Original Article
DOI: https://doi.org/10.70749/ijbr.v3i8.2097

INDUS JOURNAL OF BIOSCIENCE RESEARCH
https://ijbr.com.pk
ISSN: 2960-2793/ 2960-2807

Effects of *Linum usitatissimum* and *Trigonella foenum-graecum* on Growth, Digestion, Histology, Haematology and Immunity in Labeo rohita Fingerlings

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ARTICLE INFO

Keywords: Flaxseed, Fenugreek seed, *Labeo rohita,* Histology, Immunity responses, Growth, Digestive performance.

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Declaration
Authors' Contribution: All authors equally contributed to the study and approved the final manuscript.

Conflict of Interest: No conflict of interest.

Funding: No funding received by the authors.

Article History
Received: 03-06-2025 Revised: 28-07-2025
Accepted: 03-08-2025 Published: 20-08-2025

ABSTRACT

Aquaculture contributes nearly half of global fish production by producing food at a faster rate. Flaxseed, rich in omega-3 fatty acids and fenugreek known for bioactive compounds like saponins and flavonoids, play significant roles in improving fish health. This study investigated the effects of dietary soluble flaxseed (*Linum usitatissimum*) and fenugreek (*Trigonella foenum-graecum*) seed meals on growth, digestive performance, histology, and immunity responses in *Labeo rohita*. Sixty fingerlings were divided into four groups (15 each) and fed different diets for 30 days: control (G0), G-1 (3g flaxseed meal), G-2 (3g fenugreek meal) and G-3 (6g flaxseed-fenugreek mixture). Results showed that G-2 had significantly higher final body weight (8.35±2.07 g) and specific growth rate (11.87±7.66), while FCR was highest in G-3 (8.815±1.874). Digestive enzyme activity, particularly amylase was highest in G-2. Histological analysis revealed normal liver structure and elongated intestinal villi. Immune responses varied with WBCs highest in G-1, G-1 recorded the highest lymphocyte percentages (~82–88%), G-2 recorded the lowest values (~0.9–1.1%). These findings suggest that flaxseed and fenugreek seed meals can serve as effective, natural alternatives to synthetic additives, promoting health and growth in aquaculture species. Further research on different doses and combinations could optimize their application for large-scale production. This approach supports sustainable aquaculture and reduces dependence on chemical growth promoters.

INTRODUCTION

Aquaculture production in the world has been increased greatly over the past 40 years making it a good contribution to the global fish supply for human consumption (Akhtar and Abdullah, 2021). About half of the fish used for food in the world comes from aquaculture (Subasinghe *et al.*, 2009). Aquaculture developed mainly as a freshwater food production method in Asia but now expanded worldwide and giving fish producers extra money through this business (Šimat *et al.*, 2020). Commercial fish farming was initiated in Pakistan in 19th century and proliferated over the years (Elzaki, 2024).

Fish is a nutritious food, rich in minerals, high-quality protein and healthy omega-3 fatty acids. In warm regions fish is cheaper and easier to find than other protein sources (Mohanty *et al.*, 2019). About 40% of the animal protein consumed in underdeveloped nation comes from fish that an extremely palatable diet (Badran and Hamed, 2024). Essential vitamins and minerals including vitamin B12, vitamin D, iron and phosphorus are present in fish flesh and support overall health or development (Marković and Poleksić, 2024). Consuming fish on a daily basis can also help to avoid multiple diseases (Indriani *et al.*, 2022).

Labeo rohita commonly called as rohu, is considered as highly tasty fish specie and one of the principle carps in India among the cyprinids. Rohu is widely used for its consumption qualities and nutritional benefits (Gjerde *et al.*, 2019). Rohu is a native of South Asian such as Bangladesh, India, Nepal and Pakistan. It is a well-liked food item in these regions (Sood *et al.*, 2019). It is rich in Omega-3 fatty acids which promote heart and brain health as well as essential vitamins such as A, B and C (Akhtar and Abdullah, 2021). Its good taste and texture make it a preferred choice for consumption (Masood *et al.*, 2015).

