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The effect of incorporating Tulsi (*Ocimum sanctum* L.) into fish feed on the growth, survival rates, biochemical parameters, and histological study of *Labeo rohita* F. Hamilton, 1822 (fingerlings)

Shrutika S. Kurhekar and Y. D. Akhare

Department of Zoology, Vidya Bharati Mahavidhyalaya, C.K. Naidu Road, Camp, Amravati, Maharashtra, Pincode - 444602 (India)
Email: shrutikurhekar333@gmail.com

Abstract

The present study was conducted to investigate the effect of incorporating Tulsi (*Ocimum sanctum*) leaf extract into the diet of *Labeo rohita* fingerlings on growth performance, survival rate, biochemical responses, and histological changes. Fingerlings were fed experimental diets supplemented with graded levels of Tulsi extract like 200 mg, 400 mg and 600 mg for a specified duration of 60 days under laboratory conditions. Growth parameters such as weight gain, specific growth rate, and feed conversion ratio showed a significant improvement in treated groups compared to the control. The supplemented diets also enhanced survival percentage, indicating improved disease resistance and stress tolerance. Biochemical analysis revealed positive modulation in serum protein, carbohydrates, lipid and vitamins suggesting improved metabolic and physiological status. Furthermore, histological examination of gill, liver and intestine tissues exhibited healthier cellular architecture with reduced signs of degeneration in treated fish.

Overall, the findings demonstrate that dietary inclusion of *Ocimum sanctum* L. extract can enhance growth, improve immunity, maintain tissue integrity, and promote better survival in *Labeo rohita* fingerlings. Tulsi extract can thus be recommended as a natural, cost-effective, and eco-friendly feed additive in aquaculture to promote fish health and productivity.

Key words : *L. rohia, O. sanctum, Tulsi* extract, Growth performance, Biochemical parameters, Histological analysis, Survival rate.

Aquaculture has expanded rapidly over the last few decades to satisfy global demand for animal protein, but intensive culture systems also increase the risk of stress,

opportunistic infections, and deteriorating water quality. These pressures reduce growth efficiency and survival, and they have driven interest in sustainable, residue-free strategies to maintain fish health and productivity. Herbal and natural products are emerging as promising alternatives to antibiotics and synthetic growth promoters because many contain bioactive compounds that modulate immunity, oxidative status, and metabolism[6,12].

Among Indian carps, *Labeo rohita* (rohu) is a commercially important species because of its fast growth, consumer acceptance, and central role in polyculture systems; improving rohu growth and disease resistance therefore has direct implications for small-scale and commercial aquaculture alike. Conventional feed additives often fail to address subclinical stress and immune competence simultaneously, so dietary strategies that support growth performance and host defense are especially valuable for rohu culture.[7,11]

Ocimum sanctum (*Tulsi*, Holy Basil) is a well-characterized medicinal herb used in traditional Indian medicine; phytochemical analyses identify eugenol, ursolic acid, flavonoids, and other phenolics as principal bioactives with antioxidant, anti-inflammatory, antimicrobial, and immunomodulatory properties. These activities provide a mechanistic basis for hypothesizing beneficial effects when Tulsi preparations are included in fish diets—for example, by reducing oxidative damage, enhancing innate immune responses, and limiting pathogen colonization of the gut and gills[16,19].

Recent experimental studies and reviews have reported positive effects of basil/Tulsi and other herbal supplements on growth indices (weight gain, specific growth rate, feed conversion), hematological and biochemical markers (*e.g.*, hemoglobin, total protein, antioxidant enzymes, serum metabolites), and resistance to bacterial or viral challenge in cultured species. Histological examinations in these trials commonly serve to confirm that dietary inclusion does not cause tissue damage and, in some cases, reveal improved intestinal morphology or reduced pathology after pathogen exposure.[13,21]

Despite growing interest, species-specific data for *L. rohita* remain limited because effects depend on preparation (powder, aqueous extract, essential oil), dose, exposure duration, and fish age/size. Recent trials using Tulsi leaf extracts in rohu diets have reported improvements in growth and several biochemical indices, but standardized dose-response and histological evaluations remain scarce. This gap justifies a controlled study that measures growth performance, survival, detailed hemato-biochemical profiles, antioxidant status, and histopathology of key organs (liver, gill, intestine) to determine both efficacy and safety of Tulsi supplementation in rohu fingerlings[8,15].

