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Research Article

Use of *Ocimum sanctum* L. (Tulsi) as dietary supplement and its effect on growth and biochemical parameters in *Labeo rohita* (Hamilton, 1822)

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Abstract

The present study investigates the effect of *Ocimum sanctum* (Tulsi) as dietary supplement on growth and biochemical response in *Labeo rohita* fingerlings. Fingerlings of about 18±1 (g) were fed with a diet containing 0% (control), 1% (T1), and 3% (T2) of Tulsi diet. The sampling of the experiment was done on 18th, 36th, 54th, 72th and 90th days for growth, biochemical parameters and water quality parameters analysis. The lowest FCR value and significantly higher SGR, FCE, and percentage (%) weight gain were found in T2 group. The alterations in biochemical parameters at the end of 90th day trial indicated that *Ocimum sanctum* (Tulsi) administered through feed significantly ($p<0.05$) enhanced the serum total protein, globulin and albumin content. The physico-chemical parameters like pH, temperature, dissolved oxygen, total alkalinity, ammonia–nitrogen and phosphate-phosphorus were analyzed throughout the experimental period. The result of the present study indicated that all parameters are optimum for fish culture. These results reveal that Tulsi as a dietary supplement diet enhances growth, feed utilization and influences the innate immunity of *Labeo rohita* fingerlings.

Keywords: Biochemical, Growth, Immunity, Rohu, Tulsi

Highlights

- A significant difference ($p<0.05$) was shown during the experiment in the growth parameters of *Labeo rohita.*
- Biochemical parameters at the end of the experiment significantly ($p<0.05$) enhanced the serum total protein, globulin and albumin content.
- The Physico-chemical parameters like pH, temperature, dissolved oxygen, total alkalinity, ammonia-nitrogen and phosphate-phosphorus showed no significant difference ($p>0.05$) throughout the experiment.

INTRODUCTION

Over 14 million people in India receive assistance for their livelihood and meaningful work from the fisheries and aquaculture industry, which is a significant part of the country's food production. It also contributes to exports of agriculture. Since independence, the nation's fish output has increased steadily and sustainably attributed to its various resources, which include deep oceans, lakes in the mountain regions, and more than 10% of the world's fish and shellfish species diversity. According to the Handbook of Fisheries Statistics (2022), the overall fish output in 2021–22 will be projected to be 16.25 MMT, with approximately 75 percent of that production coming from the inland sector and an annual growth rate of 7.76%, making up roughly 8.0% of the world's total fish production.

Indian major carps (IMCs), rohu (*Labeo rohita*), catla (*Labeo catla*) and mrigal (*Cirrhinus mrigala*) are the prime carp species cultivated in inland freshwater aquaculture systems in India and other Asian countries constituting about 87% of the total production from the freshwater aquaculture of India. Among the three Indian major carp species, rohu is the most important and preferred by farmers mainly due to its higher growth rate, market demands and consumer preference (Nair and Salin, 2007). *Labeo rohita* belongs to the sub-family cyprininae. *Labeo rohita* is widely distributed in India. It is a highly preferred food fish. *L. rohita* may be identified by its subterminal mouth with fringed wide lips and its downward tapered body (Jhingran, 1991). It can be domesticated in tanks and is suitable for composite fish culture with other carp species as well as single species culture. Tanks, lakes, and other types of still water basins are ideal habitats for it. It is an "illiophage," which eats the column mostly as an herbivore. Diatoms, green algae, organic debris, and zooplankton make up the majority of its diet. It can reach a maximum length of around 100 cm but grows to around

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20 cm and 800 g in the first year. *Labeo rohita* is being cultivated along with catla (*Labeo catla*) and mrigal (*Cirrhinus mrigala*) in India.

The constraints for fish production in aquaculture systems are quality seed, feed, and diseases of infectious and non-infectious origin. It is widely demonstrated that farmed fish are more susceptible to disease agents due to overcrowding, transport, handling, poor soil and water quality, etc. (Ali*et al.*, 2003; Jobling, 2010).

