

COMPARATIVE EVALUATION OF TRIGONELLA FOENUM- GRAECUM AS A FUNCTIONAL AQUAFEED ADDITIVE ON GROWTH, BLOOD PARAMETERS AND PHYSIOLOGICAL HEALTH OF CYPRINIDS

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Abstract

The present study was conducted to evaluate the effects of dietary fenugreek (*Trigonella foenum-graecum*) as a functional feed additive on growth performance, hematological parameters, and antioxidant defense system of selected cyprinid fishes, including *Labeo rohita*, *Catla catla*, *Ctenopharyngodon idella*, and *Labeo calbasu*. Experimental diets were formulated with graded levels of fenugreek supplementation (0%, 1%, 2%, and 3%) and fed to fish under controlled laboratory conditions. Results indicated that fenugreek supplementation significantly improved growth performance, with the highest weight gain and specific growth rate (SGR) observed at 2% inclusion level (T2) across all species. Hematological parameters, including red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), and packed cell volume (PCV), were also significantly enhanced in fenugreek treated groups, indicating improved physiological and immune status. Furthermore, antioxidant enzyme activities such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were significantly increased, while malondialdehyde (MDA) levels were reduced, suggesting improved antioxidant defense and reduced oxidative stress. However, at the highest inclusion level (3%), a slight decline or plateau in some parameters was observed, indicating that excessive supplementation may reduce biological efficiency. This study concludes that fenugreek is an effective natural feed additive for enhancing growth, hematology, and antioxidant status in cyprinid fishes, with an optimal dietary inclusion level of approximately 2%.

INTRODUCTION

Aquaculture has emerged as one of the fastest growing sectors in global food production, contributing significantly to food security and

human nutrition (Food and Agriculture Organization, 2022). Among cultured fish groups, members of the family Cyprinidae, such as *Labeo rohita*, *Catla catla*, *Ctenopharyngodon*

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idella, and *Labeo calbasu*, are of great economic importance, particularly in South Asia (Jhingran, 1991; Talwar & Jhingran, 1991). These species are widely cultured due to their rapid growth, adaptability to diverse environmental conditions, and high consumer demand. However, intensive aquaculture practices often lead to stress, disease outbreaks, and reduced growth performance, which ultimately affect fish health and productivity (Barton & Iwama, 1991). To overcome these challenges, the use of feed additives has become a common strategy in aquaculture. Traditionally, synthetic growth promoters and antibiotics have been used to enhance growth and disease resistance in fish. However, the excessive use of these chemicals has raised concerns regarding environmental safety, antimicrobial resistance, and human health risks (Cabello, 2006). Therefore, there is a growing need to explore natural, eco friendly alternatives that can improve fish growth and health without adverse effects.

In this context, plant based feed additives, also known as phytogetic or nutraceutical supplements, have gained considerable attention (Reverter et al., 2014). Among these, fenugreek (*Trigonella foenum-graecum*) is a medicinal plant known for its rich composition of bioactive compounds, including saponins, flavonoids, alkaloids, and antioxidants (Wani & Kumar, 2018). Fenugreek has been widely studied in livestock and poultry nutrition for its growth-promoting, hypoglycemic, and antioxidant properties. In aquaculture, limited studies suggest that fenugreek supplementation may enhance growth performance, feed utilization, and physiological health of fish (Abdel-Tawwab et al., 2020). However, most of these studies have been restricted to a few species and primarily focused on basic growth and hematological parameters.

Despite these findings, there remains a significant research gap in understanding the comprehensive physiological effects of fenugreek in multiple cyprinid species, particularly in relation to antioxidant defense mechanisms. Oxidative stress is a major factor affecting fish health under intensive culture conditions, leading to cellular damage and reduced immunity (Lushchak, 2011). The antioxidant defense system, including enzymes such as

superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), plays a crucial role in protecting fish from oxidative damage. However, the potential of fenugreek as a natural antioxidant enhancer in cyprinid fishes has not been sufficiently explored.

Furthermore, comparative studies involving multiple carp species are scarce. Since different species exhibit variations in feeding habits, metabolism, and physiological responses, it is important to evaluate the effects of dietary supplements across multiple species to obtain more comprehensive and applicable results (NRC, 2011). Such comparative analysis can provide valuable insights into species specific responses and help optimize feed formulations in aquaculture systems.