Accordingly, the present study evaluates graded dietary levels of *Ocimum sanctum* extract in *Labeo rohita* fingerlings and examines (1) growth performance and feed utilization, (2) survival under routine culture conditions, (3) hematological and serum biochemical indicators of nutritional and immune status, (4) histological architecture of liver, gill, and intestine as markers of tissue integrity and physiological response, and (5) vitamin A. Results from this work will help

define practical, evidence-based recommendations for using Tulsi as a phytogenic feed additive in sustainable rohu aquaculture.

Experimental fish and holding conditions:

Healthy fingerlings of *Labeo rohita* (initial weight 7 - 8gm) were obtained from a disease-free hatchery named Matsotpadan Kendra, Morshi and transported to the laboratory in oxygenated containers. Fish were randomly distributed into 4 glass aquaria giving three dietary treatments and one without treatment with 15 fish per tank. Before the experiment, fish were acclimated for 14 days under laboratory conditions and fed a commercial basal diet. During acclimation and the experimental period, dissolved oxygen, temperature, pH, ammonia (NH_3 /NH_4^+), and nitrite were monitored twice weekly and maintained within suitable ranges for *L. rohita* (temperature ~26–28 °C, DO >5 mg/L, pH 7.0–8.0).

Plant material and preparation of Tulsi extract :

Fresh *Tulsi* (*Ocimum sanctum*) leaves were carefully harvested, thoroughly washed under clean running water to remove any dirt or impurities, and then ground into a fine paste using a mechanical grinder. This *Tulsi* paste was incorporated into the fish meal at varying concentrations to prepare experimental feeds. The dosages were adjusted as follows: The first tank received a diet supplemented with 200 mg of Tulsi extract, while the second and third tanks were provided with 400 mg and 600 mg, respectivelys. A separate control tank was maintained without any *Tulsi* extract to serve as a baseline for comparison. All ingredients were accurately weighed and mixed according to the formulated feed composition to ensure uniform distribution of nutrients and *Tulsi* extract throughout the feed. The prepared feed was then sun-dried, stored properly, and used for subsequent feeding trials.

Ingredients	Experimental Treatment			
	T1	**T2**	**T3**	**Control (C)**
Mustard oil cake powder	28 gm	28 gm	28 gm	28 gm
Groundnut oil cake powder	20 gm	20 gm	20 gm	20 gm
Wheat Bran	12 gm	12 gm	12 gm	12 gm
Wheat Flour	14.8 gm	14.6 gm	14.4 gm	15 gm
Rice Flour	22 gm	22 gm	22 gm	22 gm
Vitamin minerals premix	1 gm	1 gm	1 gm	1 gm
Salt	0.5 gm	0.5 gm	0.5 gm	0.5 gm
Starch (Binder)	0.5 gm	0.5 gm	0.5 gm	0.5 gm
Lime stone	1 gm	1 gm	1 gm	1 gm
***Ocimum sanctum* extract**	**0.2 gm** (for 200 mg concentration)	**0.4 gm** (for 400 mg concentration)	**0.6 gm** (for 600 mg concentration)	-

Experimental diets and feeding protocol :

A basal control diet was formulated to meet the nutrient requirements of *L. rohita* fingerlings using local feed ingredients (mustard oil cake, groundnut oil cake, wheat flour, and vitamin–mineral premix). Three experimental diets were prepared by supplementing the basal feed with Tulsi extract at 200 mg, 400 mg and 600 mg feed (designated T1, T2 and T3). The extract was thoroughly mixed into the powdered basal diet with a small amount of binder (*e.g.*, starch), pelleted through hand-formed, dried and stored in different labeled containers. The control diet received the same volume of ingredients and binder with normal diet.

Fish were fed twice daily (09:00 and 16:00) to a fixed feeding rate of 3% body weight per day, adjusted biweekly according to weight sampling. The feeding trial continued for 60 days.