Plant sources are considered as viable alternative for use

in fish feed without compromising its nutritional quality. Different amino acids, fatty acids and a vide variety of proteins that are absent from animal proteins can be found in plant products like their seeds and spices (Chakraborty *et al.*, 2014). These are primary objectives for supplementation to promote better health and growth performance in fish while reducing stress and boosting immunity by their derivatives (Citarasu, 2010). These are primary objectives for supplementation to promote better health and growth performance in fish w (Zaki *et al.*, 2012).
Flaxseed commonly called linseed known as *Linum usitatissium* which belongs to family *Linaceae* and genus *Linum*. Flaxseed is one of the oldest crops cultivated for both food and fiber since old times over 300 varieties known (Kaur *et al.*, 2018). Flaxseed meal use in animal diet is limited because of some anti-nutritional factors including phytic acid, cyanogenic glycosides and anti-vitamin B6 factor (Kakabouki *et al.*, 2021). Some flaxseed varieties are cultivated primarily for their fiber content and are used in various industrial applications (Xu *et al.*, 2022).
Fenugreek known as *Trigonella foenum graecum* also called methi, is a member of the *Leguminoseae* family. Fenugreek is a local plant specie in Northern Africa, Western Asia and Northern India (Sharma, 2021). This leguminous plant contains carbs, protein, fixed oils like lipids, vitamins A, B1and C (Roohi *et al.*, 2017). Their seeds are rich source of protein and also provides a significant amount of diosgenin, which is a beneficial compound with various health properties (Awad *et al.*, 2015). Fenugreek seed particularly at the high dosage used as a beneficial dietary supplement to increase immunity and growth production (Yılmaz *et al.*, 2012).
Growth and digestion depend on a balance between the quality of feed. How much is consumed, how well it is used by the body and the organism's internal functions (Fuentes *et al.*, 2012). Growth is controlled by multiple genes and is affected by conditions such as temperature, photoperiod, salinity and pollution (Salem *et al.*, 2023) . The beneficial effects of probiotics on animal health are becoming more commonly observed. Probiotics help improve feed intake, support digestion and promote the growth of healthy intestinal microbiota (Gong *et al.*, 2018). In addition to digestive benefits, probiotics are also believed to naturally increase the animal's antioxidant capacity (Harikrishnan *et al.*, 2011).
The immune system is a complex network of biological defenses that protects living organisms from infections. It plays an important role in maintaining overall health by identifying and eliminating harmful pathogens (Kar *et al.*, 2015). Immunostimulant effects vary among fish species. These mostly depend on many aspects such as dose, duration, delivery method (Reverter *et al.*, 2014). Immune system function begins with improved digestive enzymes which is followed by an increase in fish growth and survival rates (Priyadarsani *et al.*, 2021).
Histology is the microscopic study of the structure and features of biological cells and tissues. It involves examining thin slices of tissue using light, fluorescence or electron microscopes (Mokhtar, 2021). This helps in understanding the detailed organization and function of cells and tissues. It highlights how the structural arrangement of cells within tissues is closely related to the functions performed by both healthy and diseased tissues (Musumeci, 2014). It is a sensitive diagnostic method used to detect both direct and indirect harmful effects on animal tissues (Wolf *et al.*, 2015).
This study aimed to investigate the effect of flaxseed and fenugreek seed meal on *Labeo rohita*. By examining its effect on growth, digestive performance, histology and immune responses. This study provided valuable insights for improving fish health.

MATERIALS AND METHODS

Fishes play a vital role at both social and economic level in many countries. A vital component of the human diet is fish due to its rich content of unsaturated fatty acids. Spices, herbs and their constituents including oil, leave extract and substances that have been used as a functional meal for the benefits of fish. These compounds are beneficial for growth promoter, digestive performance, immunity responses and antioxidants. This research was performed to investigate the effect of flaxseed and fenugreek seed meal on growth, digestive performance, histology and immune responces in *Labeo rohita*.
Experimental Site: The trial was conducted in the General Laboratory, Department of Zoology wildlife and fisheries, PARS campus, University of Agriculture, Faisalabad.
Experimental Animal: Sixty fingerlings of *Labeo rohita* (rohu) each weight between 6-7g and size between 2-2.5 inches were purchased from Government fish hatchery, Faisalabad. All of the fishes were carried in plastic bags with sufficient oxygen stocked. Rohu is the freshwater fish species and cultivated in the number of countries where they are easily available.
Chemical Used: Flaxseed and fenugreek seed meal were used.
Experimental Design: Four treatment groups were established.

Table 1
Different Treatment Groups

Experiment	Specified as	Experimental diet	Duration
Treatment 0	G_0	Basal feed	30 days
Treatment 1	G_1	Basal feed+ 3g of flaxseed meal	30 days
Treatment 2	G_2	Basal feed+ 3g of fenugreek meal	30 days
Treatment 3	G_3	Basal feed+ 6g mixture of flaxseed and fenugreek seed meal	30 days

After 30 days of trial, fishes were dissected and samples were collected for analysis of different parameters.

Determination of Growth Performance and Feed Utilization

Growth performance was estimated through weekly gross weight of fingerlings from each experimental group. Feed utilization and growth performance were analysed absolute weight gain, weight gain, specific growth rate, survival rate and feed conversion ratio.

Weight Gain (%)

Weight gain of fingerlings was determined by following formula:

$$Weight\ Gain\ (\%) = Final\ Weight - Initial\ Weight\ \times 100/Initial\ Weight$$

Absolute Weight Gain (AWG)

Following formula were applied for the estimation of absolute weight gain in grams.

$$Absolute\ weight\ gain\ (g) = Final\ weight\ (g) - Initial\ weight\ (g)$$

Specific Growth Rate (SGR)

Following formula were used to analyse specific growth rate:

$$SGR\ (\%) = (Final\ Weight\ (g) - Initial\ Weight\ (g) \times 100) / (Experimental\ Duration\ (days))$$

Survival Rate (%)

$$Survival\ Rate\ (\%) = (Final\ number\ of\ fingerlings \times 100) / (Initial\ number\ of\ fingerlings)$$

Feed Conversion Ratio (FCR)

$$FCR\ (\%) = Total\ dry\ feed\ intake\ (g) \times 100 / Weight\ gain\ (g)$$

Histological Analysis

Histology analysis involves preparing, staining and examining tissue sections under a microscope to assess cellular structure, integrity and any pathological changes. Key focus areas include overall tissue architecture, cell morphology and signs of damage or inflammation.

