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The effect of incorporating Tulsi (*Ocimum sanctum*) into fish feed on the hematological parameters, growth, and survival rates of *Labeo rohita* (fingerlings).

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ABSTRACT

Natural immunosuppressants are compounds that can assist modulate or suppress the immune system; they are produced from plants, herbs, or other natural sources. Medicinal herbs are increasingly being used in aquaculture for their natural immunostimulants and growth-promoting properties. The plant Tulsi, often called holy basil (*Ocimum sanctum*), is widely used in traditional medicine, particularly in Ayurveda, because of its ability of anti-inflammatory, antibacterial, and immunomodulatory qualities. Diets containing *O. sanctum* at various levels, such as 200mg, 400mg, and 600mg, were utilized as test diets, whereas diet lacking *O. sanctum* served as the control tank (C). The experiment lasted 60 days, during which time the water quality was monitored every other day. The results showed that the innate immune response was improved when *Ocimum sanctum* was administered through different diets, primarily in T1, T2, and T3 diets. This boosted the total erythrocyte count, hemoglobin, and total leukocyte count. Over the course of the 60-day trial, growth and hematological markers were examined every 20 days. After 20 days, 40 days, and 60 days, the first, second, and final readings were taken. The current study was carried out to investigate the influence of dietary Tulsi extract on fingerlings of *L. rohita*'s growth, survival, and hematological levels.

INTRODUCTION

Aquaculture has evolved as an important component of global food security, approximately 87% of freshwater aquaculture productivity in India is derived from the most prevalent carp species cultivated there and in other Asian countries, which are Indian foremost carps (IMCs), *Labeo rohita* (Rohu) is one of the most frequently grown freshwater fish species in South Asia due to its high nutritional value and market demand. However, fish survival, growth, and health especially at the crucial fingerling stage are crucial to the viability of aquaculture operations. Research has shown that many factors, such as excessive handling, transportation, overcrowding, and inadequate soil and water quality maintenance, make cultured fish more prone to disease than wild fish. **(Jobling, 2010; Ali *et al.*, 2003).** But in the case of fish illnesses, naturally occurring compounds with biodegradable and bio-compatible qualities have drawn a lot of interest and focus.

O. *sanctum* **(Logambal et al., 2000)** has been linked to improved fish immunity and growth. Despite its recognized medicinal benefits in humans and terrestrial animals, the application of *O. sanctum* as a dietary supplement in aquaculture known for its immunomodulatory, antioxidant, and antimicrobial activities, Tulsi could potentially improve the hematological parameters, growth performance, and survival rates of fish **(Ranjana and Tripathi, 2015).** Eugenol, the main constituent of tulsi, is mostly in charge of giving the plant its distinctive scent and a number of its therapeutic qualities. Tulsi can have a different chemical makeup depending on the portion of the plant that is used, how the plant is grown, and the extraction techniques used. But a broad breakdown of some of the main ingredients in Tulsi essential oil in terms of percentages is as follows: eugenol: 40–70%; methyl eugenol: 1–10%; carvacrol: 5–15%; linalool: 3-12%; caryophyllene: 2-9%; rosmarinic acid: ~1–5%; and ursolic acid: ~1-3%. The purpose of this study is to examine the effects of feeding *L. rohita* fingerlings Tulsi in their diet. It focuses on assessing the fish's hematological characteristics, growth, and survival rates in particular.

Materials and Methods:

Experimental setup: The fish farm named matsotpadan kendra at Morshi was the source of sixty rohu fingerlings, each weighing 4-5 grams and measuring 5-6 centimeters in length. 15 of the 60 fish in total were divided across four tanks: control (C), T1, T2, and T3. Prior to the start of the experiment, the fish were fed with basal food that they were fed on the farm for 10 days to acclimatize them to the experimental environment. Three experimental tanks containing varying concentrations of O. sanctum extract (200 mg, 400 mg, and 600 mg) were set up in the tank arrangement. The last tank served as a control, with no herb extract added. The experimental duration is of 60 days.