Water parameters like pH, oxygen, carbon dioxide etc. play an important role in maintenance of the homeostasis in aquatic animals and decreases in pH are reported to cause disturbances in acid-base and ion regulation and ammonia excretion (Jensen and Brahm, 1995). Stress in fish affects its physiology as well as growth due to alterations in exchange of gases, N-excretion, acid-base equilibrium, and ionic imbalance caused by a variation in water pH (Pickering, 1981; Jeney*et al.*, 1992). The carp culture industry is currently the most important sub-sector of fisheries in India, and its rapid development has attracted considerable attention in recent years. In situations of high nutrient input, supplementary feed, manures and inorganic fertilizers are added to get higher production per unit area; however, after ponds are put into culture, the activities of plants, bacteria, and animals cause the pH to cycle diurnally. During the night, carbon dioxide accumulates and pH declines (Boyd and Tucker, 1998), and a change in water pH, either to higher or lower levels, could cause stress in fish and affect its body physiology and growth (Das *et al.*, 2006).

Ocimum sanctum is a 75 cm tall, broadly branched, upright, sturdy, and scented plant. This little herb is grown close to Hindu homes and temples and may be found all across India. This plant's seeds, roots, and leaves have all been utilised in Ayurvedic treatment. Tulsi has a very intricate chemical makeup that includes a variety of nutrients and other physiologically active substances. Tulsi standardisation has so far defied contemporary research because of the intrinsic botanical and biochemical nature of the herb. The antistress, antioxidants, hepatoprotective, immunomodulated, anti-inflammatory, antimicrobial, antifungal, antiviral, antidiabetic, antimalarial, and hypolipidemic effects of *Ocimum sanctum* have been the subject of several scientific research with a high degree of safety (Ranjana and Tripathi, 2015). To the best of our understanding, the effect of *Ocimum sanctum* (Tulsi) as a dietary supplement to *Labeo rohita* has not been studied. Therefore, the present study aimed to evaluate the effect of *Ocimum sanctum* (Tulsi) as a dietary supplement on growth and biochemical parameters in *Labeo rohita* fingerlings.

MATERIALS AND METHODS

Experimental setup: The experiment was carried out in 9 rectangular fiberglass reinforced plastic (FRP) tanks of 500 liters each. The tanks were cleaned and sun-dried for 5 days. They were filled with tap water up to 200 liters, which was maintained throughout the experimental period. The exchange of water was done after every week.

Stocking and rearing of experimental fish: The healthy fingerlings of *Labeo rohita* were procured from Sonarpur Fish Hatchery, Kolkata, India, and carefully transported to the experimental unit of the Faculty of Fishery Sciences in the prescribed condition. Before reaching the wet laboratory, they were treated with a short bath treatment with 2% potassium permanganate ($KMnO_4$) solution for 3 to 4 minutes as prophylactic measures. Then, they were kept in a 500-liter rectangular FRP tank and fed with the formulated diet for 12 days before the experiment. Feeding was stopped 24 hours before the experiment, and the fishes were kept in starvation. The average weight 18±1 g and length 14±1 cm of the fish were measured and distributed in tanks according to the experimental protocol. Stocking density of experimental fish was @10 per tank, and every treatment group consisted of triplicate with continued adequate aeration.

Collection of plant material: Fresh two-kilogram leaves of *Ocimum sanctum* were collected from the local market Chakgaria, Kolkata. The leaves were washed, shade-dried and pulverized by a grinder, passed through a mesh sieve and stored in a sealed container.

Feed preparation: The diet was prepared following the method of Talpur and Ikhwanuddin (2013) with slight modifications. Commercial ingredients such as fish meal, wheat meal, soybean meal, fish oil, vitamins and minerals (pre-mixture) and cornstarch were procured from the local market at Gangajoar, South 24 Pargana, West Bengal. The composition of the feed ingredients and the composition of formulated diets are given in Tables 1

Table 1. Composition of feed ingredients

Sl. No.	Ingredients	Percent incorporated
1	Fish meal	50
2	Wheat meal	25
3	Soybean meal	15
4	Mix. oil	5
5	Vitamin and minerals	2
6	Cornstarch	3

Table 2. Composition of experimental diet

Sl. No.	Ingredients	Control diet(C)	Treatment diet (T1) (1%)	Treatment diet (T2) (3%)
1	Fish meal	50	49	47
2	Wheat meal	25	25	25
3	Soybean meal	15	15	15
4	mix. oil	5	5	5
5	Vitamin and minerals	2	2	2
6	Cornstarch	3	3	3
7	Tulsi (T1/T2)	0	1	3

and 2, respectively. Two types of feed were prepared, which were as follows: Tulsi T1 (1%), T2 (3%) and control feed (without Tulsi powder). All the ingredients were weighed properly as per the requirement and kept in a big plastic container. The required ingredients were then mixed to form the dough by adding the necessary quantity of water. When the dough was formed, the calculated concentration of the oil was added and mixed well. The dough was then transferred to an aluminum container, which was then placed in a pressure cooker for cooking. The cooking was done for half an hour. The pressure cooker was then removed from the flame and kept aside for cooling. The steamed dough was taken out and cooled further. When the steamed dough was completely cooled, a vitamin and mineral mixture was added to prevent their loss. After incorporation, the dough was mixed properly and pressed through a hand pelletizer to get uniform-sized pellets. These pellets were spread on a sheet of paper and initially fan-dried. After that, the feed was transferred to trays and kept in sunlight for complete drying. After drying, pellets were packed in polythene bags, sealed airtight and labelled according to the treatments.