Immunity Analysis

Immunity responses involve oxidative stress and antioxidant enzyme activities. These enzymes were measured by blood chemistry, Superoxide Dismutase (SOD) and Catalase (CAT). It also determined by Jeon *et al.* (2022) through leukocytes, WBCs count, Lymphocytes, monocytes and granulocytes.

Determination of Digestive Enzyme Activity

Digestive activity analysis involves measuring enzyme activities (like amylase, protease, lipase) in digestive tissues or extracts to assess nutrient breakdown efficiency. It helps evaluate digestive health and the effects of diets or treatments on digestion.

Statistical Analysis

For statistical analysis all derived data were stated as mean ± standard deviation. The value of treatment groups was compared with control group. For data analysis One-way ANOVA were used, followed by the comparison of means following least-square design (LSD). If significant modifications were indicated, post-hoc tests (Tukey's HSD, Bonferroni) were applied to identify differentiation between the control and experimental groups. The significance level was set at 0.05 for all datasets (Okoye and Hosseini, 2024).

RESULTS

The experiment was conducted to access the effect of dietary soluble flaxseed and fenugreek seed meal on growth, digestive performance, histology and immune responses in *Labeo rohita*.

Determination of Growth Parameters

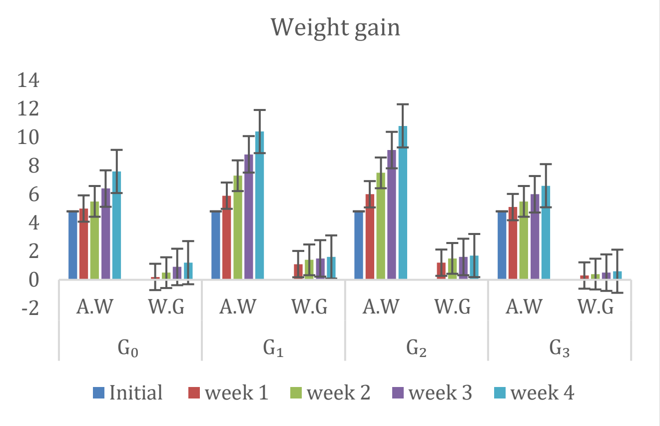
Considering the mean of weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR), SR and FCR of rohu fish, there was a significant difference among all the treatments at the end of 30 days of experiment.

Weight Gain

Graph 1 show a consistent increase in average weight (A.W) and weight gain (W.G) across all treatments over four weeks. T_2 recorded the highest A.W and W.G by week 4, followed by G_1, while G_3 showed the lowest growth performance. G_0 and G_3 exhibited comparatively smaller weight gains throughout the trial. Overall, G_2 was the most effective treatment in promoting growth, with clear weekly improvements.

Graph 1

Weekly changes in average weight (A.W) and weight gain (W.G) under different treatments (T_0–T_3)

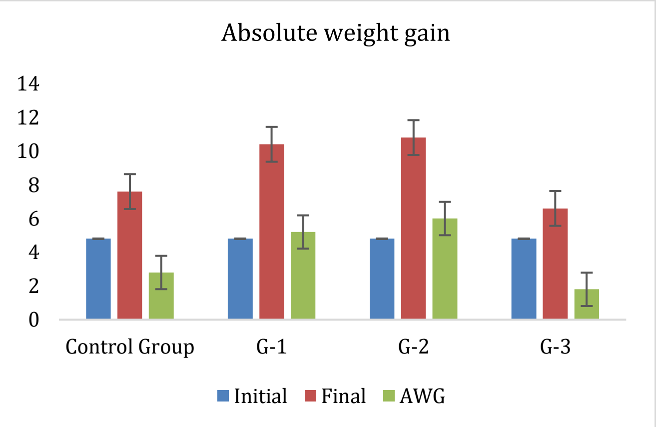


Absolute Weight Gain

Graph 2 shows changes in initial, final, and absolute weight gain (AWG) among the control and treatment groups. G-2 recorded the highest AWG, followed closely by G-1, while G-3 exhibited the lowest gain among treatments. The control group showed moderate improvement compared to treatments. Overall, G-2 was most effective in enhancing weight gain over the study period.

Graph 2

Initial, Final, and Absolute Weight Gain (AWG) in Control and Treatment Groups (G-1 to G-3)

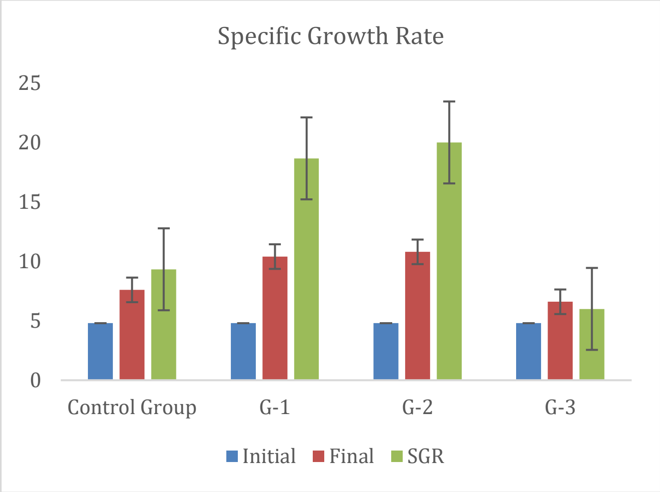


Specific Growth Rate

Graph 3 present initial, final, and specific growth rate (SGR) values for the control and treatment groups. G-2 showed the highest SGR, followed closely by G-1, while G-3 recorded the lowest growth performance. The control group displayed moderate growth compared to treatments. Overall, G-2 proved most effective in enhancing specific growth rate during the study period.