Diet Preparation: Tulsi leaves were harvested, washed, and ground into a paste. This paste was mixed with fish meal in varying dosages: 200 mg for the first tank, 400 mg for the second, and 600 mg for the third. A control tank received no tulsi extract. The ingredients were measured according to the feed formula. **(Add own reference here.)**

Ingredients	Experimental Treatment

	Expt. A	Expt. B	Expt. C	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	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)	-

Estimation of growth parameter:
Sampling was conducted every 20 days to assess fish growth, with length and weight measured using a scale and electric balance. Growth parameters, including weight gain, specific growth rate, feed conversion ratio, and survival rate, were calculated using standard formulas;

Weight gain = Final weight – Initial Weight

Length gain = Final length – Initial Length

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed given (dry weight)}}{\text{Body weight gain (wet weight)}}$$

Specific growth rate (SGR)

$$\text{SGR (\%)} = \frac{\text{(ln Final weight) - (ln of Initial weight)}}{\text{Days fed}} \times 100$$

$$\text{Survival (\%)} = (\text{Final No. of fish/initial No. of fish}) \times 100$$

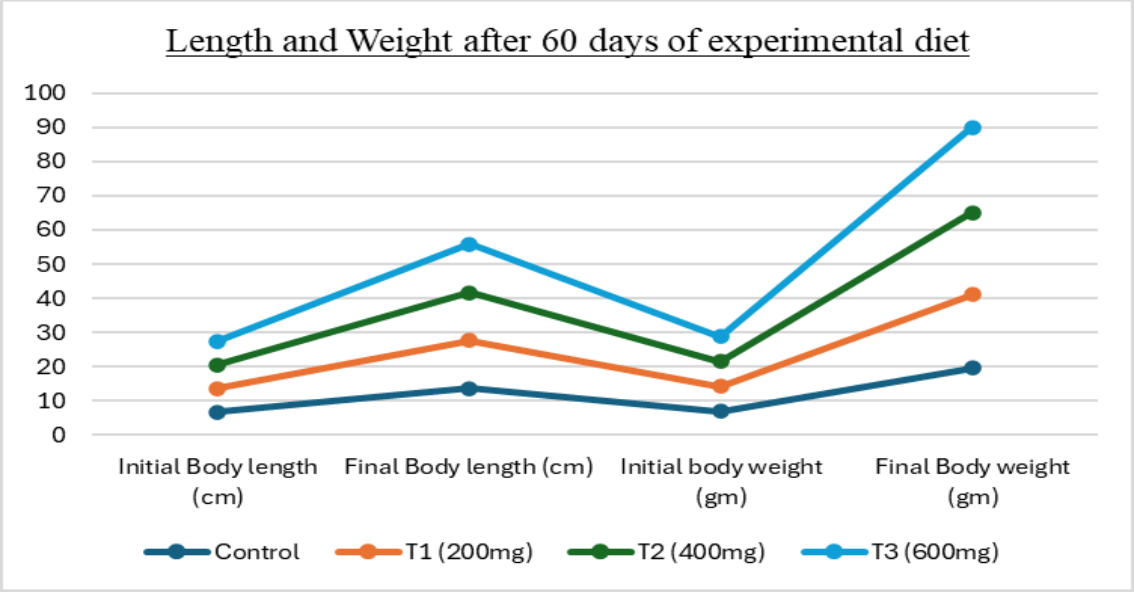
Estimation of hematological parameters:
To monitor fish growth, sampling was done every 20 days, blood samples were taken from the caudal vein of three randomly selected, anesthetized fish from each aquarium using a 3 ml sterile hypodermic syringe. And the following hematological indices were analyzed: red blood cell count (RBC), white blood cell count (WBC), and hemoglobin (Hb).

Result:
Water physio-chemical properties: Weekly analyses of water quality, including temperature, pH, dissolved oxygen (DO), and total hardness, were conducted throughout the trial. Water temperature ranged from 21 to 26°C, and pH remained between 7 and 8, both within the ideal range. Total hardness was between 220 and 325 ppm, while dissolved oxygen levels varied from 5 to 7 ppm.

