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Research article

Assessment of growth performance, blood biochemical parameters, and intestinal microbiota in common carp (*Cyprinus carpio*) following dietary *Tinospora cordifolia* root and stem powder supplementation

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Abstract

There is a growing concern regarding the use of antibiotics in aquaculture. The main problem is related to the increase of resistance to antibiotics by pathogenic bacteria. Medicinal plants can be used as an alternative to antibiotics to enhance fish health in general, but their usage is still limited. This paper aimed to evaluate the efficacy of Giloy (*Tinospora cordifolia*) as a feed supplement and its effect on growth indices, blood biochemical parameters, and intestinal microbiota in common carp (*Cyprinus carpio*). Fish were fed on *T. cordifolia* root (R) and stem (S) powder for 8 weeks at two levels (4 and 6 g kg^{-1}) and the experimental diets were labeled as 4R, 6R, 4S, and 6S, respectively. The outcome of the present study demonstrates that fish fed on *T. cordifolia* at 6 g kg^{-1} had significantly enhanced growth performance and blood biochemical indices. Furthermore, there was a significant elevation in the total bacterial count and lactic acid bacteria. In contrast, the level of blood glucose decreased significantly ($p < 0.05$) with *T. cordifolia* supplementation. The present study shows that *T. cordifolia* significantly promotes weight gain and feed utilization efficiency, the intestinal microbiota, and the general health of *C. carpio*, suggesting that it can serve as a natural alternative to antibiotics for fish in aquaculture enterprises.

Keywords: Blood biochemical parameters, Common carp, Growth performance, Intestinal microbiota, *T. Cordifolia*.

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INTRODUCTION

Fish is a vital nutritional component that contains necessary elements for human health, and it is widely available worldwide (Chen et al., 2022). Aquaculture generates 53% of the world's aquatic food (FAO, 2020). Intensive aquaculture helps to attain the goal of the Food and Agriculture Organization (FAO), but it causes problems with environmental protection and animal health (Zhao et al., 2023). Antibiotics are given in different ways, like a water bath or supplements, as part of 'prevent and treat' to ensure protection from diseases (Zhang et al., 2023).

The gut involves a complicated microbial ecosystem consisting of autochthonous (permanent) and allochthonous (transient) bacteria; colonizing the intestine epithelial surface by the permanent bacteria acts as a defense against pathogens (Gatlin III, 2015). Various factors may influence the abundance and composition of the intestinal microbiota, including life stage, diet, genetics, environmental conditions, rearing conditions, pathogen infection, and antibiotic use (Alvanou et al., 2023). The gut microbiota significantly influences growth performance, the intestinal mucosal barrier, immune responses, and nutrient digestion and absorption (Tan and Sun, 2020). The fish gut microbiome plays a crucial role in intestinal development, vitamin production, nutrient uptake, immune system strengthening, mucosal tolerance maintenance, and resistance against infectious diseases (Diwan et al., 2023).

The use of medicinal plants in the aquaculture industry is considered a safe replacement for antibiotics to improve health status and augment disease resistance (Al-Turaihi et al., 2023). Plant feed additives have positive impacts on fish health due to a variety of secondary bioactive compounds (Faehnrich et al., 2021; Kuralkar and Kuralkar, 2021). *T. cordifolia* is a shrub that belongs to the family Menispermaceae. Leaves, stems, and roots are the most widely used parts (Rushikesh et al., 2023). The basic values of *T. cordifolia* consist of protein, carbohydrate, fiber, and fat (4.5–11.2%, 61.66%, 15.9%, and 3.1%, resp.) (Nazir and Chauhan, 2018). It has several important biological properties, including hepatoprotective, anti-inflammatory, antibacterial, anti-oxidative, anti-hyperglycemic, immunomodulatory, and anti-stress characteristics (Chandel and Chintalwar, 2022).

