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Expert Opinion Article

Determination of Optimal Dietary Tryptophan Requirements for the Fingerlings of *Labeo rohita*, *Cirrhinus mrigala*, and *Catla catla* to Study Growth and Hematological Parameters under Stress.

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ABSTRACT
Dietary tryptophan, an essential amino acid, enhances stress resilience, growth performance, and hematological health in fish under temperature fluctuations, negatively impacting their growth and physiological functions. This twelve-week study investigated the effects of dietary tryptophan (TRP) supplementation on growth performance and hematological parameters in three major carp species: *Labeo rohita, Cirrhinus mrigala*, and *Catla catla*. Fish were divided into a control group (T_0, 0% TRP) and three experimental groups fed varying TRP levels: T_1 (0.39%), T_2 (1.43%), and T_3 (1.12%). Fish were acclimated to two temperature regimes (16°C and 32°C), with 96 fingerlings per species in 12 glass aquaria (n=8 per aquarium). Results showed that TRP supplementation significantly enhanced hematological profiles in all species. At high TRP levels, *L. rohita* exhibited the highest RBC counts (1.027×10^6/µL) and hemoglobin levels (8.37g/dL), while *C. mrigala* had RBC counts of 1.173×10^6/µL and Hb of 8.50g/dL, and *C. catla* had the highest RBC counts (1.227×10^6/µL) and Hb (10.20g/dL). Growth performance improved markedly with TRP, particularly at 1.43% supplementation and 32°C: *L. rohita* displayed a superior feed conversion ratio while *C. mrigala* and *C. catla* showed the maximal growth responses under these conditions. Dietary tryptophan supplementation demonstrated a significant positive correlation with enhanced growth performance and restoration of hematological parameters in freshwater carp exposed to thermal stress. Compared to the control group, TRP supplementation led to statistically significant improvements ($P < 0.05$) in weight gain, specific growth rate (SGR), and feed conversion ratio (FCR). The 1.43% inclusion level proved most effective, offering optimal mitigation of temperature stress while maximizing growth enhancement.

Keywords: Tryptophan (TRP), Hematological Parameters, Growth performance, Temperature Stress

INTRODUCTION

The global aquaculture production of cultivated aquatic animals experienced an average annual growth rate of 5.3% from 2001 to 2018. Nonetheless, in 2017, this growth rate dropped to 4 % and further decreased to 3.2% in 2018. This is according to the FAO (2020), which states that the share of world aquaculture in global fish production has risen continuously and reached 46.0% during the three years from 2016 to 2018 as opposed to just 25.7% in the year the 2000. The progress of fish rearing will depend on more specialized feeds that give exactly necessary nutrients for supporting fish life processes and their growth. However, unlike feed formulation only, this process undergoes innovations like aquaculture 4.0 that can boost industry dynamics significantly. When it comes to proteins in fish feeds, they provide essential amino acids for growth and protein synthesis. Also important are characteristics such as quality and composition of proteins as well as levels of essential amino acids, which directly affect nutrient utilization, fish growth, and immune response (Miao *et al.,* 2021).

Elevated temperatures are known to disrupt homeostasis, causing significant alterations in physiological and hematological parameters, which are vital for maintaining the health and growth of fish. Hematological changes include reductions in red blood cell (RBC) counts, hemoglobin levels, and hematocrit, impairing oxygen transport and energy metabolism (Shahjahan *et al.,* 2020). Simultaneously, elevated temperatures increase cortisol, glucose, and lactate levels, while reducing blood pH and bicarbonate concentrations, indicating stress-induced metabolic acidosis (Stewart *et al.,* 2019). Immune responses are also compromised under thermal stress, making fish more susceptible to diseases and infections. Reduced oxygen availability at higher temperatures further exacerbates physiological stress, leading to growth suppression and increased mortality risks. For example, sudden temperature drops in goldfish cause elevated cortisol, glucose, and lactic acid levels, persisting for up to 72 hours (Hur & Habibi, 2007). Similarly, rainbow trout exposed to thermal fluctuations show significant changes in hematological indices, including mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, highlighting the impact of temperature stress on oxygen transport efficiency (Docan *et al.,* 2011).