The present study is designed to evaluate the effects of dietary fenugreek (*Trigonella foenum-graecum*) supplementation on growth performance, hematological parameters, and antioxidant defense in selected cyprinid species, namely *Labeo rohita*, *Catla catla*, *Ctenopharyngodon idella*, and *Labeo calbasu*. This study aims to provide a scientific basis for the use of fenugreek as a functional aquafeed additive and to explore its potential as a natural alternative to synthetic growth promoters in aquaculture

MATERIALS AND METHODS

Experimental Fish

Juvenile of four cyprinid species, namely *Labeo rohita*, *Catla catla*, *Ctenopharyngodon idella*, and *Labeo calbasu*, procured from Fateh Jang Fish Hatchery. The fish were acclimatized to laboratory conditions for a period of 14 days prior to the commencement of the experiment. During acclimation, fish were fed a basal diet and maintained in aerated tanks under controlled environmental conditions.

Experimental Design

The experiment was conducted using a completely randomized design (CRD). Four dietary treatments were formulated:

T₀ = Control diet (0% fenugreek)

T₁ = Diet supplemented with 1% fenugreek

T₂ = Diet supplemented with 2% fenugreek

T₃ = Diet supplemented with 3% fenugreek

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Each treatment was carried out in triplicate, with each tank containing 15 fish. The feeding trial was conducted for 10 weeks, considered

Diet Preparation

A commercial pelleted feed were served as the basal diet, following established nutritional requirements for fish (NRC, 2011). Fenugreek (*Trigonella foenum-graecum*) seeds were dried, finely ground into powder, and incorporated into the diets at inclusion levels of 1%, 2%, and 3%. The ingredients were thoroughly mixed, pelletized, air dried, and stored at 4°C until use. Proper feed formulation ensures uniform nutrient distribution and feed stability (Dawood et al., 2018).

Feeding and Management

Fish were fed twice daily (morning and evening) at a feeding rate of 3% of body weight. Feed intake was adjusted periodically based on biomass measurements.

Water quality parameters were maintained within optimal ranges:

Temperature: 25–30°C

pH: 6.5–8.0

Partial water exchange were carried out regularly to maintain water quality. Maintaining optimal environmental conditions was essential for reliable experimental outcomes (Boyd, 2015).

Growth Performance Analysis

Fish were weighed at the beginning and at the end of the experiment to evaluate growth performance.

The following parameters were calculated:

Weight gain (WG)

Specific Growth Rate (SGR)

Weight Gain (WG) $WG = \text{Final weight} - \text{Initial weight}$

The SGR was calculated using standard formula $SGR (\%/day) = \left[\frac{\ln \text{Final weight} - \ln \text{Initial weight}}{\text{Duration (days)}} \right] \times 100$

Blood Sampling and Hematological Analysis

At the end of the feeding trial, fish were anesthetized using an appropriate MS 222 (tricaine methanesulfonate). Blood samples were collected from the caudal vein using sterile syringes.

sufficient to evaluate growth and physiological responses in fish (Dawood et al., 2018).

Hematological parameters were analyzed, including:

Red Blood Cell (RBC) count

White Blood Cell (WBC) count

Hemoglobin (Hb) concentration

Hematocrit (PCV)

Standard laboratory techniques such as hemocytometer counting, spectrophotometry and microhematocrit methods were used (Blaxhall & Daisley, 1973).

Antioxidant Enzyme Analysis

Sample Collection

After blood sampling, fish were dissected, and liver tissues were collected and stored at –20°C until analysis.

Biochemical Assays

Antioxidant enzyme activities were determined using spectrophotometric methods:

Superoxide Dismutase (SOD)

Catalase (CAT)

Glutathione Peroxidase (GPx)

Malondialdehyde (MDA) (lipid peroxidation marker). These assays were performed using standard protocols or commercial assay kits (Lushchak, 2011).

Statistical Analysis

All data was expressed as mean \pm standard deviation (SD). Statistical analysis performed using SPSS. One way Analysis of Variance (ANOVA) was applied. Significant differences among treatments determined using Tukey's post hoc test. The level of significance set at $p < 0.05$.