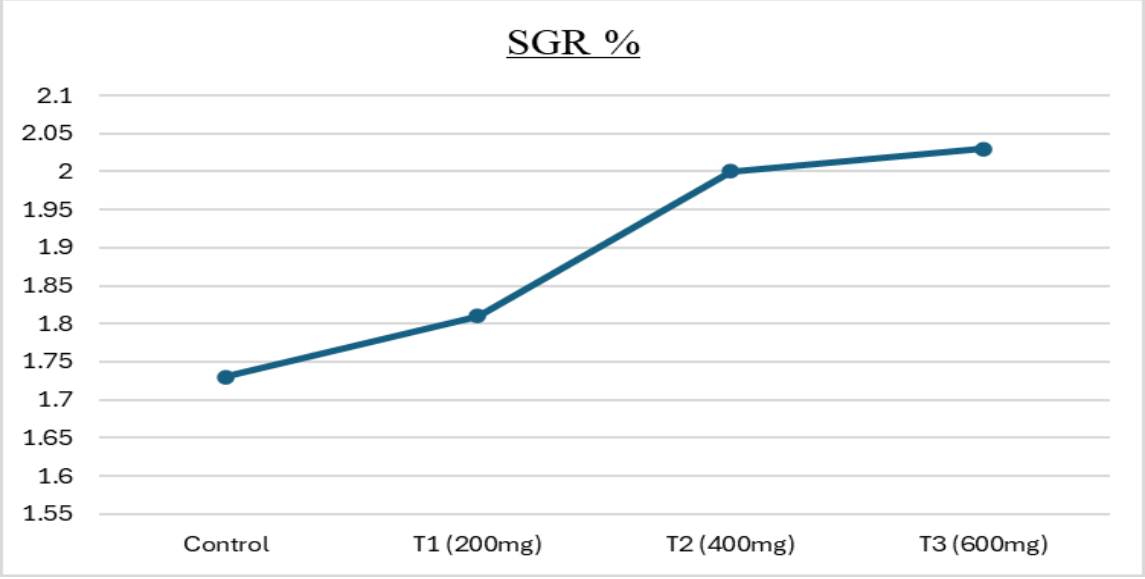
Growth indices:
The rohu fingerlings were measured for length (5–7 cm) and weight (6–8 gm) before being placed in tanks. The mean weight for each tank was recorded every two weeks. After 60 days, fingerlings in the 200 mg tank weighed 21.48 gm and measured 13.91 cm, those in the 400 mg tank weighed 24.08 gm and measured 14.03 cm, and those in the 600 mg tank weighed 24.81 gm and measured 14.17 cm. The control tank fish weighed 20.99 gm and measured 13.73 cm. Tank 3 had the highest final mean weight ($p < 0.05$), while the control tank had the lowest. The table below shows the initial and final mean weights of *L. rohita* fingerlings after 60 days. Numerous aquatic creatures have been examined for the effects of herbs. It has been claimed that feeding fish a diet enriched with herbs improves their growth. (**Kaleeswaran B et al., 2011), (Prasad G, Mukhthiraj S 2011), (Sanchez JAO et al., 2009), (Kour D et al., 2004).**
The Specific Growth Rate (SGR) is used to compare daily growth, and nutritional supplements significantly impact growth performance. After 60 days, the tank with 600 mg of *O. sanctum* extract (Tank 3) had the highest SGR ($p < 0.05$), significantly outperforming T1, T2, and the control. Tank 3 had the best SGR (1.50%), followed by Tank 2 (400 mg), Tank 1 (200 mg), and the control.