Fish metabolic rates and physiological processes are dependent on temperature, which in turn is related to their well-being. The quest for methods of reducing temperature stress in freshwater aquaculture continues, and this involves trials with different additives. It should be kept in mind that temperature changes can greatly influence the development and general health condition of aquatic organisms (Subasinghe *et al.,* 2009).

In fish, protein synthesis can be impeded if one crucial amino acid is deficient, causing decreased utilization of other amino acids. The type of fish components and species involved determine this coefficient for developing environmentally friendly and nutritionally balanced diets. It is important to make challenging appealing and easily digested purified diets to know what are the minimums of the amino acids' requirements that have been accurately determined. For instance, tryptophan is of much concern, and it tends to be limiting in plant-derived fish feed ingredients such as wheat grain (*Triticum aestivum*), mung bean *(Vigna radiata)*, hydrolyzed feather meal, meat bone meal (MBM), soybean *(Glycine max)* meal (SBM), corn gluten *(Zea mays)* meal (CGM), corn distillers dried grains with solubles (DDGS), and fish silage. Several studies have demonstrated this (Coloso *et al.,* 2004; Fatma Abidi & Khan, 2010; Ahmed, 2012; Pewitt *et al.,* 2017; Hoseini *et al.,* 2019; and Miao *et al.,* 2021).

Tryptophan is vital for protein synthesis and has a key role as a precursor for a few essential compounds such as serotonin, the hormone melatonin, and others that kynurenic acid, quinolinic acid, and make niacin. These factors have managed clinical activities of behavior and body stress management. And controlling antioxidant and immune responses in fish just to mention a few (Machado *et al.,* 2019; Miao *et al.,* 2021). Whenever the fish consume a lot of food, it leads to the elevation of the level of tryptophan in the blood system. That means the brain more easily fetches tryptophan from the blood (Johnston *et al.,* 1990; Hoseini *et al.,* 2019). The inclusion of tryptophan in fish diets has been recognized as one of the methods for the improvement of fish growth and development (Ciji *et al.,* 2015; Zaminhan *et al.,* 2017).

Increasing tryptophan in fish diets could better improve fish digestive efficiency and nutritional absorption, and consequently lead to better growth and health outcomes, especially during diagnoses of alternative environmental conditions (e.g. temperature swings) (Hakim *et al.,* 2006; Tang *et al.,* 2013; Miao *et al.,* 2021).

Many fish species, such as European seabass (*Sparus aurata*), rainbow trout (*Oncorhynchus mykiss*), channel catfish (*Ictalurus punctatus*), and sockeye salmon (*Oncorhynchus nerka*), depend on tryptophan levels. The necessary tryptophan levels for different animals have been shown in several investigations. For example, studies have been conducted on milkfish (*Chanos chanos*) (National Research Council, 2011), hybrid striped bass (*Morone chrysops* x *M. saxatilis*) (Gaylord *et al.,* 2005), mrigal (*Cirrhinus mrigala*) (Ahmed & Khan, 2005), Indian major carp (*Labeo rohita*) (Fatma Abidi & Khan, 2010), stinging catfish (*Heteropneustes fossilis*) (Ahmed, 2012; Farhat & Khan, 2014), jian carp (*Cyprinus carpio*) (Tang, *et al.,* 2013), silver catfish (*Rhamdia quelen*) (Pianesso *et al.,* 2015), Catla (*Catla catla*) (Zehra & Khan, 2015), red drum (*Sciaenops ocellatus*) (Pewitt *et al.,* 2017), Asian seabass (Lates calcarifer) (Kumar *et al.,* 2017), Nile tilapia (*Oreochromis niloticus*) (Zaminhan *et al.,* 2018; Nguyena *et al.,* 2019), hybrid catfish (*Pelteobagrus vachelli*♀ x *Leiocassis longirostris*) (Zhao *et al.,* 2019), and snakehead (*Channa argus*) (Miao *et al.,* 2021). These studies collectively underscore the importance of tryptophan in fish nutrition and its critical role in promoting growth and health across different fish species.