Experimental design: Ninety advanced healthy fingerlings of *Labeo rohita* were randomly distributed into three distinct groups. The experimental trial was conducted for 90 days. Fish were sampled on the 18th, 36th, 54th, 72nd and 90th days for analysis of blood, serum and growth. Blood and serum samples were taken for analysis of biochemical parameters. In this present experiment, the animal ethics guidelines of West Bengal University of Animal and Fishery Sciences, Kolkata, India were strictly followed.

Feeding was done @ 3% of the body weight (Nguyen, 2013). The daily ration was divided into two equal parts and was fed at 09.00 h in the morning and 17.00 h in the evening.

Growth indices: Sampling was done randomly at an interval of 18 days to assess the body weight of the fish. The weight was taken in an electric balance, and the length was measured by a scale. Growth performance was evaluated using the following formulae.

Weight gain = Final weight – Initial weight

Length gain = Final length – Initial length

Percentage weight gain:

$$\text{Weight gain (\%)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \text{ X } 100$$

Feed conversion ratio (FCR):

$$\text{FCR} = \frac{\text{Feed given (Dry weight)}}{\text{Body weight gain (Wet weight)}}$$

Feed conversion efficiency (FCE):

$$\text{FCE} = \frac{\text{Net weight gain (Wet weight)}}{\text{Feed given (Dry weight)}} \text{ X } 100$$

Specific growth rate (SGR):

$$\text{SGR (\%)} = \frac{\text{Ln final weight} - \text{Ln initial weight}}{\text{Number of days}} \text{ X } 100$$

The calculated value indicates the average increase (%) of body weight per day.

Water physio-chemical parameters: Water quality parameters, including temperature, pH, dissolved oxygen, free carbon dioxide and alkalinity, were recorded during the experimental period and determined according to the methods of APHA (2005).

Serum biochemical assay: Blood glucose was estimated by the method of Somogyi (1945) as described by Oser *et al.* (1965) by Commercial assay kits, namely Liquixx Glucose kit (BLT120235), Liquixx total protein kit (BLT00054), Liquixx total albumin kit (BLT0001), respectively were procured from Erba® Mannheim.

Blood glucose level was evaluated in mg dL^{-1}. Serum globulin was calculated using the following formulae. Serum globulin (g dL^{-1}) = Serum total protein (g dL^{-1}) - Serum albumin (g dL^{-1})

Statistical analysis: Data obtained were analyzed using the statistical package SPSS 22.0 computer program (IBM statistic 22.0). Differences in the mean value of the parameters of the test concentrations and controls were subjected to one-way analysis of variance (ANOVA), followed by Duncan's multiple range test to determine the significance level at a 5% probability level. Results were expressed as mean ± standard deviation.

RESULTS

Physico-chemical parameters of water: The physico-chemical parameters of water during the experimental period such as temperature (°C), pH, dissolved oxygen (ppm), free carbon dioxide (ppm), alkalinity, ammonia (mgL^{-1}), phosphate (ppm), nitrate (ppm) are presented in Table 3. All the physico-chemical parameters of water were observed to be within the optimum range of requirements for the fish.

Growth parameters: The results of length increment in all the treatment groups showed no significant difference ($p>0.05$). However, changes occurred significantly ($p<0.05$) in treatment T2 compared to the control group (C). The highest value of percentage of length increment was 24.062±0.7889 (%), which was found in T2 treatment group. There were significant ($p<0.05$) differences in all treatment groups Table 4.