Growth and survival measurements :

At the beginning of the experiment and at two-week intervals thereafter, the fish in each tank were collectively weighed and counted. Additionally, individual weight measurements were recorded by randomly selecting ten fish from each tank to track growth performance. At the conclusion of the trial, the following growth and performance parameters were computed:

- Weight gain (WG, gm) = final mean weight-initial mean weight.
- Length gain (LG, cm) = final mean length - initial mean weight
- Specific growth rate (SGR, %·day^{-1}) = [ln (final weight)-ln (initial weight)]×100/ days.
- Feed conversion ratio (FCR) = feed intake (dry weight) / weight gain.
- Survival (%) = (number of fish at end / number at start) × 100.

Biochemical sampling and assays :

At the end of the feeding trial, five fish per tank (n = 15 per treatment) were randomly sampled for biochemical analyses. Fish were anesthetized using chloroform prior to sampling. Blood samples (~1.0–1.5 mL) were collected from the caudal vein using non-heparinized syringes and allowed to clot in plain tubes. Serum was separated by centrifugation at 3,000 rpm for 10 minutes and stored at −20 °C until further analysis. Biochemical assays, including total protein, carbohydrates, lipids, and vitamins, were performed using commercial test kits and spectrophotometric methods following the manufacturer's protocols, with each measurement conducted in duplicate to ensure accuracy.

Vitamin A estimation :

Vitamin A was estimated by saponifying the weighed sample with ethanolic KOH and sodium ascorbate, followed by extraction of retinol into light petroleum. The combined petroleum layers were washed with water, filtered through anhydrous sodium sulfate, and evaporated to dryness under vacuum at temperatures below 40°C. The residue was then dissolved in 2-propanol, the evaporation flask was rinsed with additional 2-propanol, and the volume was made up to 20 mL. A 10-µL aliquot of this extract was injected into the HPLC system, and vitamin A concentration was calculated from peak areas using appropriate

calibration standards, with results expressed in IU or µg based on the conversion factors provided.

Tissue sampling and histological procedures:

After blood collection, fish were euthanized and tissues (liver, anterior intestine and gills) were excised, rinsed in physiological saline and fixed immediately in 10% neutral buffered formalin for 24–48 h. Fixed tissues were processed routinely for paraffin embedding, sectioned at 4–6 µm, mounted on glass slides and stained with hematoxylin and eosin (H&E). Slides were examined under a light microscope for morphological changes (*e.g.*, hepatocyte vacuolation, necrosis, gill lamellae alterations, intestinal villus integrity). Photomicrographs were taken for representative lesions and comparative assessment across treatments.

Water quality monitoring :

Water quality parameters (temperature, dissolved oxygen, pH, total ammonia nitrogen, nitrite, total hardness) were monitored twice weekly using standard portable meters and colorimetric test kits. Any major deviations from acceptable ranges were corrected by partial water exchange or aeration. Records of temperature and DO were maintained throughout the trial.

Statistical analysis :

Data are presented as mean ± standard error (SE). One-way analysis of variance (ANOVA) was used to compare means among treatments; when significant differences were detected ($p < 0.05$).

Ethical considerations :

All experimental procedures involving animals were conducted according to institutional animal care guidelines and were approved by the relevant ethics committee. Measures were taken to minimize stress and suffering, and humane endpoints were established.

Growth performance and survival :