T. cordifolia is an eco-friendly feed supplement for poultry and humans since it does not impact bone marrow, blood lymphocytes, or DNA integrity (Chandrasekaran et al., 2009), Recently, Ninama et al. (2022) reported that the *T. cordifolia* plant has healing properties with no harmful ingredients or adverse consequences. Many studies investigated the impact of medicinal plants on aquaculture. In this regard, El Basuini et al. (2022) found that *T. cordifolia* elevated Nile tilapia (*Oreochromis niloticus*) health by improving tolerance to hypoxia, augmenting the activation of mucus and serum immune functions, and strengthening the antioxidant response. Omar (2023) found that rosemary *Rosmarinus officinalis* leaf powder enhances *C. carpio* health by improving growth performance and blood biochemical parameters without altering body composition. Additionally, Ibrahim et al. (2024) discovered that feeding *O. niloticus)* a diet supplemented with red pepper (*Capsicum annuum*) extract can improve growth, digestive enzyme activities (amylase and protease), and gene expression. Also, Wei et al. (2024) showed that *Curcuma longa* leaf powder enhanced *Clarias gariepinus* health by elevating growth performance, increasing the activity of digestive enzymes, and boosting antioxidant enzyme activity. This study aimed to find out how *T. cordifolia* stem and root powder may affect growth indices, blood biochemical parameters, and intestinal microbiota in *C. carpio*.

MATERIALS AND METHODS

Ethical approval

This study has been in accordance with the ethical protocol and guidelines for experimental animals approved by the Department of Fisheries and Marine Resources, College of Agriculture, University of Basrah, Iraq (UOB\COA.0002-IQ10-10-2023).

Preparing the experiment diet

A mercantile aquafeed (made in Iran) was utilized as a basal diet. The percentage of its components of protein, fat, ash, fiber, and moisture was 35.50%, 12.20%, 16.60%, 24.4%, and 10%, respectively. The basal diet pellets were ground into a fine powder. *T. cordifolia* root (R) and stem (S) powders were then incorporated into separate batches at two concentrations: 4 and 6 g per kg of feed. The resulting experimental diets were labeled as follows: 4R: 4 g/kg root powder; 6R: 6 g/kg root powder; 4S: 4 g/kg stem powder; 6S: 6 g/kg stem powder. The control diet (T1) remained unsupplemented. Each experimental diet was prepared by thoroughly mixing the respective *T. cordifolia* powder concentration with the ground basal diet and warm water to form a homogeneous dough. Using a meat machine, the dough was made into 2 mm thick pellets and stored in plastic containers in the refrigerator until use.

Fish husbandry and experimental condition

The experiment was conducted at the fish nutrition laboratory of the College of Agriculture, University of Basrah, Iraq. After fifteen days of fish acclimatization, 150 fish (initial weight 12.58±0.05 g) were distributed among fifteen plastic aquariums (a semi-closed recirculation system), with each aquarium having a capacity of 30 L of water with 10 fish. The feeding trial consisted of five treatments with three replicates for each one. During the experiment, fish were fed at 3% of their body weight and weighed every fifteen days after a 24-hour starvation period. Water quality parameters, that is, temperature, dissolved oxygen, pH, salinity, and ammonia, were monitored daily.

Growth indices

At the end of the feeding experiment, the growth indices: weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER), were computed as follows:

WG = final body weight (FW) – initial body weight (IW)

SGR (% day^{-1}) = 100 × (Ln FW – Ln IW) / experimental days (ED)

FCR = feed intake (FI) / WG

PER = wet weight gain (g) / protein intake (g)

Blood biochemical parameters analysis

For blood sample collection, six fish per treatment were sedated with 5 mg/L lidocaine (lidocaine hydrochloride), and the tail-cutting method was applied according to Duman et al. (2019). The number of red blood cells (RBC), packed cell volume (PCV%), and hemoglobin (Hb) concentrations (g/dL) were estimated according to Witeska et al. (2022). Blood samples were centrifuged at 3000×g for 10 minutes to obtain the serum for evaluating the total serum protein (TP) and albumin (ALB) at wavelengths of 550 nm and 630 nm, respectively using a Mindray BS-230 kit. The serum globulin (GLB) level was measured according to Wolf and Darlington (1971), while the levels of glucose (GLU) in the blood were measured by a glucose meter (VivaCheck™).

Intestinal microbiota analysis

The second-third of the intestine from six fish per treatment was obtained to assess the intestinal microbiota. The Standard Plate Count (SPC) was conducted using a series of decimal dilutions. To prepare the dilutions, 1 g of fish intestines was homogenized with 9 ml of sterile 0.1% peptone solution. Total bacterial count (TBC) was measured using the nutrient agar medium, following Andrews (1992), and lactic acid bacteria (LAB) were enumerated using Man Rogosa Sharpe agar medium according to Speck (1984).

Statistical analysis

All data were exhibited as means ± standard deviation (SD) and statistically significant at $p < 0.05$. Statistical analyses were carried out using SPSS (version 24). A one-way analysis of variance (ANOVA) followed by least significant difference (LSD) was performed to evaluate statistical differences.