Parameter	Control	T1 (200mg)	T2 (400mg)	T3 (600 mg)
Initial Total Body Length (cm)	6.81 ± 0.56	6.92 ± 0.36	6.85 ± 0.69	6.93 ± 0.22
Final Total Body Length (cm)	13.73 ± 0.54	13.91 ± 0.51	14.03 ± 0.51*	14.17 ± 0.58**
Initial Total Body Weight (gm)	6.93 ± 0.41	7.28 ± 0.46	7.26 ± 0.45	7.36 ± 0.53
Final Total Body Weight (gm)	19.57 ± 0.53	21.48 ± 0.47	24.08 ± 0.22**	24.81 ± 0.61**
SGR % in 60 days	1.73 ± 0.096	1.81 ± 0.12*	2.00 ± 0.11**	2.03 ± 0.13**
FCR % in 60 days	1.89 ± 0.093	1.81 ± 0.093*	1.67 ± 0.073**	1.66 ± 0.083**
Survival %	40%	53.33%*	73.33%*	86.67%**

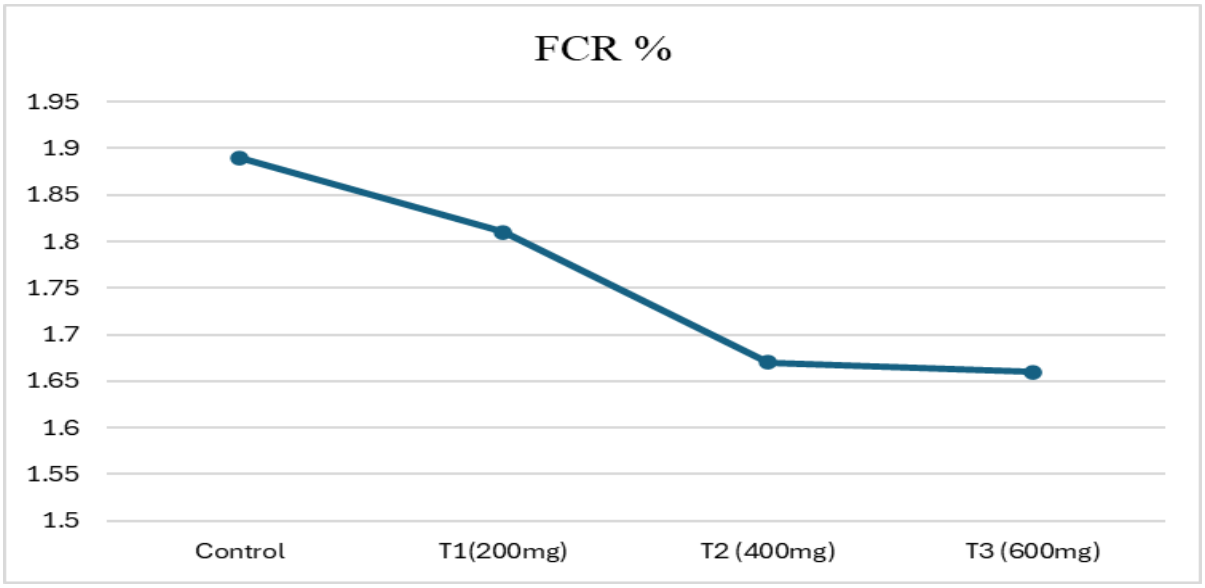
Mean length (cm) and weight (gm) of *L. rohita* after 60 days of fed tulsi-supplemented diets during the experiment (n=5 fish, Mean ± SD) showed significant differences ($P<0.05$) between values with different superscripts. * indicates moderate significance, and ** indicates high significance. Specific growth rate (%) at the end of the experiment is also presented (Mean ± SD).