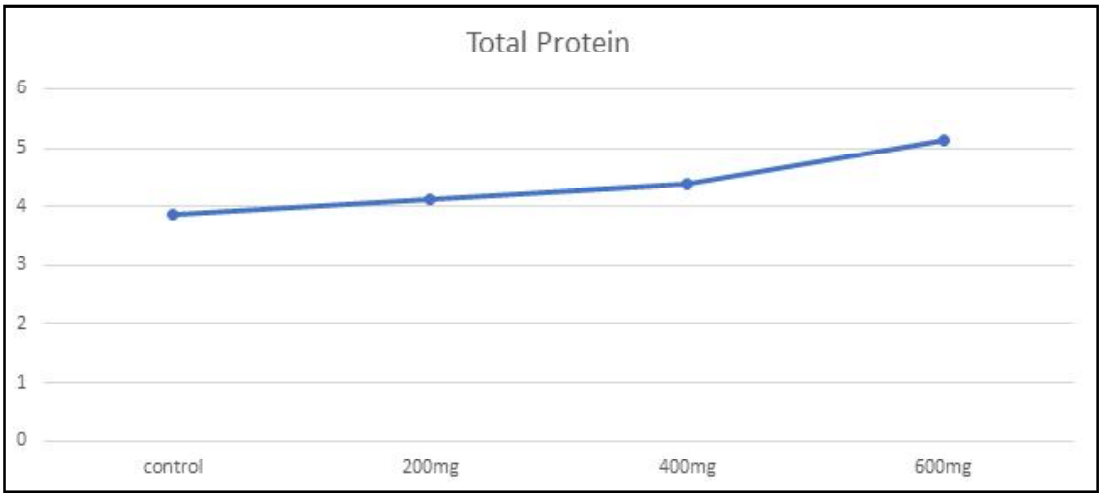
Fingerlings of *Labeo rohita* responded positively to the dietary inclusion of *Ocimum sanctum* extract throughout the 60-day trial. Fish fed Tulsi-supplemented diets exhibited progressively higher final body weights and specific growth rates when compared to the control group. Among all treatments, the 600 mg diet produced the maximum growth response, followed closely by the 400 mg inclusion level. Feed conversion ratio (FCR) improved significantly in the supplemented groups, indicating more efficient utilization of feed. Survival percentage remained above 90% in all treatments, with the highest survival recorded in the maximum-dose group (600mg). No abnormal mortality or external signs of stress were observed.

Biochemical parameters :

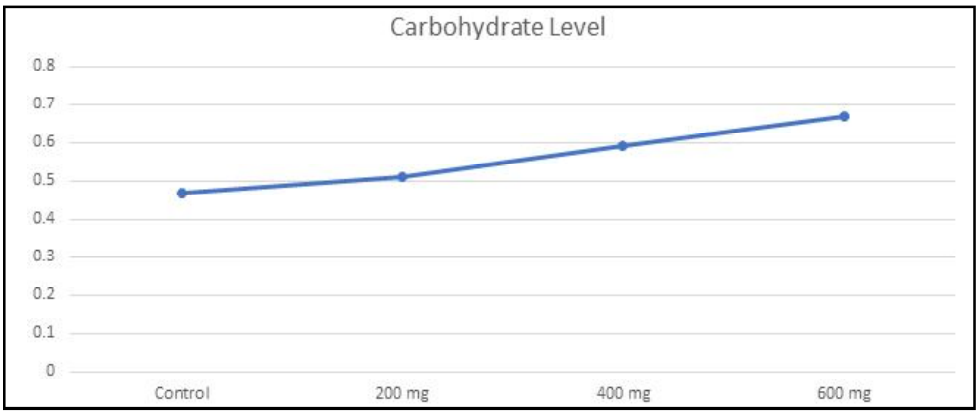
Serum biochemical markers reflected improved metabolic status in Tulsi-fed fish. Total protein, lipid, carbohydrate level was highest in fish fed the higher and medium dosages of the extract, indicating better health and improved liver function. Serum glucose and cholesterol showed a downward trend in the supplemented groups compared to the control, suggesting reduced metabolic stress.

Parameter	Control	T1 (200mg)	T2 (400mg)	T3 (600 mg)
Initial Total Body Length (cm)	6.83 ± 0.56	6.72 ± 0.36	6.95 ± 0.69	6.91 ± 0.22
Final Total Body Length (cm)	13.63 ± 0.54	13.97 ± 0.51	14.05 ± 0.51*	14.15 ± 0.58**
Initial Total Body Weight (gm)	6.63 ± 0.41	7.18 ± 0.46	7.22 ± 0.45	7.38 ± 0.53
Final Total Body Weight (gm)	19.37 ± 0.53	21.28 ± 0.47	24.18 ± 0.22**	24.88 ± 0.61**
SGR % in 60 days	1.72 ± 0.096	1.91 ± 0.12*	2.08 ± 0.11**	2.19 ± 0.13**
FCR % in 60 days	1.69 ± 0.093	1.91 ± 0.093*	1.71 ± 0.073**	1.54 ± 0.083**
Survival %	40%	53.43%*	73.23%*	86.87%**