The main carp, *C. catla, L. rohita* and *C. mrigala*, are Pakistan's most important and predominant freshwater fish species. And being industrially in great demand they are a popular choice for aquaculture in this area as the environment in Pakistan is favorable for culture (Hayat, 2009). Big carp (Maulu *et al.,* 2021) plays a significant role in aquaculture output, food security and economic growth. Their rapid development, environmental tolerance, and capacity to use various feed resources make them extremely valuable. Aquaculture growers like all three because of their strong market acceptability (Tachibana *et al.,* 2020).

However, there is not enough information available on the dietary tryptophan requirements of freshwater carp species. So, the main objective of this study was to find out the optimal amount of tryptophan required and also to investigate the potential of varying levels of tryptophan as feed additive by fingerlings of *L. rohita, C. mrigala,* and *C. catla* by measuring their growth parameters and hematological indices, and to reduce temperature stress.

EXPERIMENTAL PROCEDURES

Study Site and Fingerling Collection: The research trial was conducted at the Toxicology Laboratory at the Fisheries Research Farm, which is part of the Department of Zoology, Wildlife and Fisheries at the University of Agriculture in Faisalabad, Pakistan. For the study, fish fingerlings were acquired from the Satiana Fish Seed Hatchery, located near Faisalabad.

Acclimatization: Before the start of the experiment, fish species were acclimatized in glass tanks measuring 60×30×45 cm for 14 days. During this period, the water temperature was that of room temperature (approximately 25 °C), and the oxygen concentration was maintained above a DO level of 80% saturation with a continuous aeration system. One-tenth of the water was replaced daily. Throughout the experimental period, the fish were fed once daily (9:00 am) to satiation using commercially available floating fish feed. After preacclimation, 120 fish from each species were randomly selected and assigned to two acclimation temperature treatment groups (16 °C and 32 °C). Next, the water temperature of the rearing system was either decreased or increased by 1 °C per day to reach 16 °C or 32 °C. In each aquarium, the temperature of the water was fixed using Automatic (Warm Tone; WT-300) aquarium heaters. The rearing conditions were identical to those of the preacclimation period except for water temperature.

Experimental Setup and Diet: A total of one hundred and ten fish were randomly distributed and reared. Four diet groups were prepared with different levels (0%, 0.39%, 1.43%, and 1.12%) of TRP (Bio Basic Inc.). Earlier studies (Akhtar *et al.,* 2013 and Tejpal *et al.,* 2014) have shown that TRP supplementation increases growth, feed efficiency, and overall fish health, the concentrations of which have been chosen in the present study. Additionally, a TRP of 1.44% reduced stress measures and improved immuno-hematological parameters in *Labeo rohita* with high temperature stress, according to Kumar *et al.* (2018). Our study's preliminary trials validated the selected concentrations by demonstrating a favorable association between dietary TRP levels and critical hematological and growth variables at 16°C and 32°C, two distinct temperature ranges. These temperatures were chosen to represent a number of conditions commonly seen in areas in which these carp species are reared. Both the higher temperature (32°C) matches increased temperature linked to climate change and warming of aquaculture habitats, and the lower temperature (16°C) reflects cooler circumstances that may happen throughout climate improve. In each aquarium, the temperature of the water was fixed using Automatic (Warm Tone; WT-300) aquarium heaters. The fish were fed a diet containing 5% of their body weight. Daily maintenance included siphoning the aquaria to remove waste and uneaten food, followed by a partial water change of 50% with fresh water to maintain water quality.

Diet preparation and Feeding program: Four diets with varying quantities of dietary tryptophan were prepared for the experiment using fresh fish feed components, using Pearson's Square Method (Wagner and Stanton, 2012). The dietary items were homogeneously blended with fish oil using a food mixer to ensure a uniform mixture. Subsequently, the prepared pellets were desiccated under ambient conditions and stored at -20◦ C in hermetically sealed polythene bags before their use in the experiment (Table 1). The proximate content was analyzed by using the A.O.A.C. (2005) standard technique.

