Original Research Article

# Effect of Jeevamruth and Millet-Based Biofloc on Hepatosomatic and Intestinal Somatic Indices of *Labeo rohita* in an Integrated Fish cum Horticulture Model

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## Abstract

The present study evaluated the effects of Jeevamruth and biofloc-based systems on the organosomatic indices of *Labeo rohita* cultured under five treatments: Control (C), 0.5% Jeevamruth (T1), 1% Jeevamruth (T2), biofloc (T3), and biofloc + 0.5% Jeevamruth (T4). The Hepatosomatic Index (HSI) and Intestinosomatic Index (ISI) were assessed after a 60-day culture period to determine the physiological response and metabolic efficiency of fish. Results revealed non-significant differences in HSI among treatments, with values ranging from 1.19 ± 0.26% (T3) to 1.62 ± 0.09% (Control), indicating stable hepatic function across all groups. However, a significant reduction in ISI was observed in T4 (6.29 ± 0.37%) compared to the control (9.57 ± 0.07%), suggesting improved intestinal efficiency and nutrient assimilation. The combination of biofloc and Jeevamruth promoted a healthier microbial environment, enhancing digestion and minimizing intestinal stress. These findings demonstrate that the synergistic use of Jeevamruth and biofloc fosters optimal physiological balance, efficient nutrient utilization, and improved fish health, supporting its potential as a sustainable strategy for eco-friendly aquaculture.

*Keywords: Jeevamruth; biofloc technology; organosomatic indices; hepatosomatic index (HSI); intestinosomatic index (ISI); Labeo rohita; gut health; nutrient assimilation; sustainable aquaculture*

## 1. INTRODUCTION

Physiological indices such as the Hepatosomatic Index (HSI) and Intestinal Somatic Index (ISI) are widely employed in aquaculture to assess the metabolic condition and organ development of cultured fish. These indices serve as reliable indicators of nutritional status, energy storage, and digestive capacity under varying dietary or environmental conditions (Manzoor et al. 2020; Yadav et al. 2025). The liver, being a major site of lipid and glycogen metabolism, responds sensitively to feed formulation and water quality, while intestinal mass and morphology reflect digestive efficiency and nutrient absorption (Gupta et al. 2025; Ramasubburayan & Prakash 2025). In Labeo rohita (rohu), one of India's most important aquaculture species, HSI and ISI have been used to monitor the effects of probiotic supplementation, plant extracts, and biofloc-mediated nutrition on fish health and performance. For instance, dietary supplementation with chia seed oil improved hepatosomatic and intestinal indices while modulating gut microbiota toward beneficial genera such as *Lactobacillus* and *Bacillus* (Gupta et al. 2025). Similarly, Khejri (*Prosopis cineraria*) seed extract enhanced metabolic enzyme activities and raised HSI and ISI values, indicating improved energy utilization and gut development (Yadav et al. 2025). The integration of microbial inputs either as probiotics, synbiotics, or through biofloc systems has been shown to support hepatointestinal health by promoting balanced gut flora, stabilizing water parameters, and enhancing nutrient recycling (Nayak 2021; Khanjani & Sharifinia 2024). Probiotics such as *Bacillus subtilis*, *Lactobacillus plantarum*, and *Pseudomonas fluorescens* improve liver morphology and intestinal histometrics, supporting better feed conversion and immunity (Ferdous et al. 2025; Sharna et al. 2025). Monitoring organosomatic indices like HSI and ISI can therefore provide early insights into physiological adaptation to alternative aquaculture inputs, including natural biofertilizers such as Jeevamruth. Given its rich microbial consortium of nutrient-cycling bacteria, Jeevamruth may influence hepatointestinal physiology by optimizing digestion, detoxification, and microbial balance. The present study was thus undertaken to evaluate the impact of Jeevamruth supplementation on HSI and ISI of Labeo rohita, aiming to elucidate how natural microbial amendments affect organ-level health and energy allocation in aquaculture system.

