D-glucan (RR1) compound, which stimulates the creation of several immune-stimulating cytokines **(Alsuhaibani & Khan, 2017)**. Arabinogalactan polysaccharide (G1-4A) is isolated from all parts (leaves, stems, and roots) of *T. cordifolia* and activated macrophages by a classical pathway **(Das, 2022)**. Our results agree with common carp's digestive enzymes, growth performance, serum antioxidant enzyme, lysozyme, and complement were all increased by dietary costmary (*Tanacetum balsamita*) as a supplementary feed additive **(Yousefi *et al*., 2023)**.

CONCLUSION

The present study demonstrated beneficial effects on the overall health of common carp fed a diet supplemented with 6g/ kg of *T. cordifolia* leaf powder. This dietary group exhibited optimal improvements in growth performance, hemato-biochemical parameters, and antioxidant and LYZ activities compared to non-supplemented groups. These findings revealed the potential for developing fish feeds incorporating *T. cordifolia* leaf powder as a natural agent to meet nutritional requirements, improve fish health, and promote growth. Further research could explore incorporating other parts of *T. cordifolia*, such as the stems and roots as aquafeed ingredients to determine their effects and optimal inclusion levels. Analyzing different formulations with combinations of leaf, stem, and root powders may provide insights into the effects of various bioactive compounds in *T. cordifolia* on health and production outcomes in farmed fish species.

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