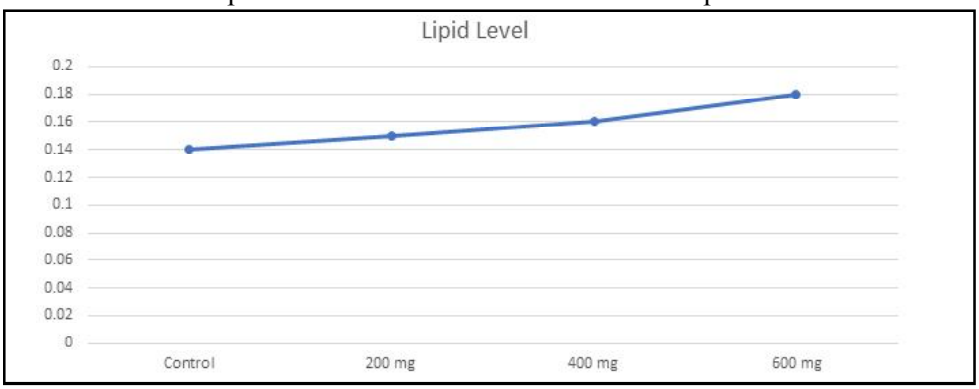
	Control	T1 (200 mg)	T2 (400 mg)	T3 (600 mg)
Total Protien(gm/dl)	3.86 ± 0.3	4.11 ± 0.32	4.38 ± 0.54	5.12 ± 0.6
Lipid(gm/dl)	0.14 ± 0.048	0.15 ± 0.061	0.16 ± 0.062	0.18 ± 0.086
Carbohydrate(gm/dl)	0.47 ± 0.05	0.51 ± 0.06	0.59 ± 0.08	0.67 ± 0.12
Vitamin A (mg/100gm)	28	29	31	34



Graph 1. Total Protien value (gm/dl) of L. rohita fed Tulsi supplemented diets were compared to control fish at the end of the experiment.

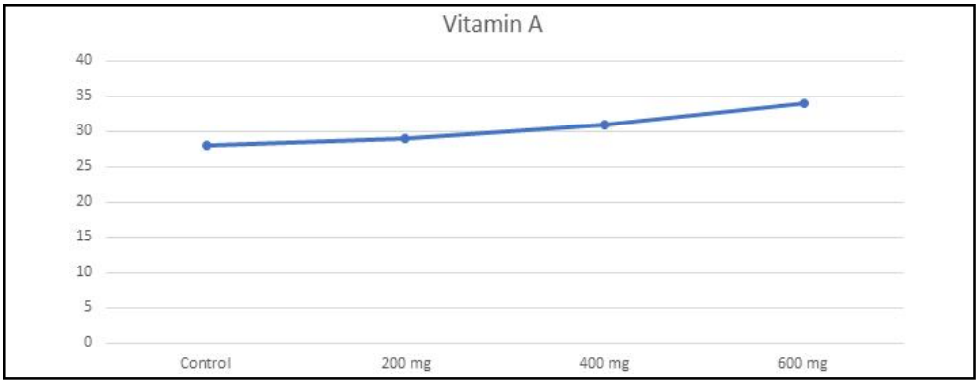


Graph 2. Carbohydrate level (gm/dl) of L. rohita fed Tulsi supplemented diets were compared to control fish at the end of the experiment.



Graph 3. Lipid level (gm/dl) of L. rohita fed Tulsi supplemented diets were compared to control fish at the end of the experiment.

Vitamin A Estimation :



Graph 4: Vitamin A (mg/100gm) of L. rohita fed Tulsi supplemented diets were compared to control fish at the end of the experiment.

Vitamin A concentration in the muscle tissue of *Labeo rohita* showed a gradual increase with increasing levels of Tulsi extract in the diet. The control group recorded a mean retinol value of 28 mg/100gm, while fish in 200 mg tank T1 exhibited a slight increase to 29 µg/g. A more notable enhancement was observed in the higher supplementation groups, where the 400 mg tank T2 resulted in 31 mg/ 100gm of vitamin A, and the 600 mg tank T3 group showed the highest concentration at 34 mg/100gm. This dose-dependent improvement indicates that dietary inclusion of Tulsi extract enhances vitamin A deposition in muscle tissue, reflecting improved metabolic and antioxidant status in treated fish.