Table 1. Feed ingredients and preparation of purified test diets with varying L-Trp levels (% dry matter)

	T_0	T_1	T_2	T_3
Ingredients	**Inclusion level %**			
Fish Meal	48	36	36	22
Soybean Meal	-	12	12	22
Wheat Flour	13	13	12	13
Wheat Bran	18	16	16	16
Rice Bran	15	13.5	12.5	13.5
Soy oil	-	4	4	4
Fish oil	4	-	-	-
Vit. and Min.	2	2	2	2
L-tryptophan	-	0.39	1.43	1.12
Proximate Composition%				
Moisture[a]	91.8	91.8	91.8	91.8
Crude Protein[b]	51.7	51.7	51.7	51.7
Ether extract[c]	10.5	10.5	10.5	10.5
Total Ash[d]	15.4	15.4	15.4	15.4
Crude fiberr[e]	1.3	6.5	13.5	0.2
Energy (Kcal/Kg)[f]	20.0	18.7	14.5	18.1

Please note that each kilogram of vitamin and mineral mixture includes the following vitamins and minerals: vitamin A, vitamin E, vitamin B1, vitamin B2, folic acid, choline chloride, potassium iodide, ferrous sulfate, 100 milligrams of potassium, and 600 milligrams of vitamin D3. The carbohydrate content was determined by subtracting the following from 100: (protein + fat + ash + moisture). The Bomb Calorimeter, manufactured by Parr Instrument Company in Moline, USA, was used to measure energy. items (a–d) Carried out three times.

Growth Measurement: Growth was calculated by the procedures of (Chowdhury *et al.,* 2020; Panase and Mengumphan 2015). Growth parameters were recorded by using the given formulas.

FCR% = Total weight that fish gain × 100
Final weight – Initial weight
SGR % = Ln (Final Wet Body Wt.) – Ln (Initial Wet Body Wt.) × 100
Duration in days
Weight gain (g) = Final Wt. – Initial Wt. × 100
Initial Wt.
Length Gain = Final Length (mm) - Initial Length (mm)
Condition Factor (K) = W x 10^5 / L^3

Where: - W represents the wet fish's body weight in grams (g),
- L represents the total length of the fish in centimeters (cm).

Hematological indices:

Blood Sampling: After each phase, blood was collected to be analyzed for hematological examination. The fish was anesthetized using clove oil at a concentration of 5mg/L. A 23-gauge, heparin-coated needle with a 2.5 mL syringe was used to puncture the caudal vein after anesthesia to collect blood samples under sterile circumstances.

Hematology: The hematological parameters under investigation included:

Red Blood Cells and White Blood Cells: Using a hemocytometer, red blood cell as well as white blood cell counts were calculated (Stevens, 1997).

Hemoglobin: The amount of hemoglobin was determined using the cyanohemoglobin method. Lee *et al.,* 1998).

Hematocrit: The microhematocrit technique was used to determine the hematocrit content. In 1971, Goldenfarb *et al.* developed this method.

Red Blood Cell Indices. Calculation: The study by Lee *et al.* (1998) determined the following formulae were used to compute them:

MCH =Hb*10/RBC
MCHC = Hb × 10/Hct (32-36%)
MCV = Hct×10/RBC (84-96fL)

Statistical Analysis: The data were analyzed using three-way ANOVA followed by Tukey's multiple comparison tests. The p-value was set at 0.05. The results are presented as means ± SD to show the variability and central tendency of the data.

RESULTS

In aquaculture, optimizing fish diets with essential nutrients like tryptophan enhances growth performance and improves the overall health and quality of cultured fish, contributing to better nutrition for consumers.

Hematological Findings: The analysis of blood characteristics showed that fish that were fed diets enriched with tryptophan were more resilient to stress. These fish had increased levels of hemoglobin and red blood cells, which suggests enhanced oxygen transportation ability. Furthermore, they also showed a balanced white blood cell count, indicating a stronger immune response that helps maintain the overall health of the fish.