Graph 3

Initial, Final, and Specific Growth Rate (SGR) in Control and Treatment Groups (G-1 to G-3)

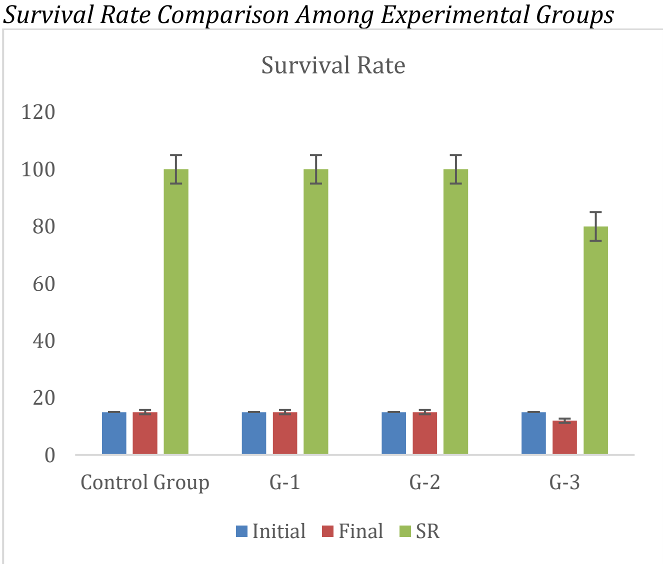


Survival Rate

The bar chart illustrates the initial count, final count, and survival rate (SR) of subjects in the control group and three experimental groups (G-1, G-2, G-3). The initial and final counts remain close in the control, G-1, and G-2 groups, resulting in high survival rates near 100%. In contrast, G-3 shows a noticeably lower survival rate, around 80%, despite similar initial counts to other groups. Error bars indicate variability in measurements, with slight differences across groups.

Graph 4

Survival Rate Comparison Among Experimental Groups

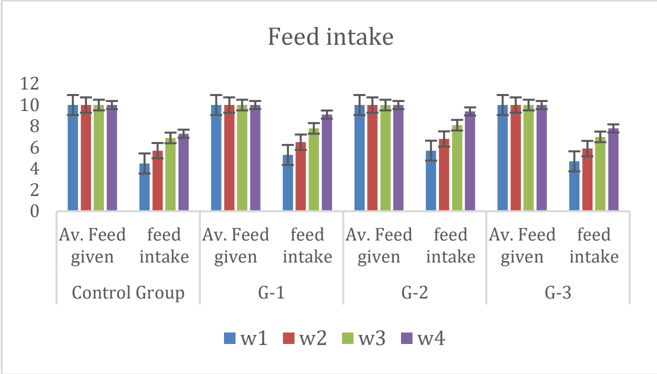


Feed Intake

The bar chart compares the average feed given and actual feed intake over four weeks (w1–w4) for the control and experimental groups (G-1, G-2, G-3). In all groups, average feed given remains consistent, while feed intake gradually increases from week 1 to week 4. G-1 and G-2 show similar intake patterns, with steady rises across weeks, whereas G-3 records slightly lower intake levels overall. Error bars indicate variation in feed measurements across weeks and groups.

Graph 5

Weekly Feed Intake and Average Feed Given Across Groups

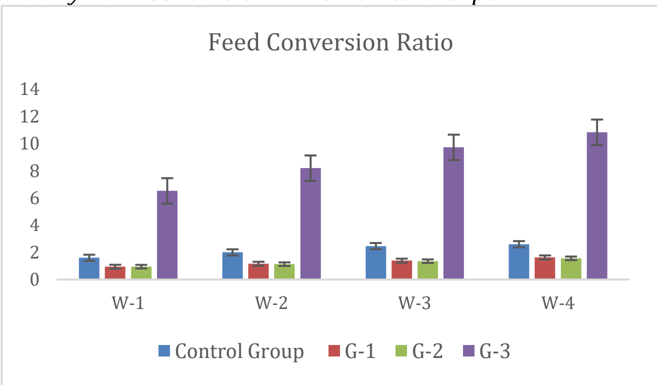


Feed Conversion Ratio

The bar chart presents the feed conversion ratio (FCR) over four weeks (W-1 to W-4) for the control and experimental groups (G-1, G-2, G-3). Control, G-1, and G-2 maintain relatively low and stable FCR values throughout the study period. In contrast, G-3 exhibits significantly higher FCR values, increasing steadily from week 1 to week 4. Error bars indicate variation in FCR measurements across groups and weeks.