Histological observations :

Histological examination of liver, gills, and intestinal tissues showed distinct improvements in structural integrity in fish fed Tulsi-based diets. Liver sections from the control group showed mild

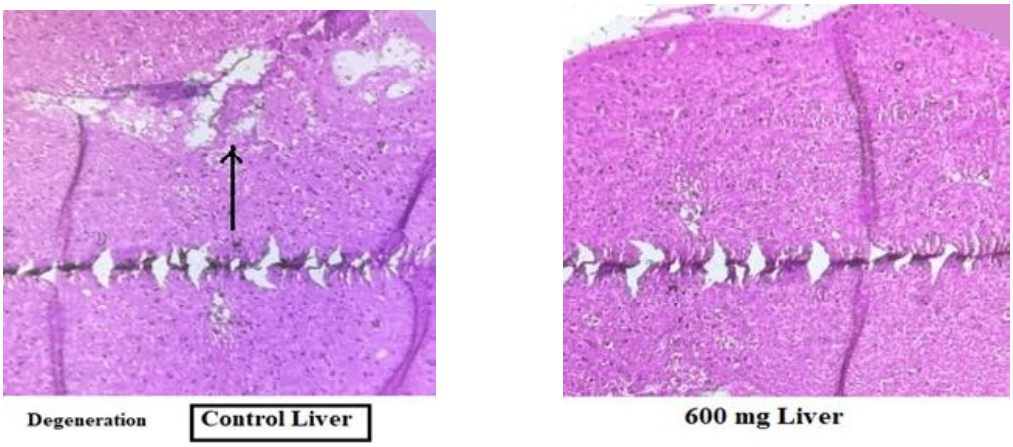


Figure 1. Histological comparison of liver sections from the control group and fish administered 600 mg of herbal extract for 60 days.

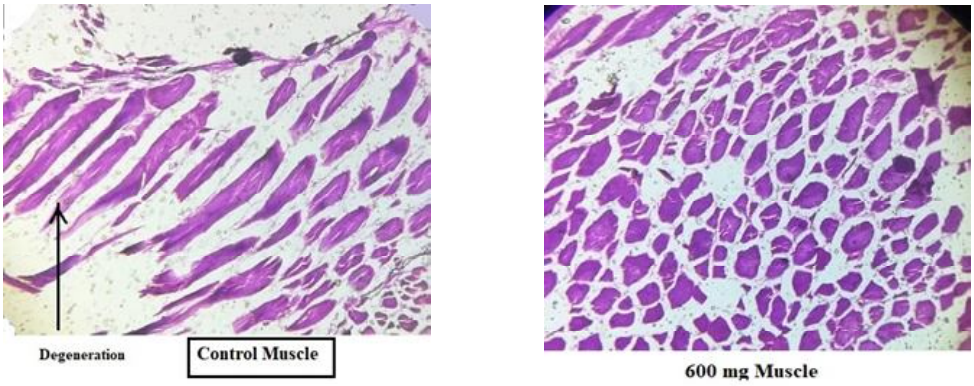


Figure 2. Histological comparison of intestine sections from the control group and fish administered 600 mg of herbal extract for 60 days.