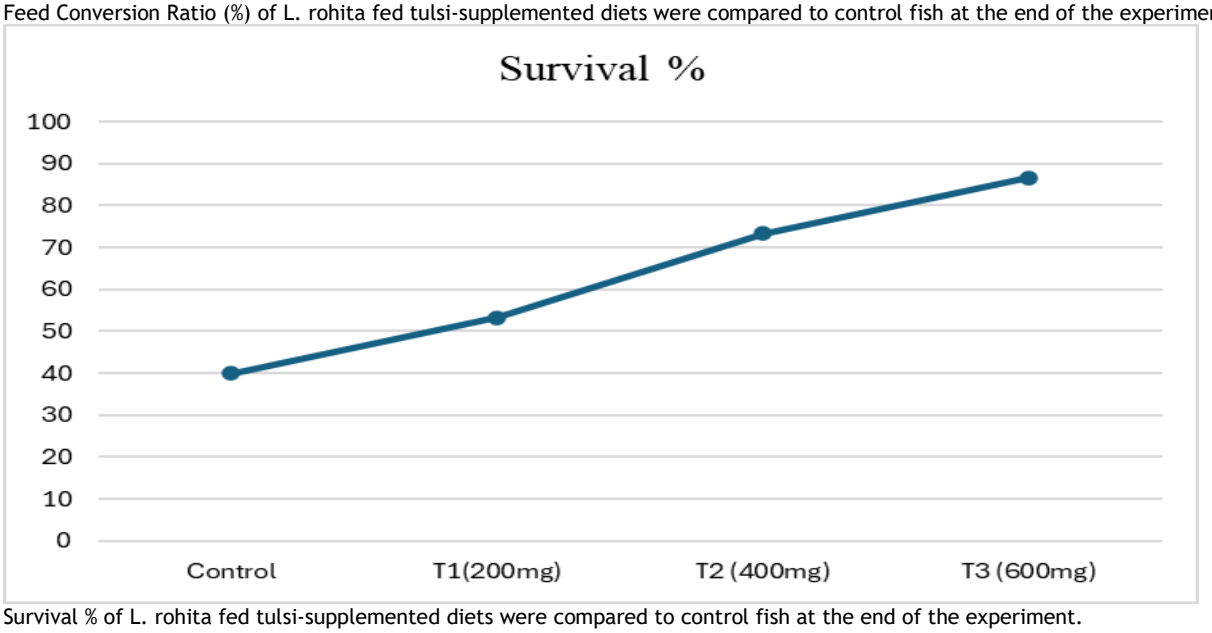
Mean weight (g), length (cm), and specific growth rate (%) of L. rohita fed tulsi-supplemented diets were compared to control fish at the end of the experiment.



Specific growth rate (%) of L. rohita fed tulsi-supplemented diets were compared to control fish at the end of the experiment.



Feed Conversion Ratio (%) of L. rohita fed tulsi-supplemented diets were compared to control fish at the end of the experiment.



Survival % of L. rohita fed tulsi-supplemented diets were compared to control fish at the end of the experiment.

Hematological parameters:

The blood extracted from the fish's caudal vein was meticulously moved into 1.5 ml EDTA-heparinized tubes that were stored on ice. However, some scientists **(Faggio et al., 2013)** claimed that EDTA was more reliable as an anticoagulant for fish hematological investigation.

Haematological analysis were carried out after 60 days of feeding trial. This involved the estimation of hemoglobin, total leucocyte count (WBCs) and red blood cell count (RBCs). Using a hemocytometer, total leucocyte and hemoglobin levels were examined.

Initial reading (first day of experiment):

Hematological parameter	Control	T1 (200 mg)	T2 (400 mg)	T3 (600 mg)
RBC (×10^6 cells/µL)	2.01 ± 0.51	2.21 ± 0.29	2.16 ± 0.55	2.24 ± 0.35
Hb (g/dL)	5.06 ± 0.35	5.11 ± 0.29	5.14 ± 0.28	5.13 ± 0.29
WBC (×10^3 cells/µL)	14.84 ± 0.53	14.82 ± 0.47	14.91 ± 0.44	14.88 ± 0.53

Initial reading (after 20 days):

Haematological parameter	Control	T1 (200mg)	T2 (400mg)	T3 (600mg)
RBC (×10^6 cells/µL)	2.06 ± 0.38	2.22 ± 0.47	2.19 ± 0.21	2.27 ± 0.32*
Hb (g/dL)	5.06 ± 0.58	5.12 ± 0.28	5.17 ± 0.30*	5.16 ± 0.39
WBC (×10^3 cells/µL)	14.86 ± 0.64	14.87 ± 0.58	14.92 ± 0.48*	14.91 ± 0.45