Graph 6

Weekly Feed Conversion Ratio Across Groups

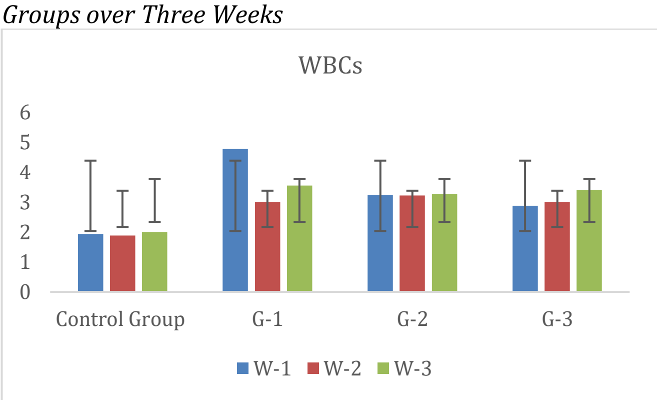


Determination of Haematology WBC

The control group maintained low WBC levels (~2.0 $\times 10^3/\mu L$) with no notable change over time. G-1 showed the highest WBC count in W-1 (~4.8 $\times 10^3/\mu L$) followed by a decrease in W-2 and slight recovery in W-3. G-2 remained stable (~3.2 $\times 10^3/\mu L$) across all weeks. G-3 showed a gradual increase from ~2.9 $\times 10^3/\mu L$ in W-1 to ~3.2 $\times 10^3/\mu L$ in W-3.

Graph 7

White Blood Cell (WBC) Count in Control and Experimental Groups over Three Weeks

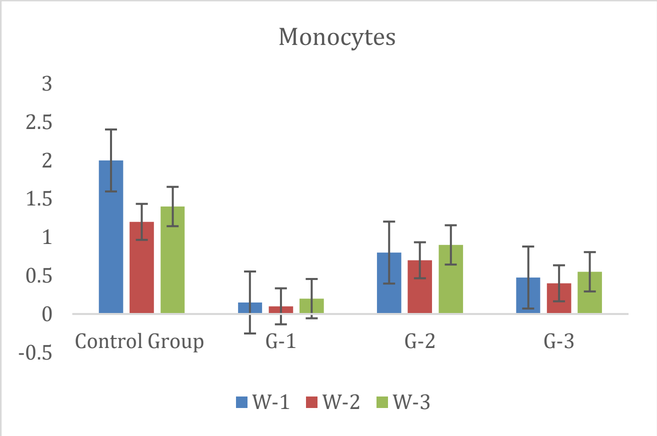


Monocyte

The control group showed the highest monocyte levels in W-1 (~2.0%) with a decline in W-2 and partial recovery in W-3 (~1.4%). G-1 maintained very low monocyte levels (~0–0.2%) across all weeks. G-2 showed moderate levels (~0.8–0.9%) with minimal fluctuation. G-3 recorded slightly lower values (~0.4–0.6%) and remained stable over time.

Graph 8

Monocyte Levels in Control and Experimental Groups over Three Weeks

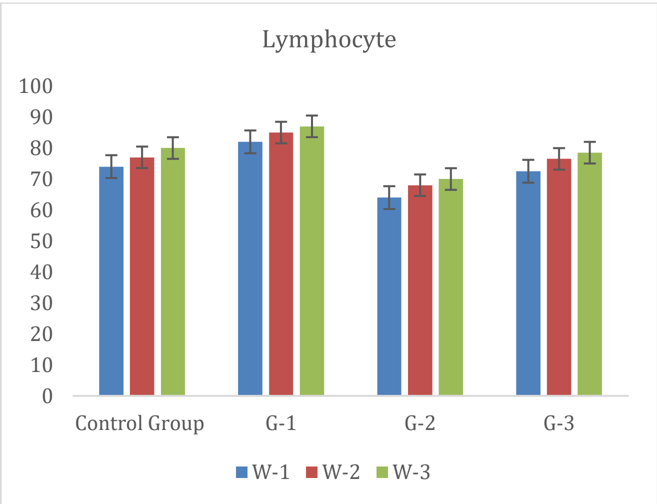


Lymphocyte

The control group showed a gradual increase in lymphocyte levels from ~74% in W-1 to ~78% in W-3. G-1 recorded the highest lymphocyte percentages (~82–88%) with a steady upward trend. G-2 had the lowest values (~64–70%) but showed consistent weekly increases. G-3 maintained intermediate levels (~73–79%) with a gradual rise over time.

Graph 9

Lymphocyte Levels in Control and Experimental Groups over Three Weeks

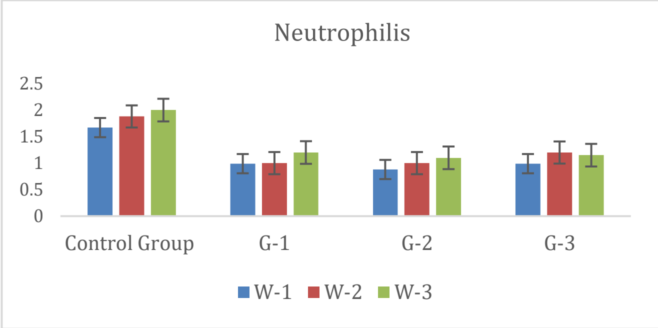


Neutrophil

The control group showed an increase in neutrophil levels from ~1.7% in W-1 to ~2.0% in W-3. G-1 maintained lower levels (~1.0–1.2%) with minimal variation over time. G-2 recorded the lowest values (~0.9–1.1%) but showed a slight upward trend. G-3 displayed moderate levels (~1.0–1.2%) with a peak in W-2 before a slight decline in W-3.