The research presented the impact of dietary L-tryptophan levels and temperature on hematological parameters in three fish species: *L. rohita, C. mrigala,* and *C. catla*. Notably, *C. catla* demonstrated the highest RBC count under treatment T_2 at 32°C (1.227±0.022 10^6/μL), indicating that *C. catla* had the most optimal hematological response to this specific combination of TRP and temperature. *C. mrigala* and *L. rohita* also showed increased RBC under the same treatment but to a lesser extent. WBC counts varied significantly across species and treatments. All three species showed increased WBC under higher TRP levels especially, under T_2. *C. catla* showed the highest WBC count under T_2 at 32°C (0.663±0.009 10^3/μL) which indicates a strong immune response (Fig. 2). Hb was highest in *C. catla* under T_2 at 32°C (10.20±0.15 g/dL) which indicates that this species has better oxygen-carrying capacity. *C. mrigala* and *L. rohita* also showed increased Hb under T_2 but *C. catla* had the highest values (Fig. 3). MCV of *C. catla* was highest under T_2 at 32°C (115.57±0.64 fl) which means this species produces larger RBC under these conditions. *C. mrigala* and *L. rohita* also had increased MCV under similar treatments, *C. mrigala* had the second highest MCV (Fig. 4).

MCHC was highest in *C. catla* under T_2 at 16°C (42.26±0.58 g/dL) which means higher concentration of hemoglobin per unit volume of RBC (Fig. 1). *C. mrigala* followed this trend, *L. rohita* had a similar concentration. MCH was highest in *C. catla* under T_2 at 16°C (66.54±0.68 pg) which means higher hemoglobin per cell. *C. mrigala* and *L. rohita* showed similar patterns under treatment T_2, but with slightly lower MCH values compared to *C. catla*. Overall, these findings suggest that optimizing dietary L-tryptophan levels, especially under elevated temperatures, can greatly improve the hematological health of these fish species in aquaculture.

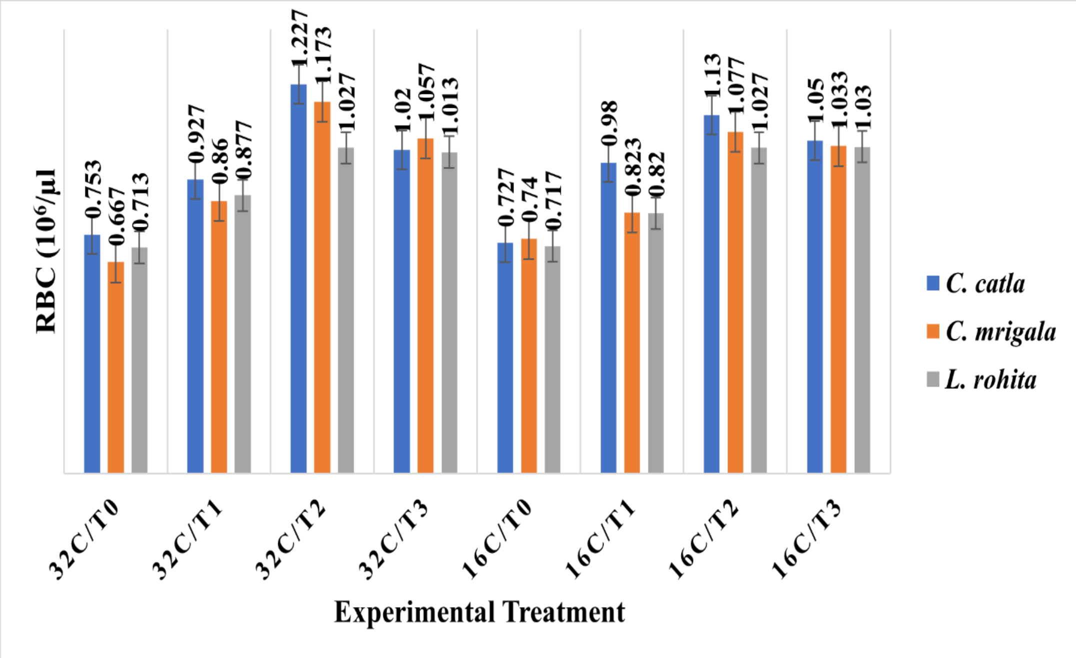

Figure 1. Impact of Dietary Tryptophan Supplementation on Red Blood Cell Levels in Fish Species (*Catla catla, Cirrhinus mrigala, Labeo rohita*) Reared at 16°C and 32°C

Figure 1. Red Blood Cell (RBC) count (10^6/µl) of *C. catla, C. mrigala,* and *L. rohita* under different tryptophan treatments at 32°C and 16°C. All species reached their peak RBC values when given the L-trp supplements, which resulted in elevated RBC counts in T_2. The RBC count of *C. catla* achieved the highest level at 32°C when it reached 1.227 10^6/µl. RBC counts were lower among fish maintained at 16°C when compared to those maintained at 32°C due to temperature effects. The combination of TRP supplements led to the highest increase in RBC numbers, where T_2 proved optimal for this enhancement.