## 2. MATERIALS AND METHODS

The study was conducted at the College of Fisheries Science (CoFS), Kamdhenu University, Rajpur (Nava), Himmatnagar, Gujarat, India. All aquaculture experiments were performed in the wet-laboratory facility, while water quality analyses were carried out in the Soil and Water Chemistry Laboratory. The experiment lasted 60 days (March-May,2025). A Completely Randomized Design (CRD) was used comprising one control and four treatments, each in triplicate. Each 1000-L FRP tank was hydraulically connected to three respective horticulture plots to enable nutrient transfer for the integrated fish–horticulture model. Healthy *Labeo rohita* (rohu) fingerlings (8.8 ± 0.2 g) were procured from the Department of Aquaculture, CoFS, Kamdhenu University. Fish were disinfected in 0.05 % $KMnO_4$ for 2 min and acclimatized for two months under laboratory conditions. Fish were stocked at 1 $g\ L^{-1}$ density (approximately 500 fish $tank^{-1}$) and maintained under continuous aeration. Tanks were filled with bore-well water (previously quality tested), and water losses were compensated daily. The control received a 10 % water exchange per day; all other treatments were operated as zero-water-exchange biofloc systems. Fish were fed a commercial floating feed containing 32 % crude protein twice daily at 5 % body weight $day^{-1}$ for the first 20 days. Biofloc was prepared following Avnimelech (1999) and Hargreaves (2013). Tanks were filled to 500 L, aerated for 5 days, and inoculated with a commercial probiotic mixture (5 g $tank^{-1}$). Millet flour was used as a carbon source containing approximately 64 % C (Lukiwati et al., 2018). The C:N ratio was adjusted to 20:1 by adding ~100 g millet $tank^{-1}\ day^{-1}$, calculated from the protein content (32 %) of feed assuming 16 % N in protein and 75 % feed waste contribution. Floc maturity was confirmed when floc volume reached 10-15 $mL\ L^{-1}$ and total ammonia nitrogen < 0.5 $mg\ L^{-1}$, measured with Imhoff cones and spectrophotometric tests (Avnimelech & Kochba, 2009). Jeevamruth was prepared per the NITI Aayog (2019) formulation: 2 kg cow dung, 1 L cow urine, 400 g jaggery, 400 g pulse flour, 200 g native soil, 10 g lime, and water up to 40 L. The mixture was fermented aerobically for 7 days with daily stirring and then filtered through cloth. The filtrate was applied to treatment tanks at 0.5 % ($T_2$) and 1 % ($T_3$) concentrations every 10 days; the combined treatment ($T_4$) received 0.5 % Jeevamruth along with millet-based biofloc. A 200 m² field adjacent to the aquaculture setup was divided into 15 plots (3 replicates $treatment^{-1}$). Cluster bean (*Cyamopsis tetragonoloba*) was grown as the test crop. Each plot was irrigated with corresponding aquaculture effluents through a mild-slope pipeline system ensuring gravitational flow. Physico-chemical parameters were measured every 10 days using standard methods (APHA, 1998). Soil samples were collected pre- and post-experiment from 0-15 cm depth using a zig-zag pattern, composited, air-dried, and analyzed for pH, EC, and N-P-K status (Jackson, 1973). Every 20 days, 15 fish $tanks^{-1}$ were sampled to determine growth indices (Hopkins, 1992) At the end of the experimental period, three fish were randomly collected from each tank for organosomatic measurements. Fish were anesthetized with MS-222 (100 $mg\ L^{-1}$) before dissection to minimize stress. Each fish was carefully dissected to remove the liver and intestine, which were rinsed in distilled water and blotted dry with absorbent paper to remove surface moisture. The organs were immediately weighed to the nearest 0.001 g using a calibrated electronic balance (Aczet CY220). The Hepatosomatic Index (HSI) and Intestinal Somatic Index (ISI) were calculated as per Shearer (1994) and Manzoor et al. (2020):

$$\text{HSI (\%)} = \frac{\text{Liver weight (g)}}{\text{Total body weight (g)}} \times 100$$

$$\text{ISI (\%)} = \frac{\text{Intestine weight (g)}}{\text{Total body weight (g)}} \times 100$$

All data were subjected to One-way ANOVA using SPSS v30, and mean differences were evaluated by Tukey's HSD at $p < 0.05$. Results are presented as mean ± standard error.