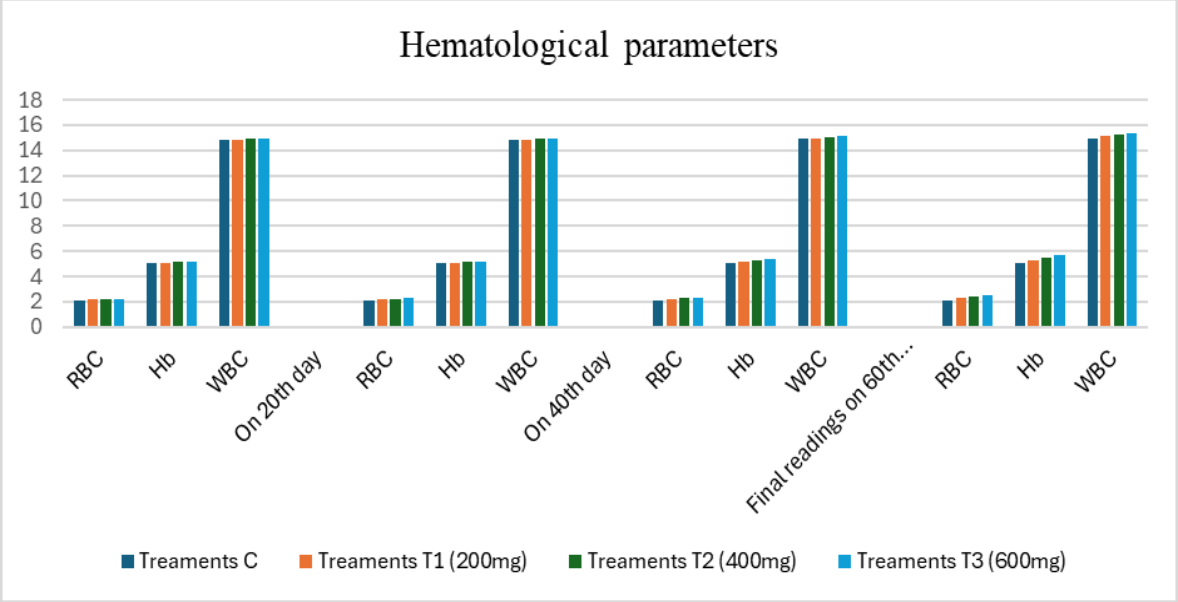
Initial reading (after 40 days):

Haematological parameter	Control	T1 (200mg)	T2 (400mg)	T3 (600mg)
RBC (×10^6	2.07 ± 0.58	2.24 ± 0.42	2.29 ± 0.38	2.35 ± 0.34*

cells/µL)				
Hb (g/dL)	5.08 ± 0.36	5.19 ± 0.39	5.27 ± 0.16	5.36 ± 0.31*
WBC (×10^3 cells/µL)	14.88 ± 0.38	14.96 ± 0.55	15.09 ± 0.24	15.12 ± 0.26*

Final reading on 60th day:

Haematological parameter	Control	T1 (200mg)	T2 (400mg)	T3 (600mg)
RBC (×10^6 cells/µL)	2.11 ± 0.49	2.27 ± 0.48	2.41 ± 0.46*	2.54 ± 0.56**
Hb (g/dL)	5.12 ± 0.38	5.28 ± 0.39	5.45 ± 0.31*	5.68 ± 0.18**
WBC (×10^3 cells/µL)	14.92 ± 0.36	15.19 ± 0.26	15.28 ± 0.31*	15.39 ± 0.23**



Total red blood cells (RBCs), hemoglobin (Hb) and white blood cells (WBCs) of L. rohita fed tulsi-supplemented diets were compared to control fish at the interval of 20 days from starting day of the experiment.

Highest number of RBC count was observed in those fish fed with O. sanctum diet at T3 tank fed over the control. Significant decrease ($p < 0.05$) was observed in control group. Similarly, haemoglobin content was higher in T3 followed by T2 and T1. The hemoglobin levels were in T3 showed the highest Hb concentration (5.68 g/dL), followed by T2 (5.45 g/dL) and T1 (5.28 g/dL), compared to the control (5.12 g/dL). Higher hemoglobin levels suggest enhanced oxygen-carrying capacity and overall better physiological status of the fish. Total leucocyte count in T3 Tulsi fed fishes was significantly ($p < 0.05$) higher than the control and other treatment groups. WBC counts increased significantly in Tulsi fed fish, indicating a stronger immune response. T3 had the highest WBC count (15.39 × 10^3 cells/µL), followed by T2 (15.28 × 10^3 cells/µL), T1 (15.19 × 10^3 cells/µL), and the control (14.92 × 10^3 cells/µL). The higher WBC counts in the Tulsi groups suggest that Tulsi enhances the fish's immune defense against infections.