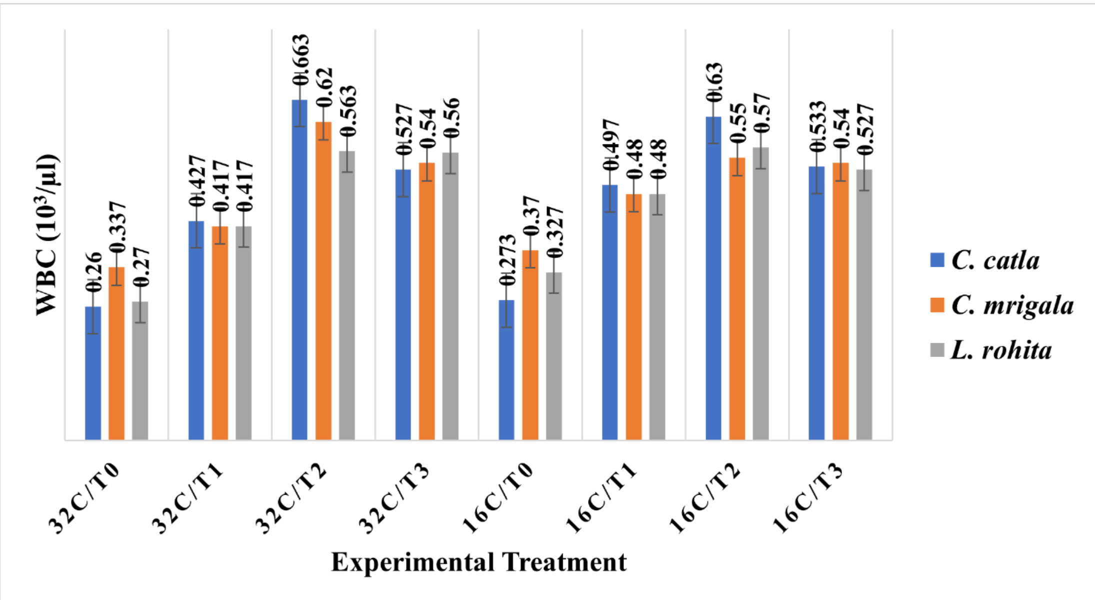

Figure 2. Impact of Dietary Tryptophan Supplementation on White Blood Cell Levels in Fish Species (*Catla catla, Cirrhinus mrigala, Labeo rohita*) Reared at 16°C and 32°C

Figure 2. White Blood Cell (WBC) count (10^3/µl) of *C. catla, C. mrigala,* and *L. rohita* under different tryptophan treatments at 32°C and 16°C. The addition of tryptophan led to elevated WBC numbers, and T_2 yielded the highest figures for each aquatic species. The largest improvement in blood cell levels occurred in *C. mrigala* when observed at 32°C, resulting in 0.663 10^3/µl. The WBC counts of fish decreased when the fish temperature at 16°C compared to 32°C. The WBC levels of fish in the T_2 group demonstrated the highest positive change after receiving tryptophan supplementation.