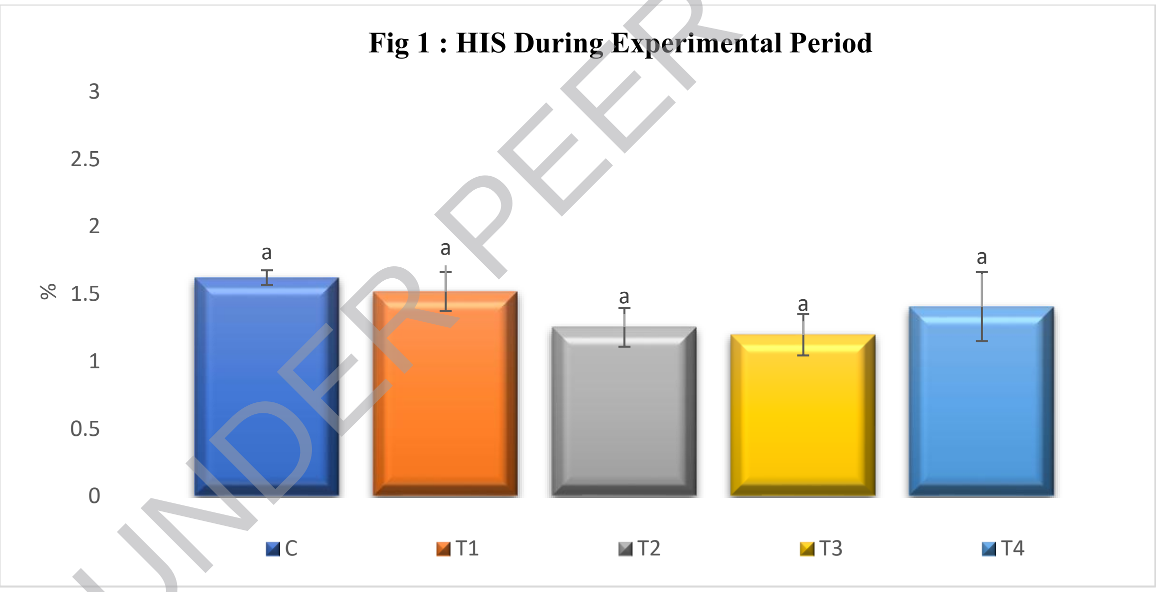
## 3. RESULT AND DISCUSSION

Organosomatic indices such as the Hepatosomatic Index (HSI) and Intestinosomatic Index (ISI) are vital indicators of fish metabolic status, nutrient utilization, and general health. In the present study, HSI and ISI values varied slightly among treatments but remained within physiological norms for *Labeo rohita*. The variations observed can be attributed to differences in microbial activity, nutrient assimilation, and metabolic efficiency associated with Jeevamruth and biofloc supplementation.

**Table 1 : Hepatosomatic index recorded during experimental period.**

| Parameters | Treatments | Mean ± SE |
|---|---|---|
| **HSI%** | C | $1.6197\pm0.09^a$ |
| | T1 | $1.5173\pm0.25^a$ |
| | T2 | $1.2527\pm0.24^a$ |
| | T3 | $1.1977\pm0.26^a$ |
| | T4 | $1.4053\pm0.44^a$ |

(*Note HIS = Hepatosomatic index, T1= Bio-floc (20:1); T2= 0.5% Jeevamruth; T3=1% Jeevamruth, T4=Biofloc (20:1) + 0.5% Jeevamruth; The mean values followed by the different superscript letters in each factor indicate significant difference at 0.05.)



The HSI values ranged from 1.19 ± 0.26% (T3) to 1.62 ± 0.09% (Control), with no significant differences (p > 0.05) among treatments. The relatively stable HSI across treatments indicates that none of the organic amendments induced hepatic stress or lipid overload in *L. rohita*. A slight reduction in HSI in Jeevamruth and biofloc treatments compared to the control may reflect enhanced metabolic efficiency and reduced hepatic fat deposition due to better nutrient utilization. Similar results were reported by (Zaki et al., 2024) and (Panigrahi et al., 2018), who noted that fish reared in biofloc or probiotic-enriched systems exhibited balanced liver weights owing to efficient lipid mobilization and lower hepatocellular lipid accumulation. The marginally lower HSI in T4 (1.40 ± 0.44%) suggests that the combination of millet-based carbon and Jeevamruth enhanced microbial degradation of organic waste, providing cleaner water and stable metabolic conditions. According to (Lal et al., 2024) and (Ramasubburayan et al., 2025), well-regulated microbial environments help reduce oxidative and nutritional stress in fish liver tissue by maintaining favorable water quality and nutrient turnover. The non-significant difference in HSI also implies that Jeevamruth, even at 1%, did not impose metabolic load or toxicity on hepatic function, consistent with the findings of (Said et al., 2022), who reported that natural microbial stimulants improve liver condition by reducing ammonia toxicity and lipid oxidation. Hence, the overall HSI trend in the present experiment suggests that organic and microbial amendments contributed to stable hepatic metabolism and improved energy allocation efficiency.

**Table 2 : Intestinal somatic index recorded during experimental period.**

| Parameters | Treatments | Mean ± SE |
|---|---|---|
| ISI% | C | $9.57 \pm 0.07^{a}$ |
| | T1 | $8.51 \pm 0.36^{ab}$ |
| | T2 | $9.56 \pm 0.86^{a}$ |
| | T3 | $7.90 \pm 0.72^{ab}$ |
| | T4 | $6.29 \pm 0.37^{b}$ |

(*Note ISI = Intestinal somatic index T1= Bio-floc (20:1); T2= 0.5% Jeevamruth; T3=1% Jeevamruth, T4=Biofloc (20:1) + 0.5% Jeevamruth; The mean values followed by the different superscript letters in each factor indicate significant difference at 0.05.)