Graph 10

Neutrophil Levels in Control and Experimental Groups over Three Weeks



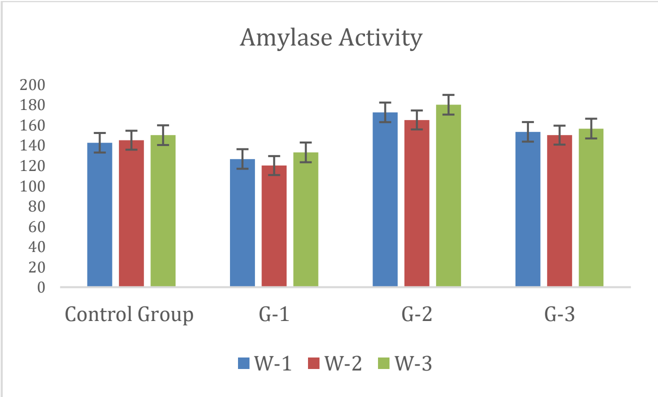
Analysis of Digestive Enzyme performance

Amylase Activity

The control group showed consistent amylase activity across W-1 to W-3 (~140–150 U/mL). G-1 had the lowest activity among all groups, particularly in W-2 (~120 U/mL). G-2 displayed the highest activity, peaking in W-3 (~180 U/mL). G-3 maintained intermediate activity levels (~150–155 U/mL) with minimal variation over time.

Graph 11

Amylase Activity in Control and Experimental Groups over Three Weeks

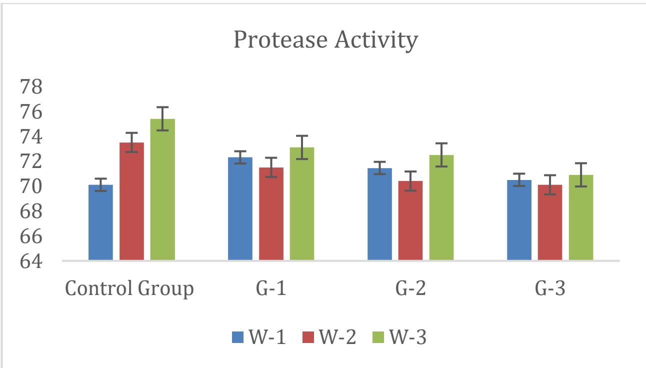


Protease Activity

The control group showed a steady increase in protease activity from W-1 (~70 U/mL) to W-3 (~75 U/mL). G-1 maintained moderate activity, with a slight dip in W-2 before rising in W-3 (~74 U/mL). G-2 followed a similar trend, dipping in W-2 and recovering in W-3 (~73 U/mL). G-3 had the lowest activity overall (~70–71 U/mL) with minimal changes over time.

Graph 12

Protease Activity in Control and Experimental Groups over Three Weeks



Histopathological Analysis

Histopathology is the microscopic examination of organs. Liver and intestine are used to analyses histo-patterns of *Labeo rohita*.

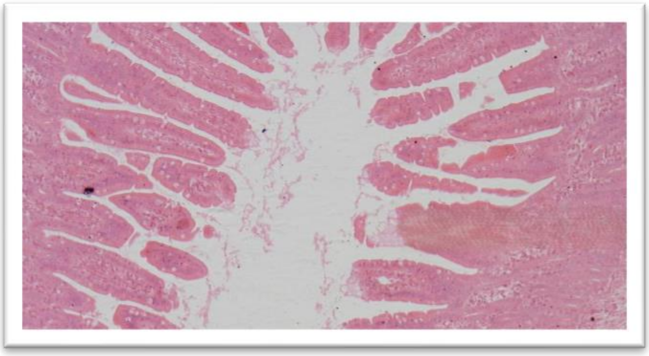
Histology of Intestine Tissues

Histological analysis of the intestinal tissue of *Labeo rohita* fed with diets supplemented with flaxseed and fenugreek seed meal showed significant improvements in intestinal architecture and mucosal integrity. The intestinal villi appeared elongated, slender, and well-organized, with an increased villus height and surface area indicating enhanced absorptive capacity. The epithelial lining was intact displaying a healthy arrangement of columnar epithelial cells with clearly visible brush borders (microvilli) suggesting effective nutrient uptake.

Goblet cells were moderately distributed along the mucosal surface, reflecting a balanced mucin production for mucosal protection and lubrication. The overall improvement in intestinal histoarchitecture indicates a beneficial effect of flaxseed's omega-3 fatty acids and fenugreek's bioactive components (saponins, flavonoids, alkaloids) on gut health, immune modulation and digestive efficiency.

Figure 1

Representation of Normal intestinal villi in rohu from Control Group



The intestinal villi of fish in the G-1 group (fed with 3g of flaxseed) showed normal morphology with moderate elongation and compact arrangement. The epithelial lining remained intact, consisting of simple columnar cells interspersed with goblet cells, which appeared slightly increased compared to the control. This suggests improved mucus secretion and protection of the intestinal lining.