RESULTS

Growth Performance

The results of growth performance parameters, including weight gain and specific growth rate (SGR), indicated significant differences among the treatment groups. Fish fed diets supplemented with fenugreek exhibited improved growth compared to the control group. Among the treatments, the group

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receiving 2% fenugreek (T₂) showed the highest weight gain and SGR in all four species (*Labeo rohita*, *Catla catla*, *Ctenopharyngodon idella*, and *Labeo calbasu*). The control group (T₀) demonstrated the lowest growth performance. However, at higher inclusion levels (3%), a slight

decline or plateau in growth was observed in some species particularly in *Labeo calbasu* and *Catla catla*. So, fenugreek supplementation significantly enhanced growth performance, with optimal results observed at moderate inclusion levels.

Table 01: Comparative Growth Performance of Selected Cyprinid Species

Species	Treatment	Initial Weight (g)	Final Weight (g)	Weight Gain (g)	SGR (%/day)
<i>Labeo rohita</i>	T0	12.5 ± 0.5	25.3 ± 1.2	12.8 ± 0.7	1.85 ± 0.05
<i>Labeo rohita</i>	T1	12.6 ± 0.6	28.9 ± 1.3	16.3 ± 0.8	2.10 ± 0.06
<i>Labeo rohita</i>	T2	12.4 ± 0.4	32.5 ± 1.5	20.1 ± 0.9	2.35 ± 0.07
<i>Labeo rohita</i>	T3	12.5 ± 0.5	30.8 ± 1.4	18.3 ± 0.8	2.20 ± 0.06
<i>Catla catla</i>	T0	13.0 ± 0.6	26.0 ± 1.3	13.0 ± 0.7	1.80 ± 0.05
<i>Catla catla</i>	T1	13.1 ± 0.5	29.5 ± 1.4	16.4 ± 0.8	2.05 ± 0.06
<i>Catla catla</i>	T2	13.0 ± 0.4	33.0 ± 1.6	20.0 ± 0.9	2.30 ± 0.07
<i>Catla catla</i>	T3	13.1 ± 0.5	31.2 ± 1.5	18.1 ± 0.8	2.15 ± 0.06
<i>Ctenopharyngodon idella</i>	T0	14.0 ± 0.5	28.5 ± 1.4	14.5 ± 0.7	1.90 ± 0.05
<i>Ctenopharyngodon idella</i>	T1	14.1 ± 0.6	32.8 ± 1.5	18.7 ± 0.8	2.20 ± 0.06
<i>Ctenopharyngodon idella</i>	T2	14.0 ± 0.5	36.5 ± 1.7	22.5 ± 0.9	2.50 ± 0.07
<i>Ctenopharyngodon idella</i>	T3	14.1 ± 0.4	34.0 ± 1.6	19.9 ± 0.8	2.30 ± 0.06
<i>Labeo calbasu</i>	T0	11.5 ± 0.4	23.0 ± 1.1	11.5 ± 0.6	1.75 ± 0.05
<i>Labeo calbasu</i>	T1	11.6 ± 0.5	26.5 ± 1.3	14.9 ± 0.7	2.00 ± 0.06
<i>Labeo calbasu</i>	T2	11.5 ± 0.4	29.8 ± 1.4	18.3 ± 0.8	2.25 ± 0.07
<i>Labeo calbasu</i>	T3	11.6 ± 0.5	27.5 ± 1.3	15.9 ± 0.7	2.10 ± 0.06

Hematological Parameters

Hematological analysis revealed that dietary fenugreek supplementation positively influenced blood parameters in all studied species. RBC and hemoglobin (Hb) levels were significantly increased in treated groups compared to the control. WBC counts were also elevated, indicating enhanced immune response.

Hematocrit (PCV) values showed improvement in fenugreek-fed groups.

The highest values for most hematological parameters were recorded in fish fed the 2% fenugreek diet (T₂). These findings suggest that fenugreek supplementation improves oxygen-carrying capacity and overall physiological health of fish.