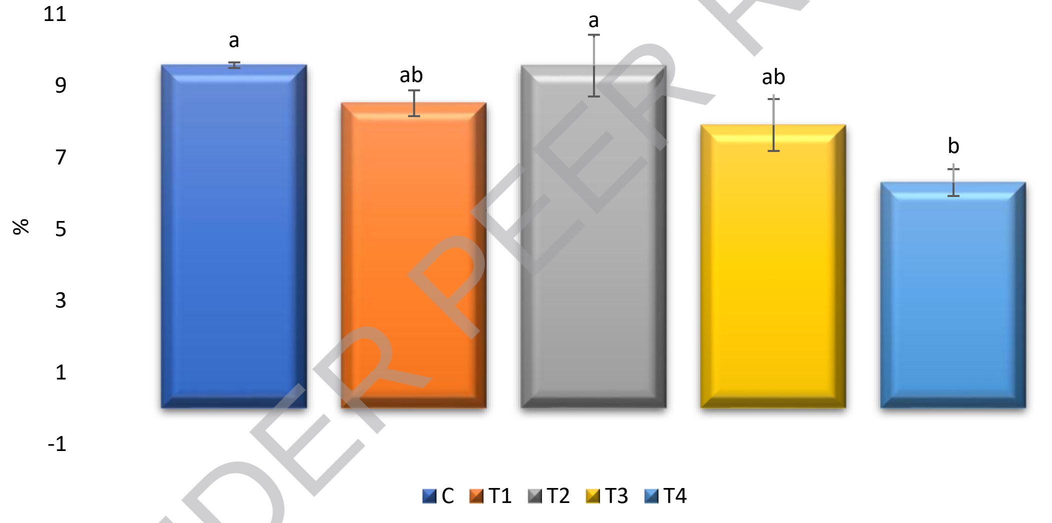


**Fig 2 : ISI During Experimental Period**

The ISI values showed more pronounced variations across treatments, ranging from 9.57 ± 0.07% in the Control to 6.29 ± 0.37% in T4, with the lowest ISI observed in the biofloc + Jeevamruth treatment. A lower ISI typically indicates improved nutrient digestibility and reduced intestinal hypertrophy, as the digestive system functions more efficiently with better microbial support and enzyme activity. The decrease in ISI in T4 and T3 suggests that microbial inoculation and carbon enrichment promoted the proliferation of beneficial gut microbiota, enhancing digestion and absorption processes. Similar findings were reported by (Hersi et al., 2023), who observed that probiotic-enriched biofloc diets significantly improved gut histomorphology and reduced intestinal mass in *Oreochromis niloticus*. (Banu et al., 2024) also found that microbial diversity and enzymatic profiles in biofloc-fed fish correlated with enhanced intestinal nutrient transport and reduced intestinal weight proportion. The synergistic effect in T4, combining Jeevamruth's microbial richness with the nutrient-dense biofloc environment, likely optimized gut microbial communities, thereby lowering ISI through improved digestive efficiency. The control and single Jeevamruth treatments (T1 and T2) exhibited comparatively higher ISI values (8.51–9.56%), implying that although Jeevamruth alone improves microbial availability, the absence of a carbon-rich biofloc substrate may limit microbial diversity and enzymatic interaction. This observation agrees with (Nguyen et al., 2021) and (Wu et al., 2024), who emphasized that combined carbon–microbial systems promote higher nutrient assimilation and intestinal function compared to single-source inoculants. The observed ISI reduction in T4 also aligns with the trend of improved feed conversion efficiency (FCE = 0.66) and protein efficiency ratio (PER = 2.07) in the same treatment, confirming that better nutrient assimilation translates into reduced intestinal mass and improved digestive economy. Furthermore, studies by Zaki et al. (2024) and Cuzon et al. (2004) reported that balanced microbial ecosystems improve gut enzyme activity, reducing the need for structural enlargement of the intestine.

## 4. CONCLUSION

The organosomatic assessment of *Labeo rohita* clearly indicated that integrating Jeevamruth with a biofloc system enhances fish physiological performance without causing metabolic stress. While HSI values remained stable across treatments, the significantly lower ISI in the biofloc + Jeevamruth group (T4) confirmed enhanced digestive efficiency and better nutrient assimilation. These outcomes suggest that Jeevamruth, a natural microbial stimulant, when combined with carbon-based biofloc, promotes beneficial gut microbiota and supports balanced organ development. The overall physiological stability and improved intestinal function observed in T4 reflect the superior metabolic environment created by microbial synergy. Thus, the integration of Jeevamruth with biofloc technology provides a cost-effective, sustainable approach for maintaining fish health and improving production efficiency in intensive freshwater aquaculture systems

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