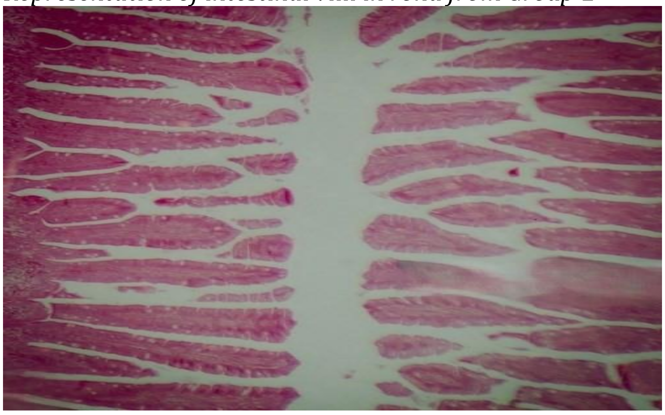
Figure 2

Representation of intestinal villi in rohu from Group-1

The intestinal villi exhibited well-developed, elongated and densely packed structures in G-2. The epithelium was intact and thick with a notable increase in goblet cells, indicating enhanced mucus production and mucosal defense. The lamina propria appeared healthy with no evidence of infiltration or degeneration. The increased villi height and surface area suggest improved nutrient absorption, highlighting the positive effects of fenugreek seed meal on intestinal histomorphology and digestive efficiency.

Figure 3

Representation of intestinal villi in rohu from Group-2



The intestinal tissue in the G-3 group showed shortened, irregularly arranged villi compared to G-1 and G-2. Some regions displayed slight epithelial disruption and mild blunting of the villi tips. The goblet cells were present but less prominent and the lamina propria showed mild leukocytic infiltration, indicating a subtle inflammatory response. These findings suggest that the combined supplementation may have led to digestive stress or negative synergistic effects at the given concentration, resulting in compromised villi structure and reduced absorption surface.

Figure 4

Representation of intestinal villi in rohu from Group-3

DISCUSSION

Medicinal plants have long been used as natural feed additives, range of plants along with their extracts, essential oils and bioactive compounds used as functional feed supplementation in aquaculture (Pandey *et al.*, 2012). Bioactive compounds like flavonoids, saponins and alkaloids in plants such as garlic, fenugreek, flaxseed, neem and turmeric helped to boost up digestive efficiency and antioxidant activity. Overall, medicinal plants serve as natural eco-friendly alternatives to synthetic additives in aquaculture (Stratev *et al.*, 2018). These natural substances have been shown to promote fish growth, immune system, enhancement of digestive property, regulate hormonal activities and helpful in metabolism (Akhtar *et al.*, 2022).

The present study was conducted to investigate the effect of dietary supplementation with soluble flaxseed and fenugreek seed meal on the growth performance, digestive efficiency, histological architecture and immune response of *Labeo rohita*, commercially important freshwater fish species in aquaculture (Attia *et al.*, 2024; Mohammed *et al.*, 2024). It has been found that biological process like growth, immunity, feed intake, antibacterial and anti-antistress characters in fish culture (Velmurugan *et al.*, 2010). Flaxseed powder includes omega-3 fatty acids, fibbers, fats and protein (Sorour *et al.*, 2022). Fenugreek is leguminous plant, its biological properties include anti-inflammatory, antidiabetic and antioxidant properties (Mehboob *et al.*, 2017).

The results of present study indicating maximum results of weight gain shown in G_2 treatment fed with 3g of fenugreek seed meal (8.35±2.07) (1.500±0.216) and significantly ($p>0.05$) decreased as compare to control group (6.125±1.141) (0.700±0.440). The improvement in growth performance might be attributed to high content of omega-3 fatty acids in flaxseed and the presence of saponins, flavonoids and essential amino acids in fenugreek while minimum in control group. These findings are also reported by Mir *et al.* (2021); (Dyková *et al.*, 2022) documented similar growth-promoting effects of flaxseed and fenugreek in other freshwater species. Similar results observed by Díaz-Vázquez *et al.* (2025) who investegated the effect of flaxseed meal as a source of Omega-3 Fatty Acids on growth, muscle composition, fatty acid profile and productive performance in tilapia.

Results regarding FCR showed that maximum value in G_3 (8.815±1.874) treated with combination of 6g flaxseed and fenugreek seed meal and minimum value was seen in G_2 treatment (1.248±0.265) fed with 3g of fenugreek seed meal. The improved feed intake and a further reduction in FCR is likely due to the digestive stimulatory effect of fenugreek compounds. Minimum FCR value estimated in G_2 group which means that feed intake is higher in rate and increased body weight present in same group. Decreased FCR value is more efficient. The same results shown by Sallam *et al.* (2021) observed significant improvement in feed conversion efficiency and nutrient digestibility in Nile tilapia fed a combination of plant-based additives including flaxseed. Similarly, Tonial *et al.* (2009) found that fenugreek inclusion in the diet of *Cattla cattla* led to a marked increase in feed intake and a decrease in FCR confirming the results obtained in the present study.