The result of body weight gain (g) and percentage of body weight gain of the experimental fishes are represented in Table 5. There were significant ($p<0.05$) differences in the body weight gain among the various treatment groups C, T1, and T2. However, no significant difference ($p>0.05$) was observed in the treatment T2. The inclusion of the *Ocimum sanctum* extract diet showed no significant ($p>0.05$) effect on the percentage body weight gain of *Labeo rohita* in the T1 and T2. The inclusion of *Ocimum sanctum* leaf extract in the diet showed no significant ($p>0.05$) effect on the SGR of *Labeo rohita*, presented in Table (5). The most perfect value of FCR was 2.528±0.046, found in the T2 treatment group. There was a significant difference ($p<0.05$) in FCR values observed between treatment groups (Table 5). The most perfect value of FCE was 0.396±0.007, which was found in T2 treatment group. In all treatment groups showing a significant difference ($p<0.05$) in the value of FCE (Table 5).

Table 3. Physio–chemical parameters of water during the experiment period

Parameters	Experimental groups		
	C	T_1	T_2
Temperature (°C)	25.5±2.0172	26.2±2.017	26.1±2.041
pH	8.03±0.205	7.9±0.295	7.733±0.287
Dissolved oxygen (ppm)	5.6±0.295	5.93±0.205	5.8±0.245
Free CO_2(ppm)	4.53±0.236	4.033±0.125	4.33±0.287
Total alkalinity (ppm)	179.56±1.759	179.03±1.212	179.8±1.606
Ammonia –N (ppm)	0.051±0.0013	0.05±0.0016	0.048±0.0016
Phosphate –P (ppm)	0.038±0.0016	0.033±0.0016	0.036±0.002

Table 4. Growth study of *Labeo rohita* during the experiment period for different groups (Data expressed as Mean ± SD)

Treatments	Growth study (1st day to 90th days)					
	Initial length	Final length	Length increment	% length increment	Initial weight	Final weight
C	14.45±0.0408	17.072±0.0906	2.622±0.149[a]	18.149±1.085[a]	18.802±0.035	33.166±0.0266
T1	14.408±0.221	17.359±0.2439	2.951±0.174[a]	20.489±1.329[ab]	17.725±0.102	35.953±0.3896
T2	14.297±0.0386	17.737±0.097	3.440±0.112[b]	24.062±.7889[b]	18.222±0.125	37.689±0.268

Identical alphabetical superscripts along the column indicate no significant difference ($p>0.05$)

Table 5. Growth study of *Labeo rohita* during the experiment period for different groups (Data expressed as Mean ± SD)

Treatments	Growth study (1st day to 90th days)				
	Wt. gain	% Wt. gain	SGR	FCR	FCE
C	14.363±0.082[a]	76.389±0.575[a]	0.631±0.004[a]	3.535±0.027[a]	0.283±0.003[a]
T1	18.228±0.314[b]	102.835±1.439[b]	0.786±0.008[b]	2.626±0.037[b]	0.381±0.005[b]
T2	19.467±0.268[c]	106.836±1.904[b]	0.808±0.011[b]	2.528±0.046[b]	0.396±0.007[b]

Identical alphabetical superscripts along the column indicate a significant difference ($p<0.05$)

Biochemical assay: The total protein in the serum can be divided into two groups, albumin and globulin. They are the major proteins which play an important role in the immune response of fish (Kumar *et al*., 2007). A rise in the total protein, albumin and globulin levels is thought to be associated with a stronger non-specific immune response in fish (Wiegertjes *et al*., 1996). The effect of Tulsi on the blood glucose level of *L. rohita* is represented in Fig. 1. The findings of this present study indicated that the blood glucose content decreased in different treatments as compared with the control. There was a significant difference ($p<0.05$) in serum glucose level among the respective sampling days (18th, 36th, 54th, 72th and 90th) in each of the experimental groups.

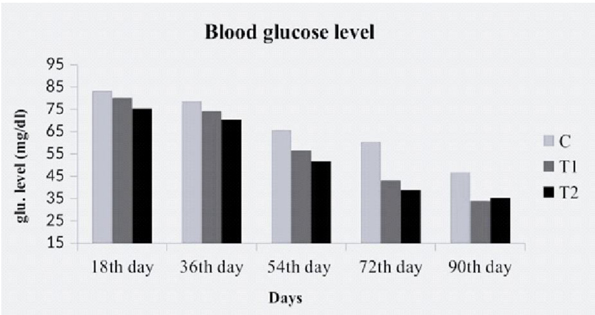

Fig. 1. Blood glucose (mg/dL) level of *Labeo rohita* during the experimental period of different groups