DISCUSSION

Several studies have shown that adding herbs to the diets of aquatic animals greatly improves the growth and general development of fish. When compared to traditional diets, researchers have found that certain herbal supplements can improve weight gain and feed conversion ratios in a variety of fish species by maximizing nutrient absorption, bolstering immunological function, and promoting metabolic efficiency **(Kaleeswaran B et.,al 2011, Prasad G, Mukhthiraj S 2011, Sanchez JAO et.,al 2009, Kour D et.,al 2004).** When *Labeo rohita* were fed *Utrica dioica* which is known as stinging nettle, **(Ngugi et.,al 2015)** saw an increase in growth rate and SGR in comparison to the control. Weight gain and SGR values were shown to have increased in the current investigation, which was consistent with a study conducted by **(Abdel-Tawwab et.,al 2018)** in which *Clarias gariepinus* were provided a nutritional supplement of Clove basil leaf extract for a period of twelve weeks. The current investigation shown that adding powdered *Ocimum sanctum* leaves to the meal improved the growth performance of *Labeo rohita*, which is consistent with a study conducted by **(Shalaby et.,al 2006)** on *Nile tilapia* fed a diet supplemented with garlic extract for 84 days. This investigation demonstrated that adding Tulsi leaf powder to the diet improved *Labeo rohita* growth performance and FCR value, which was found to be connected with the research of **(Jagadeesh et.,al 2014)** in *Etroplus maculatus* when fed *Marigold oleoresin* with supplemented feed for 45 days. According to **(Mamta et.,al 2017)**, the antioxidant qualities of Tulsi leaves, which function as a growth stimulant, are the reason why fish showed improved growth performance. Additionally, because the fish grew properly and gained greater body weight, they discovered that Tulsi leaf extract decreased oxidative stress in the fish's body.

Hematology is an important biomarker for determining the nutritional value of fish diets. It provides essential information about fish health and diet efficiency. The current study was similar with **(Sahu *et.,al* 2007)** that in *Labeo rohita* fingerlings fed *Magnifera indica* the RBC counts were recorded higher when compared to control. The usage of dietary supplements containing herbal immune stimulants increased the quantity of WBCs in common carp fish, according to **(Abasali H, Mohamad S. 2010)**. This implies that fish with plant-based additions may have stronger immune responses and be more disease-resistant. Numerous research have demonstrated how herbal supplements can alter hematological parameters, promoting the general health and development of fish. Furthermore, bioactive substances that promote blood cell development and improve metabolic processes may be present in plant extracts. On several treatment days, the hemoglobin content of *Cyprinus carpio* fed a meal made with plant extract of *Andrographis paniculata* gradually increased **(Muthu R et.,al 2015)**. Phagocyte cells are

known to be stimulated by a number of medicinal plants, including *Zingiber officinale, Curcuma longa, and Allium sativum* **(Alambra JR et.,al 2012)**. These herbs are useful for boosting fish immunity since they have immunomodulatory and antibacterial qualities. According to (**Pavaraj et.,al 2011)** leaf extract from *Ocimum sanctum* increases *C. carpio*'s phagocytic activity.

CONCLUSION

Herbs have long been utilized in India to treat and prevent illnesses as well as to promote health. Tulsi (*Ocimum sanctum*) supplementation in fish feed, particularly at 600 mg, significantly improves hematological parameters in *Labeo rohita* fingerlings, leading to better health and immune function. The results suggest that Tulsi can be a beneficial natural additive in fish diets to enhance overall fish welfare and performance in aquaculture. Further studies are recommended to explore the long-term effects and optimal dosages for other fish species.

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