RESULTS

Growth indices

Table 1 displays the growth indices. The study demonstrated that fish groups fed diets supplemented with *T. cordifolia* root and stem powder exhibited significantly greater improvement compared to the control groups in terms of WG, SGR, FCR, and PER. The best growth parameters of the mentioned standards were recorded in fish fed 6 g kg^{-1} *T. cordifolia* stem powder (6S), followed by fish fed 6 g kg $^{-1}$ root powder (6R). Significant differences ($p < 0.05$) were observed between the two groups.

Table 1 Growth indices in common carp after 8 weeks of feeding on experimental diets.

	T. cordifolia root and stem powder supplemented diet (g kg $^{-1}$)				
Parameters	T1	4R	6R	4S	6S
IW (g)	12.33±0.07	12.75±0.06	12.42±0.03	12.90±0.01	12.52±0.07
FW (g)	32.95±0.17^{e}	42.91±0.05^{d}	47.63±0.64^{b}	45.60±0.40^{c}	50.50±0.09^{a}
WG (g)	20.62±0.10^{e}	30.16±0.05^{d}	35.21±0.67^{b}	32.70±0.40^{c}	37.99±0.16^{a}
FCR	1.67±0.01^{a}	1.48±0.01^{b}	1.42±0.03^{c}	1.39±0.01^{c}	1.36±0.00^{d}
SGR (% day^{-1})	1.64±0.01^{e}	2.02±0.01^{d}	2.24±0.03^{b}	2.10±0.02^{c}	2.32±0.02^{a}
PER	0.58±0.0^{e}	0.85±0.0^{d}	0.99±0.02^{b}	0.92±0.01^{c}	1.07±0.01^{a}

Significant differences between groups are indicated by different superscript letters. Values are expressed as mean ± SD.
Note: T1 (control group without supplement diet), 4R (fish groups fed dietary supplements containing 4 g/kg of *T. cordifolia* root powder), 6R (fish groups fed dietary supplements containing 6 g/kg of *T. cordifolia* root powder), 4S (fish groups fed dietary supplements containing 4 g/kg of *T. cordifolia* stem powder), and 6S (fish groups fed dietary supplements containing 6 g/kg of *T. cordifolia* stem powder).

Blood biochemical parameters

All values for blood biochemical parameters data are presented in Table 2. The study illustrated that the RBC count, PCV%, Hb, TP, ALB, and GLB were enhanced significantly ($p < 0.05$) in *T. cordifolia*-supplemented diet groups compared to the control group. The highest values for the mentioned standards were registered in fish fed 6 g kg^{-1} of stem powder, followed by fish fed 6 g kg^{-1} of root powder. On the other hand, glucose levels in the blood of all supplement groups of *T. cordifolia* root and stem powder decreased significantly ($p < 0.05$) compared with the control group.

Table 2 Blood biochemical parameters of common carp after 8 weeks of feeding on experimental diets.

Parameters	*T. cordifolia* root and stem powder supplemented diet (g kg $^{-1}$)				
	T1	4R	6R	4S	6S
RBC ×10^6 / µl	1.37±0.04^c	1.54±0.03^b	1.57±0.05^{ab}	1.55±0.05^{ab}	1.60±0.04^a
PCV	25.49±1.72^c	27.51±1.35^b	31.95±1.10^a	27.88±1.51^b	32.66±1.75^a
Hb g/ dL	9.88±0.83^d	13.84±0.60^c	17.36±0.59^a	14.95±0.71^b	17.77±0.64^a
TP g/ dL	2.18±0.03^d	2.45±0.08^c	2.65±0.06^a	2.52±0.06^b	2.71±0.03^a
ALB g/ dL	1.27±0.03^c	1.43±0.05^b	1.60±0.04^a	1.48±0.04^b	1.62±0.09^a
GLB g/ dL	0.91±0.04^b	1.02±0.07^a	1.60±0.04^a	1.04±0.07^a	1.09±0.09^a
GLU mg/dL	67.83±2.32^a	57.38±1.75^b	56.20±3.31^b	56.17±4.27^b	54.90±2.43^b

Significant differences between groups are indicated by different superscript letters. Values are expressed as mean ± SD.
Note: T1 (control group without supplement diet), 4R (fish groups fed dietary supplements containing 4 g/kg of *T. cordifolia* root powder), 6R (fish groups fed dietary supplements containing 6 g/kg of *T. cordifolia* root powder), 4S (fish groups fed dietary supplements containing 4 g/kg of *T. cordifolia* stem powder), and 6S (fish groups fed dietary supplements containing 6 g/kg of *T. cordifolia* stem powder).