The serum total protein in *L. rohita* advanced fingerlings of different experimental groups is presented in Fig. 2. The findings of this present study indicated that the serum total protein content increased in different treatments as compared with the control group. There were significant differences ($P<0.05$) in total serum protein content among the various treatment groups (C, T1 and T2) on each sampling day. There was a significant difference ($p<0.05$) in serum total protein among the respective sampling days (18th, 36th, 54th, 72nd and 90th) in each treatment group (C, T1 and T2). The albumin content in different fingerlings of *L. rohita* of different experimental groups is presented in Fig. 3. The findings of this present study indicated that albumin content increased in different treatments as compared with the control group. In each day sampling there were significant differences ($p<0.05$) in the content of albumin for the various treatment groups (C, T1 and T2). In the treatment of the treatment group (C, T1 and T2), there was no significant difference ($p>0.05$) in albumin levels within the respective sampling days (18th, 36th, 54th, 72nd and 90th). In *L. rohita*, the effects of Tulsi on its globulin are presented in Fig. 4. The findings of this present study indicated that globulin content increased in different treatments as compared with the control group. In each of the treatment group (C, T1 and T2) within the respective sampling days (18th, 36th, 54th, 72nd and 90th). There was no significant difference ($p>0.05$) in globulin content during the study.

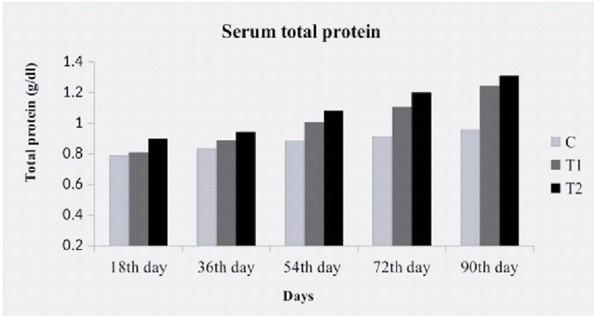

Fig. 2. Serum total protein (g/dL) level of *Labeo rohita* during the experimental period of different groups

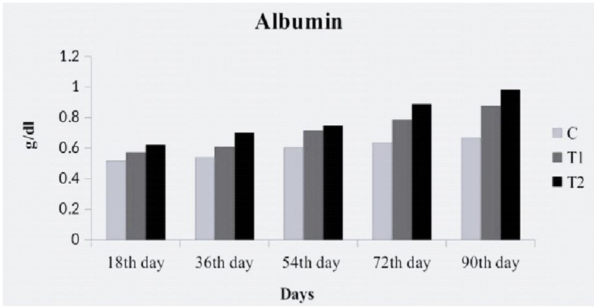

Fig. 3. Albumin (g/dL) level of *Labeo rohita* during the experimental period of different groups

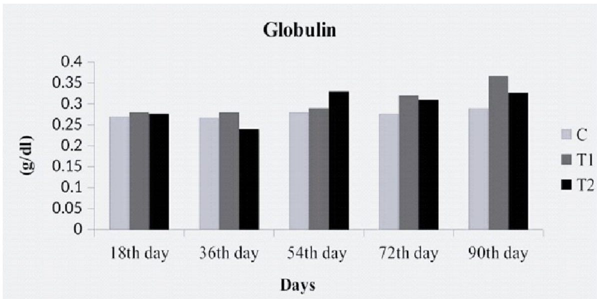

Fig. 4. Globulin (g/dL) level of *Labeo rohita* during the experimental period of different groups

DISCUSSION

The findings of the present study are similar to the study of Rao *et al.* (2006); they used *Achyranthes aspera* extract in the growth study *of Labeo rohita*. Equivalent results have also been detected in *Oreochromis mossambicus* by administering *Ocimum basilicum* extract in feed for growth as reported by Karpagam and Krishnaveni (2014). Ngugi *et al*. (2015) found a rise in growth rate and SGR compared to control in *Labeo rohita* fed with stinging nettle (*Utrica dioica*). In the current study, the value of the weight gain and SGR was found to be increased, which was correlated with the study of Abdel-Tawwab *et al.* (2018) in *Clarias gariepinus* fed with a dietary supplement of Clove basil leaf extract for 12 weeks. The present study showed that inclusion of a powder form of *Ocimum sanctum* leaf in the diet has a positive effect on the growth performance of *L. rohita* which is in agreement with the study of Shalaby *et al.* (2006) in Nile tilapia when fed with garlic extract with supplemented diet for 12 weeks.