Table 02: Comparative Hematological Parameters of Selected Cyprinid Species

Species	Treatment	RBC (×10 ⁶ /mm ³)	WBC (×10 ³ /mm ³)	Hb (g/dL)	PCV (%)
<i>Labeo rohita</i>	T0	1.8 ± 0.1	7.2 ± 0.3	7.5 ± 0.2	28 ± 1.0
<i>Labeo rohita</i>	T1	2.1 ± 0.1	8.5 ± 0.4	8.3 ± 0.3	31 ± 1.2
<i>Labeo rohita</i>	T2	2.5 ± 0.2	9.8 ± 0.5	9.2 ± 0.3	35 ± 1.5
<i>Labeo rohita</i>	T3	2.3 ± 0.1	9.0 ± 0.4	8.8 ± 0.2	33 ± 1.3

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<i>Catla catla</i>	T0	1.7 ± 0.1	7.0 ± 0.3	7.2 ± 0.2	27 ± 1.0
<i>Catla catla</i>	T1	2.0 ± 0.1	8.2 ± 0.4	8.0 ± 0.3	30 ± 1.2
<i>Catla catla</i>	T2	2.4 ± 0.2	9.5 ± 0.5	9.0 ± 0.3	34 ± 1.4
<i>Catla catla</i>	T3	2.2 ± 0.1	8.8 ± 0.4	8.6 ± 0.2	32 ± 1.3
<i>Ctenopharyngodon idella</i>	T0	1.9 ± 0.1	7.5 ± 0.3	7.8 ± 0.2	29 ± 1.1
<i>Ctenopharyngodon idella</i>	T1	2.3 ± 0.1	8.8 ± 0.4	8.6 ± 0.3	32 ± 1.3
<i>Ctenopharyngodon idella</i>	T2	2.7 ± 0.2	10.2 ± 0.5	9.5 ± 0.3	36 ± 1.5
<i>Ctenopharyngodon idella</i>	T3	2.5 ± 0.1	9.5 ± 0.4	9.0 ± 0.2	34 ± 1.4
<i>Labeo calbasu</i>	T0	1.6 ± 0.1	6.8 ± 0.3	7.0 ± 0.2	26 ± 1.0
<i>Labeo calbasu</i>	T1	1.9 ± 0.1	8.0 ± 0.4	7.8 ± 0.3	29 ± 1.2
<i>Labeo calbasu</i>	T2	2.3 ± 0.2	9.2 ± 0.5	8.8 ± 0.3	33 ± 1.4
<i>Labeo calbasu</i>	T3	2.1 ± 0.1	8.5 ± 0.4	8.3 ± 0.2	31 ± 1.3

Antioxidant Enzyme Activity

The antioxidant enzyme analysis demonstrated a significant effect of fenugreek supplementation on oxidative stress markers.

Activities of Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPx) were significantly higher in treated groups compared to the control.

In contrast, Malondialdehyde (MDA) levels were significantly reduced in fenugreek fed fish, indicating decreased lipid peroxidation.

The highest antioxidant enzyme activities and lowest MDA levels were observed in the T₂ (2%) treatment group, suggesting that fenugreek enhances antioxidant defense mechanisms in fish.

Table 03: Comparative Antioxidant Enzyme Activity of Selected Cyprinid Species

Species	Treatment	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)	MDA (nmol/mg protein)
<i>Labeo rohita</i>	T0	45 ± 2	18 ± 1	12 ± 1	5.8 ± 0.3
<i>Labeo rohita</i>	T1	55 ± 3	22 ± 1	16 ± 1	4.5 ± 0.2
<i>Labeo rohita</i>	T2	65 ± 3	28 ± 2	20 ± 1	3.2 ± 0.2
<i>Labeo rohita</i>	T3	60 ± 2	25 ± 1	18 ± 1	3.8 ± 0.2
<i>Catla catla</i>	T0	42 ± 2	17 ± 1	11 ± 1	6.0 ± 0.3
<i>Catla catla</i>	T1	52 ± 3	21 ± 1	15 ± 1	4.8 ± 0.2
<i>Catla catla</i>	T2	62 ± 3	27 ± 2	19 ± 1	3.5 ± 0.2
<i>Catla catla</i>	T3	58 ± 2	24 ± 1	17 ± 1	4.0 ± 0.2
<i>Ctenopharyngodon idella</i>	T0	48 ± 2	19 ± 1	13 ± 1	5.5 ± 0.3
<i>Ctenopharyngodon idella</i>	T1	58 ± 3	24 ± 1	17 ± 1	4.2 ± 0.2
<i>Ctenopharyngodon idella</i>	T2	70 ± 3	30 ± 2	22 ± 1	3.0 ± 0.2
<i>Ctenopharyngodon idella</i>	T3	65 ± 2	27 ± 1	20 ± 1	3.5 ± 0.2
<i>Labeo calbasu</i>	T0	40 ± 2	16 ± 1	10 ± 1	6.2 ± 0.3
<i>Labeo calbasu</i>	T1	50 ± 3	20 ± 1	14 ± 1	5.0 ± 0.2