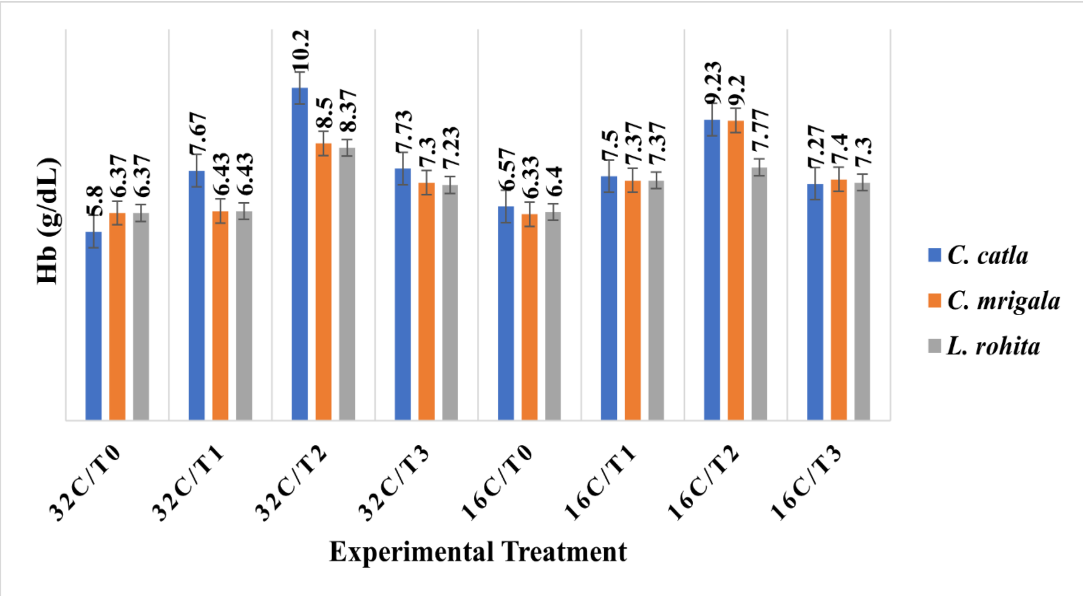

Figure 3. Effect of Dietary Tryptophan Supplementation on Hemoglobin (Hb) Levels in Fish Species (*Catla catla, Cirrhinus mrigala, Labeo rohita*) Reared at 16°C and 32°C

Figure 3. Hemoglobin (Hb) measurements at g/dL density determined the quantity in *C. catla, C. mrigala,* and *L. rohita* fish treated with differing tryptophan levels at 32°C and 16°C. The fish groups received the best Hb level results in treatment T_2 across all species. The Hb concentration of *C. catla* increased the most, resulting in 10.2 g/dL at 32°C. The Hb levels in fish reached their highest point at 16°C compared to 32°C. This indicates that temperature affects protein levels. Research showed that T_2 produced the maximum beneficial impact on Hb levels in fish blood.

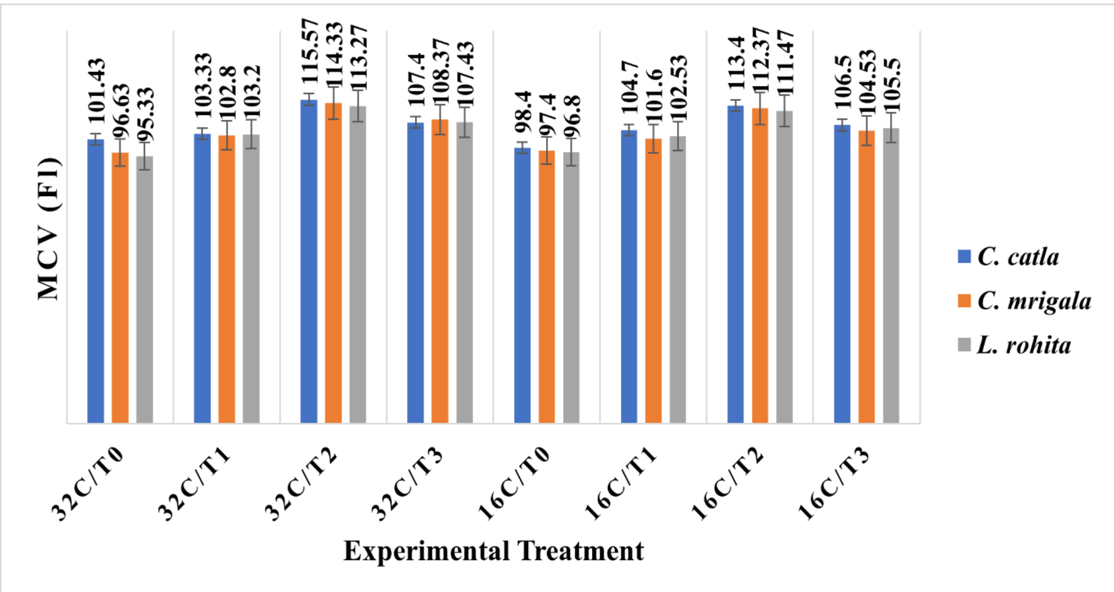

Figure 4. Effect of Dietary Tryptophan Supplementation on Hematocrit Volume (HCV) Levels in Fish Species (*Catla catla, Cirrhinus mrigala, Labeo rohita*) Reared at 16°C and 32°C