Intestinal microbiota

The intestinal microbiota of *C. carpio* after 8 weeks of feeding on fed *T. cordifolia* root and stem powder is shown in Table 3 and Figure 1. The study showed that all experimental groups fed on the *T. cordifolia* supplement diet displayed a significantly higher ($p < 0.05$) improvement in the TBC and LAB counts than the control group. Fish fed on *T. cordifolia* stem powder at level 6 g kg^{-1} recorded the best TBC value, followed by fish fed on a diet supplemented with *T. cordifolia* root powder at level 6 g kg^{-1}. However, no significant differences ($p > 0.05$) had been recorded between the two groups. For LAB, fish fed on 6 g kg^{-1} stem powder (6S) recorded the highest value, followed by fish fed on 6 g kg^{-1} root powder (6R). Moreover, significant differences ($p < 0.05$) were observed between the two groups.

Table 3 Intestinal microbiota in common carp after 8 weeks of applying a *T. cordifolia*-supplemented diet.

Parameter	*T. cordifolia* root and stem powder supplemented diet (g kg $^{-1}$)				
	T1	4R	6R	4S	6S
TBC (cfu/g)	4.95±0.15 ^{d}	5.33±0.06 ^{c}	5.78±0.08 ^{a}	5.45±0.06 ^{b}	5.84±0.10 ^{a}
LAB (cfu/g)	2.21±0.02 ^{d}	2.65±0.07 ^{c}	2.91±0.03 ^{b}	2.74±0.08 ^{c}	3.13±0.18 ^{a}

Values in each row with different letters are significantly different at $p < 0.05$ (mean ± SD).
Note: T1 (control group without supplement diet), 4R (fish groups fed dietary supplements containing 4 g/kg of *T. cordifolia* root powder), 6R (fish groups fed dietary supplements containing 6 g/kg of *T. cordifolia* root powder), 4S (fish groups fed dietary supplements containing 4 g/kg of *T. cordifolia* stem powder), and 6S (fish groups fed dietary supplements containing 6 g/kg of *T. cordifolia* stem powder).

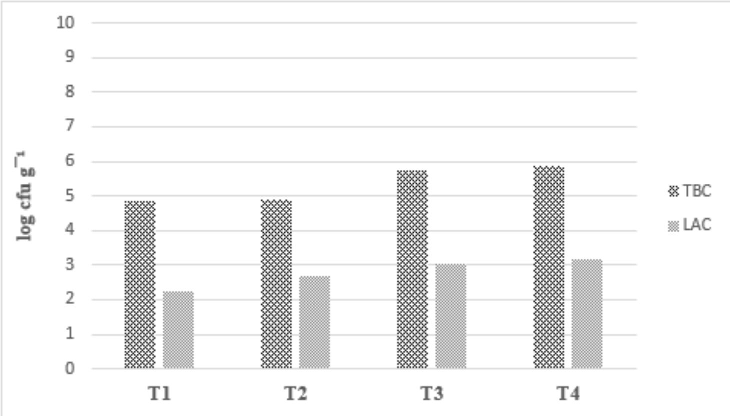

Figure 1 Intestinal microbiota total bacterial count (TBC) and lactic acid bacteria (LAB) of common carp fed a *T. cordifolia* supplemented diet for 8 weeks.

DISCUSSION

Growth performance estimation through weight gain, specific growth rate, feed intake, and protein efficiency ratio is fundamental for evaluating the effectiveness of feed additives (Abdel Rahman et al., 2021). The current study showed that *T. cordifolia* root and stem supplementation improved *C. carpio* growth indices compared to the control group. This result could be attributed to the presence of the polysaccharide (starch) in the stem and root parenchyma (Khosa and Prasad, 1971). Polysaccharides in *T. cordifolia* involve glucose, xylose, arabinose, galactose, mannose, rhamnose, and galactose as the ratio 98.0%, 0.8%, 0.5%, 0.3%, 0.2%, and 0.2%, respectively (Jahfar, 2003). Starch provides energy, thereby improving the digestion process (Mia et al., 2009). Adequate levels of polysaccharides can raise protein retention and benefit from it to improve growth criteria (Ma et al., 2019; Arenas et al., 2021). Similarly, dietary *T. cordifolia* stem powder supplementation significantly improved the body weight gain, feed intake, and feed conversion ratio in broiler chickens (Singh et al., 2018). In the same context, Ramezanzadeh et al. (2021) reported that barberry root (*Berberis vulgaris*) promotes growth index and improves flesh quality in rainbow trout fingerlings (*Oncorhynchus mykiss*).