Mamta *et al.* (2017) justified that an increasing growth performance was observed in fish due to the antioxidant properties of Tulsi leaf, which acts as a growth promoter. They also found that Tulsi leaf extract reduced the oxidative stress in the fish body as a result of its proper growth, and more body weight gain was shown. Kelm *et al.* (2000) reported that *O. sanctum* contains several compounds like carnosol, ursolic acid, rosmarinic acid, apigenin, cirsimaritin, and all of them were found to have potent redox/antioxidant properties as well as anti-inflammatory activity.

The most important source of energy in the animal body is said to be glucose. It is generally considered a stress indicator by physical factors (Manush *et al.*, 2005). The anti stress factor present in *Ocimum sanctum* may show a significant decrease in serum glucose level (Citarasu *et al.*, 2006). The levels of serum glucose found to be low during the respective days, which conforms to the findings of Sahu *et al.* (2007) in *Labeo rohita* fed with *Magnifera indica* supplemented, *Lates calcarifer* fed with neem supplemented diet, Talpur and Ikhwanuddin (2013) in Asian sea bass. Similar to the present study, align found in *Labeo rohita* fingerlings (Sahu *et al.*, 2007) and black tiger shrimp, *Penaeus monodon* (Citarasu *et al.*, 2006) that glucose level was lower after feeding with herbal Immuno-stimulant diets.

In fishes, the serum protein level increase is said to be allied with a stronger innate response (Magnadóttir, 2006). The T2 group was found to have the highest protein concentration being supplemented with marigold than in the other groups. This is consistent with the findings of Sahu *et al.* (2007), Basha *et al.* (2013) and Kumar *et al.* (2013) in *L. rohita*. Similarly, a significant increase in total protein was recorded in *C. carpio* fed diets containing 0.5% and 1% Chinese herbal medicine (Yuan *et al.*, 2007). Since serum proteins include various humoral elements of the non-specific immune system, measurable total protein, globulin and albumin levels suggest that high concentrations are likely to be a result of the enhancement of the non-specific immune response of fishes(Citarasu *et al.*, 2006).

The albumin content was observed to be increased in all treatment groups in the study. Serum albumin values were always higher in fish treated with different immuno-stimulants than in the control (Choudhury *et al.*, 2005). In the current study, serum albumin values in the fishes treated with Tulsi were significantly higher than in the control group, which was supported by Kaleeswaran *et al.* (2012), who observed that serum protein, albumin and globulin were significantly higher in the fish fed with *C. dactylon* ethanol extract *in C. catla*. Increases in the serum protein, albumin and globulin levels are thought to be associated with a stronger innate response in fishes (Citarasu *et al.*, 2006). Similarly, Yin *et al.* (2006) identified an immunostimulant effect of Astragalus root and Scutellaria herbal extracts in *O. niloticus*. Manju and Nair (2004) have reported an increase in serum albumin content in *A. testudineus* fed with aqueous leaf, stem and root extracts of *A. marmelos*.

In this study, the T2 group supplemented with Tulsi has the highest globulin concentration than the control group. This is consistent with the findings of Sahu *et al.* (2007) and Kumar *et al.* (2013) in *L. rohita*. In globulin, the largest portion is made of gamma fraction. Das *et al.* (2015) justified that immune response is enhanced due to the large portion of globulin made up of gamma fraction. Rakus *et al.* (2003) reported that elevations in globulin levels are believed to be related to a stronger innate immune response of fish and are vital fractions for sustaining the strongest immune system (Jha *et al.*, 2007).

In India, herbs have long been used for the promotion of health, prevention and treatment of diseases. *Ocimum sanctum* L., which belongs to the Lamiaceae family, is commonly known as 'Tulsi', 'holy basil', 'Queen of plants' and 'The mother medicine of nature'. The above names are given to Tulsi plant due to its enormous medicinal properties like anti-inflammatory and antipyretic activity, antimicrobial activity, antitubercular activity, immunomodulatory activity, endocrinological effects, hypoglycaemic activity, antistress activity, antiasthmatic effect. However, from the foregoing results of the preliminary study, it can be concluded that, among all the concentrations, Tulsi 3% (T2) was found to give more fruitful results for all the evaluating parameters. So, incorporating Tulsi extract @ 3% in the feed may influence the innate immunity and growth performance of fish.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: BC: Conceptualization, investigation, methodology, formal analysis, original draft preparation, writing- reviewing and editing; SAD: Methodology, validation, writing, reviewing and editing; SK: Data curation, writing, reviewing and editing; SKR: Supervision, methodology, validation and editing; VCK-Reviewing, editing and statistical analyses; EAG: Conceptualization, methodology, software; GSP: Conceptualization, methodology and software.

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