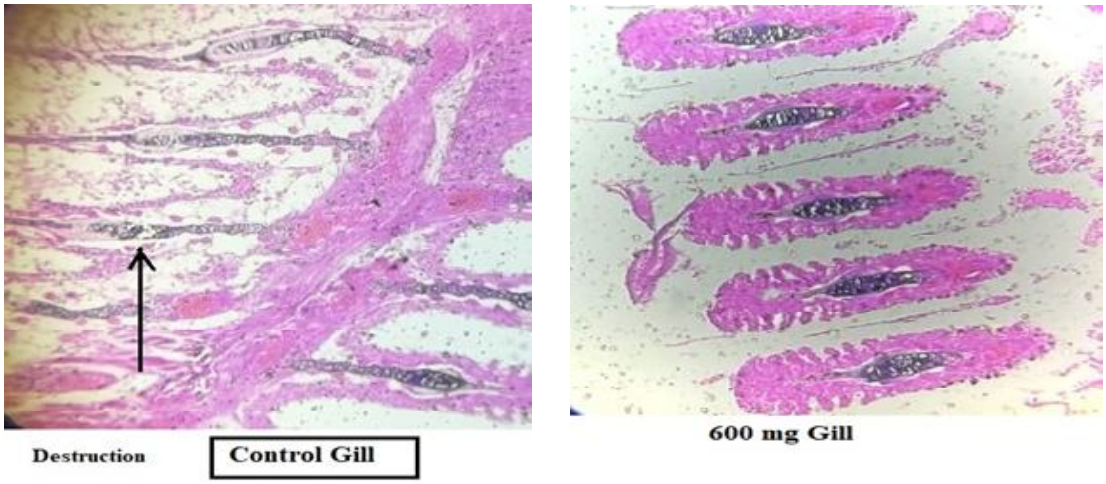


Figure 3. Histological comparison of gill sections from the control group and fish administered 600 mg of herbal extract for 60 days.

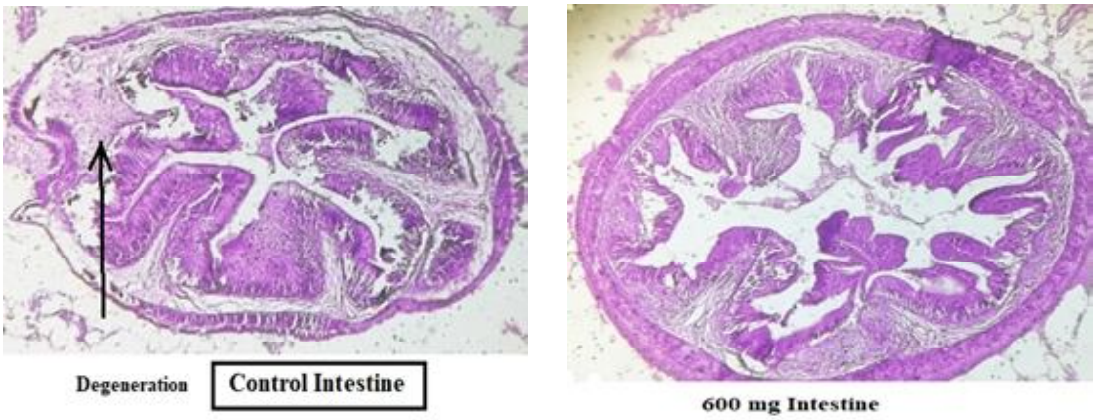


Figure 4. Histological comparison of Intestine sections from the control group and fish administered 600 mg of herbal extract for 60 days.

vacuolation and occasional hepatocyte degeneration, whereas the treated groups displayed normal cellular arrangement with fewer signs of inflammation. Intestinal tissue from supplemented groups exhibited well-defined villi, increased mucosal folds, and enhanced goblet cell presence, suggesting improved nutrient absorption. Gill lamellae appeared intact with reduced epithelial lifting in Tulsi-fed fish compared to the control.

Overall findings :

Overall, the dietary incorporation of *Ocimum sanctum* extract demonstrated beneficial effects on growth performance, feed

utilization, survival, and physiological status of *L. rohita* fingerlings. The 600 mg inclusion level consistently produced the strongest positive responses, while the 200mg and the control tank fishes showed no adverse effects. These results suggest that Tulsi extract can serve as a natural, effective feed additive for improving health and productivity in freshwater aquaculture.

The findings of the present study clearly demonstrate that dietary incorporation of *Ocimum sanctum* extract exerts a substantial positive influence on the growth, feed utilization, and survival of *Labeo rohita* fingerlings. The dose-dependent improvements, particularly at 400 mg and 600 mg, indicate that Tulsi's phytochemicals may enhance digestive efficiency and metabolic regulation, ultimately supporting superior weight gain and specific growth rate. These outcomes align with earlier reports on carp species where Tulsi extract promoted growth performance through its antioxidant-rich bioactive compounds, which minimize oxidative stress and enhance nutrient assimilation. Habib *et al*.[10] documented significant improvements in weight gain and immune biomarkers in *Cyprinus carpio* fed Tulsi extract, suggesting that the herb operates through conserved physiological pathways across cultured species. Similar growth-enhancing effects of Tulsi were also noted in *L. rohita* by Chhaba *et al*.,[5], who attributed the improvements to better feed conversion and enhanced metabolic activities. Bhatnagar and Mann[2] further reported that Tulsi supplementation improved digestive enzyme activity in *Cirrhinus mrigala*, supporting the present observation that Tulsi promotes efficient nutrient assimilation. Such consistency reinforces Tulsi's potential as a reliable phytogenic feed additive suitable for freshwater aquaculture systems.