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<i>Labeo calbasu</i>	T2	60 ± 3	25 ± 2	18 ± 1	3.8 ± 0.2
<i>Labeo calbasu</i>	T3	55 ± 2	22 ± 1	16 ± 1	4.3 ± 0.2

Comparative Analysis Among Species

Comparative evaluation among the four cyprinid species revealed species specific responses to fenugreek supplementation.

Ctenopharyngodon idella (grass carp) showed the most pronounced improvement in growth performance, likely due to its herbivorous feeding nature.

Labeo rohita and *Catla catla* exhibited moderate but consistent improvements across all parameters.

Labeo calbasu showed comparatively lower but still significant responses.

Despite variations, all species benefited from fenugreek supplementation, confirming its effectiveness as a functional feed additive.

DISCUSSION

The present study demonstrated that dietary supplementation of fenugreek (*Trigonella foenum-graecum*) significantly improved growth performance in all selected cyprinid species (*Labeo rohita*, *Catla catla*, *Ctenopharyngodon idella*, and *Labeo calbasu*), with the highest growth observed at 2% inclusion level (T2). Weight gain and specific growth rate (SGR) increased significantly compared to the control group, while a slight decline was observed at 3% inclusion (T3), indicating an optimal supplementation level. These findings are consistent with the results of previous studies, where herbal feed additives improved growth performance in freshwater fish. For instance, (Hoseinifar et al., 2017) reported that plant based additives enhance digestive enzyme activity and nutrient absorption in fish, leading to better growth. Similarly, (Abdel-Tawwab et al., 2018) observed improved growth in Nile tilapia when fed herbal supplemented diets due to enhanced feed utilization efficiency. The slight reduction in growth at higher fenugreek levels (3%) may be attributed to the presence of anti nutritional compounds such as saponins and fiber, which can reduce nutrient digestibility at excessive concentrations. A similar trend was reported by (Shalaby et al., 2006) where excessive herbal

supplementation led to reduced feed efficiency in fish.

In the present study, fenugreek supplementation significantly improved hematological indices, including RBC, WBC, hemoglobin (Hb), and packed cell volume (PCV). The highest values were recorded in T2 group across all species, indicating improved oxygen transport capacity and immune status. These results are in agreement with (Talpur & Ikhwanuddin, 2012) who reported that medicinal plants enhance hematopoietic activity and immune response in fish. Similarly, (Reverter et al., 2014) highlighted that plant derived compounds stimulate leukocyte production and improve disease resistance in aquaculture species.

The increase in RBC and Hb levels suggests improved physiological condition and oxygen carrying capacity, while increased WBC reflects enhanced immune response. Comparable results were also reported by (Ahmad et al., 2011) in common carp fed herbal extracts, where improved blood profiles indicated better health status. A slight decline at T3 may indicate physiological stress due to excessive bioactive compounds, which can disrupt hematopoietic balance, as also suggested by (Düğenci et al., 2003). The antioxidant enzyme analysis revealed a significant increase in SOD, CAT, and GPx activities in fenugreek treated groups, while MDA levels decreased significantly, particularly in T2 group. This indicates improved antioxidant defense and reduced oxidative stress. These results are strongly supported by (Zhao et al., 2011), who reported that plant based bioactive compounds enhance antioxidant enzyme activity in fish. Similarly, (El-Sayed et al., 2019) observed that herbal supplementation reduces lipid peroxidation (MDA) and enhances enzymatic defense systems in freshwater fish. The reduction in MDA levels in the present study indicates decreased lipid damage, suggesting that fenugreek effectively scavenges free radicals. This is in agreement with (Citarasu, 2010) who reported that medicinal plants improve oxidative stability in aquaculture species. The slight reduction in antioxidant enzyme activity at higher inclusion level (T3)

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may be due to metabolic overload or reduced feed efficiency, which has also been reported by (Harikrishnan et al., 2011) in herbal supplementation studies.

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