Hematological parameters are utilized to estimate the health and immunity status (Dorucu et al., 2009). The result of the current study showed a rise in RBC, PCV%, and Hb levels in *C. carpio* fed a diet supplemented with *T. cordifolia* root and stem powder. This might be attributed to *T. cordifolia* having bioactive compounds like Magnoflorine and Tinocordiside which belong to alkaloids and glycosides respectively, that elevate phagocytic activity and enhance nitric oxide and reactive oxygen species production in macrophages (Sharma et al., 2012). Likewise, Sharma et al. (2022) found an improvement in the immunity and hematological parameters in snow trout *Schizothorax richardsonii* when fed a diet supplemented with a powder consisting of *T. cordifolia*, *Allium sativum*, *Aloe barbadensis,* and *Withania somnifera*.

Nutrient absorption, metabolism, health, and immunity status in fish can be determined by blood biochemical parameters (Ban et al., 2022; Pastorino et al., 2022). In the current study, the fish ingesting the *T. cordifolia* root and stem powder supplement diet showed increased biochemical parameters compared to the control group. *T. cordifolia* is abundant with phytochemical compounds that belong to many classes such as phenolics, flavonoids, alkaloids, glycosides, polysaccharides, and others, which play a crucial role in biological activities (Anjum et al., 2023). Tungmunnithum et al. (2018) declared that phenolic and flavonoid substances have immune and antioxidant effects.

In this study, supplementation with *T. cordifolia* root and stem powder has been shown to lower blood glucose levels in *C. carpio* compared to the control group. This might be attributed to *T. cordifolia* having compounds belonging to groups like steroids, alkaloids, and cardiac glycosides, which are known to control blood sugar levels by stimulating insulin production and blocking gluconeogenesis (Rawal et al., 2004). In addition, the alkaloid Magnoflorine in *T. cordifolia* is an effective inhibitor of ά -glucosidase by the closing of glucose co-transporters Glut-2 and Glut-4, which are considered the major transporter of glucose into the blood from the gut (Sharma et al., 2015). Our findings are consistent with those of Savin et al. (2023) who found that *C. carpio* fed on a diet supplemented with licorice *(Glycyrrhiza glabra)*, echinacea (*Echinacea purpurea)*, and wild thyme (*Thymus serpyllum)* showed enhancement in biochemical and hematological parameters and lowered glucose levels.

The digestion process, nutrition absorption, and immunity status significantly depend on gut health (Ramena et al., 2020). The gut microbiota enhances immunity in many ways, including by boosting physical barriers and managing physiological processes that regulate innate immunity to protect the body against infections (Qi et al., 2019). The current study demonstrated that *T. cordifolia* root and stem powder supplementation enhanced the intestinal microbiota in *C. carpio* compared to the control group. This effect could be attributed to the chemical components of *T. cordifolia,* specifically polysaccharides (Upadhyay et al., 2010). It is well known that plant polysaccharides elevate the number of beneficial bacteria and limit the growth of several pathogenic bacteria, leading to an increase in the balance of the gut microbiota (Li et al., 2017a; Li et al., 2017b). At the same time, many types of intestinal microbiota ferment polysaccharides into short-chain fatty acids; these last products supply energy for epithelial cell proliferation and enhance intestinal barrier function (Qi et al., 2018). These results are in line with a previous investigation conducted by He et al. (2022), who indicated the capability of *Artemisia annua* to modify the intestinal microflora, enhance hepatic enzymes, and increase the number of gut villi in largemouth bass (*Micropterus salmoides*).

CONCLUSIONS

Introducing natural feed additives to improve fish health is considered a promising, available, cost-effective, and eco-friendly way to expand the aquaculture industry. The experiment showed that fish fed on a diet supplemented with 6 g kg^{-1} of *T. cordifolia* root and stem powders exhibited enhancements in growth performance indices, blood biochemical parameters, and intestinal microbiota. Both powders have good therapeutic effects, yet the stem is more available than the root. We recommend further research into the applications of *T. cordifolia* nanoparticles to control disease in intensive aquaculture, as well as potential effects on water quality.

AUTHOR CONTRIBUTIONS

Zahraa Mohammed Ridha Al-Turaihi: Methodology, Resources, Data Curation.
Khalidah Salim Al-Niaeem and Arafat Rajab Ahmed: Supervision.
All authors: Writing—original draft, and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors declare that no conflicts of interest exist.

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