Biochemical responses in Tulsi-fed fish provide deeper insight into the physiological mechanisms contributing to improved performance. Higher serum protein, lipids, and carbohydrate levels, coupled with reduced liver enzyme activities (ALT and AST), indicate reduced hepatic stress and enhanced metabolic homeostasis. This pattern reflects improved liver function, possibly due to the hepatoprotective nature of Tulsi's compounds such as eugenol and ursolic acid, which are known for stabilizing cellular membranes and suppressing inflammatory processes. Comparable studies on Nile tilapia demonstrated that Tulsi supplementation minimized hepatic damage and improved antioxidant status under toxic stress, findings consistent with those reported by Saad *et al*.[17] in cadmium-exposed *Oreochromis niloticus*. In support of the present results, Biswas *et al*.[4] found that *Tulsi* administration effectively reduced cortisol levels and improved biochemical stability in tilapia subjected to stress-inducing conditions. The elevated vitamin A deposition observed in muscle tissue also supports the hypothesis that Tulsi enhances antioxidant protection, allowing better retention and stability of fat-soluble vitamins under normal metabolic processes.

The histopathological improvements recorded in liver, gill, and intestinal tissues further confirm Tulsi's protective impact. Control groups exhibited mild vacuolation, disrupted gill lamellae, and less-developed intestinal villi—typical indicators of stress or compromised nutrition—whereas Tulsi-fed fish showed compact hepatocytes, clearer lamellar

structure, and more pronounced intestinal villi with abundant goblet cells. Such structural enha-ncements suggest improved digestive efficiency and stronger barrier functions, which may contribute to the better growth and health outcomes observed. These findings closely resemble those of earlier investigations where Tulsi and basil extracts reduced tissue degeneration and improved gut morphology in stressed fish species, similar to the enhanced intestinal histo-architecture described by Bhatnagar and Mann[2]. Furthermore, the synergic effects of Tulsi and probiotics on mucosal health in *Cirrhinus mrigala*, demonstrated by Singh *et al*.[19], parallel the present observation that Tulsi strengthens tissue integrity and mitigates cellular degeneration. The maintenance of healthier microstructures across tissues underscores the herb's multi-faceted role as an immunomodulator, antioxidant, and anti-inflammatory agent.

Considering the combined improvements in growth response, serum biochemistry, vitamin A deposition, and histological architecture, Tulsi emerges as a valuable natural feed additive for enhancing the overall robustness of *L. rohita*. Its cost-effectiveness, ease of incorporation into diets, and eco-friendly nature make it an excellent candidate for sustainable aquaculture practices. The results from this controlled experiment establish a scientific basis for recommending Tulsi extract at 400–600 mg/kg for optimal performance. These findings are in agreement with the recommendations of Chhaba *et al*.[5], who emphasized the practicality of herbal supplements for carp culture. However, future studies should focus on larger-scale pond trials, assessments under pathogen-challenge conditions, and detailed molecular analyses of immune and antioxidant pathways to precisely define long-term impacts and refine dosage strategies. Such efforts will help integrate Tulsi into mainstream aquaculture nutrition, supporting healthier fish stocks and reducing dependence on synthetic growth promoters.

The present study concludes that dietary supplementation of *Ocimum sanctum* extract significantly enhances growth, feed efficiency, survival, metabolic status, and tissue health of *Labeo rohita* fingerlings. Higher inclusion levels, especially 400 mg and 600 mg, produced the most favorable improvements, indicating a strong dose-dependent response. Enhanced biochemical profiles and healthier liver, gill, and intestinal structures further demonstrate Tulsi's antioxidant, hepatoprotective, and immunomodulatory roles. Given its natural origin, affordability, and safety, Tulsi extract can be recommended as an effective phytogenic feed additive for improving fish health and supporting sustainable aquaculture practices. Future research should expand these findings under field conditions and explore molecular mechanisms to strengthen its application in commercial aquafeeds.

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