Specific growth rate was maximum in G_2 treatment (11.87±7.66) fed with 3g of fenugreek seed meal and significantly ($p>0.05$) decreased SGR value was observed in T_3 treatment (5.800±0.917) fed with combination feed. Supplementation of fenugreek diet might be attributed to the antioxidant by neutralizing free radicals, antimicrobial by inhibition of pathogenic growth and gut health, antibacterial by reducing infections and enhanced digestive property. The same observation was discussed by Gherescu *et al.* (2023) who explained that flaxseed meal have valuable impact on growth parameters like weight gain, feed conservation and specific growth rate. Similar results was observed by Dokdok *et al.* (2020) who investigated the effect of fenugreek seed meal on growth performance and feed utilization of red tilapia. Same observations indicated by Akhtar *et al.* (2024) who discussed the impact of plant based dietary fat level on growth, feed utilization and body composition in *Labeo rohita.*

Survival rate of fish showed 100% survival rate in treatment G1, G2 and control group as compare to treatment G3 (80%) treated with 6g of flaxseed and fenugreek seed meal. Minimum survival rate might be showed due to increasing oxidative stress by excess free radicals damaging cells when antioxidant defenses are insufficient. Fish are less likely to suffer from cellular damage and saponin effect of fenugreek seed powder in water. Similar results observed by Banerjee *et al.* (2023) used flaxseed oil cake in diet of rohu to investigate overall health of fish and gut bacterium significantly ($p<0.05$) improved crude protein along with amino acids. Results of the present study resembles with Shahzad *et al.* (2022) indicated that probiotics and linseed addition showed significant improvement in fish health.

The present study indicated the increase in amylase activity was highly significant ($p<0.01$), indicating a strong enhancement in starch digestion efficiency, maximum results showed in treatment G_2 (172.50±7.50) and minimum in G_1 (126.50±6.50). The results improved in G_2 group may be attributed to the appetizing and aromatic effect of fenugreek seed meal. Fenugreek acts as a prebiotic, supporting beneficial gut bacteria, which further stimulate enzyme secretion and overall digestive performance. Similar results shown by Mueed *et al.* (2022) reported that dietary phytogenic significantly enhanced digestive enzyme secretion in Indian major carps. Same results demonstrated by Hano *et al.* (2013) who showed that to determine the potential inhibitory effect on α-glucosidase and α-amylase activities of flaxseed extract.

Results of recent study suggested that maximum proteases were shown in T_0 treatment (73.00±2.69) fed with basal diet as compare to G_1 treatment (72.300±0.800) fed with 3g of flaxseed, G_2 treatment (71.450±1.050) fed 3g fenugreek seed meal and G_3 treatment (70.500±0.400) fed with 6g mixture of fenugreek and flaxseed meal powder. The significant increase in protease activity in the G0 group suggested that the basal diet composition was easily digestible and balanced in terms of protein content and quality. Results are similar with the findings of Pradhan *et al.* (2021) who discussed that no significant differences were observed for somatic and serum parameters between treatments. Same findings were observed by Logarušić *et al.* (2020) who discussed that highest proteolytic activity, while the produced flaxseed protein hydrolysate exhibited the strongest antioxidant potential.

The present study investigated the effect of flaxseed and fenugreek seed meal and their combination in histopathological analysis of *Labeo rohita*. Analysis of the intestine revealed that the G1 and G2 groups had moderately elongated villi and a higher goblet cell density than the control, supporting better nutrient absorption and mucosal integrity. There were no negatively effected signs in organs. Similar enhancement shown by Banerjee *et al.* (2023) observed improved gut histology and enzyme activity in fish fed with phytogenic additives. Same studies showed by Alkhaledy and Mohammad (2024) emphasized the role of phytogenic feed additives in maintaining organ health and preventing tissue damage in fish under various stress conditions.

In present study G1 group fed with flaxseed meal showed elevated leukocyte count, moderate increase in WBCs (3.773±0.906) and lymphocytes (84.67±2.52) compared to the control group. The immunostimulatory effect refers to the ability of certain compounds such as saponins, flavonoids, and omega-3 fatty acids found in fenugreek and flaxseed to activate and enhance the fish immune system. Similar findings observed by Abou Zaid *et al.* (2025) showed that herbal supplements like flaxseed and fenugreek enhanced leukocyte proliferation and improved disease resistance in fish.

Results observed that maximum count of monocytes calculated in G0 group (1.533±0.416) demonstrated a significant rise due to basal diet and not effected by supplementary feed. G0 group displayed the highest values across all immune cell types neutrophils and monocytes reflecting a comprehensive and synergistic immune boost. Similar findings observed by Diab *et al.* (2023) who investigate dietary effect of fenugreek seed meal on immunity responses of tilapia. Similar study highlights by Singh *et al.* (2025) suggested the potential of seed mucilage as an effective prebiotic that improves gut microbiome composition, immune activity and metabolic outcomes.

CONCLUSION

This study presented that dietary supplementation with 3 g/kg fenugreek seed meal significantly improved growth performance, feed efficiency, intestinal and liver health in *Labeo rohita*. Flaxseed supplementation also showed positive effects but was less effective than fenugreek. The combined supplementation (G3) led to reduced growth and mild tissue stress, likely due to overloading of bioactive compounds or anti-nutritional effects. Overall, the findings support the use of plant-based supplements for improved fish health when used at appropriate levels. Fenugreek seed meal recommended as the optimal dose for enhancing growth performance, feed utilization and tissue integrity.

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