Figure 4. Mean Corpuscular Volume (MCV) (fL) of *C. catla, C. mrigala*, and *L. rohita* under different tryptophan treatments at 32°C and 16°C. An increase in MCV levels occurred from dietary supplementation, and the maximum MCV readings appeared in T_2 among all studied species. The quantity of red blood cells expanded the most in *C. catla* fish, which increased to 115.57 fL under 32°C temperature conditions. The fish kept at 16°C showed enlarged MCV values compared to those at 32°C, probably because of temperature dependence. The data demonstrate that MCV receives favorable influence from tryptophan consumption, but T_2 delivers the greatest enhancements.

Growth Performance: The study revealed that adequate addition of tryptophan to the feed leads to significant growth enhancement among *L. rohita, C. mrigala, and C. catla*. Where the effects of dietary tryptophan (TRP) supplementation on growth performance and feed efficiency were compared in the broodstock and the fingerlings of *C. catla*, *C. mrigala*, and *L. rohita*, the responses were varied. Specifically, this was true between the two temperatures (32°C and 16°C). Tryptophan (TRP) at the highest concentration, T_2 (1.43%), was the most consistent and best supplemental TRP and resulted in better growth performance, especially when supplemented with dietary TRP in *L. rohita* throughout the two temperatures. At 32°C, *L. rohita* had the best feed conversion ratio (FCR) of 1.73 ± 0.02, the highest final body weight (FBW) of 83.80 ± 2.10 g, the maximum weight gain (AWG) of 12.71 ± 0.75 g, and the FCR rate of 2.71 ± 0.55 g. The specific growth rate (SGR) at T_2 was also the highest (2.284±0.01), with significant improvements in condition factor (C.F. 1.95±0.12) and length gain (TLG: 29.22±3.41 mm). At 16°C, although the overall growth was lower compared to 32°C, T_2 still provided the best growth outcomes with an FBW of 82.86±1.25 g, AWG of 12.55±0.80 g, and FCR of 1.79±0.02, confirming that TRP supplementation led to enhanced growth and feed efficiency, especially at higher TRP concentrations.

Cirrhinus mrigala also showed positive growth responses to TRP supplementation, particularly at 32°C, where T_2 (1.43% TRP) resulted in the highest FBW of 81.50±2.14 g, AWG of 12.30±0.82 g, and the best FCR of 1.96±0.041. The SGR at T_2 was also the highest (2.270±0.008), with notable improvements in length gain (TLG: 28.07±4.40 mm) and condition factor (C.F: 1.63±0.086) compared to the control. At 16°C, where growth was generally slower, T_2 still showed strong performance with FBW of 79.47±2.27 g, AWG of 12.04±0.84 g, and FCR of 1.98±0.016, indicating that TRP positively influenced growth and feed efficiency, even under cooler conditions.

Catla catla also benefited from dietary TRP, particularly at 32°C, where T_2 (1.43% TRP) led to an FBW of 77.50±3.64 g, AWG of 11.66±0.87 g, and the best FCR of 1.82±0.041. The SGR was also highest at T_2 (2.240±0.015), and both the condition factor (C.F: 1.57±0.072) and length gain (TLG: 24.90±3.15 mm) were enhanced. However, at 16°C, although growth was lower compared to 32°C, T2 still showed better performance with FBW of 74.20±1.99 g,

Table 2. Effects of dietary tryptophan supplementation on growth performance and feed efficiency (mean ± SD) in *Catla catla* reared at 32°C

Parameters	T_0 (Control)	T_1	T_2	T_3
IBW (g)	7.82±0.34	7.99±0.38	7.95±0.41	7.90±0.41
FBW (g)	68.30±2.87a	71.60±2.29b	77.50±3.64b	72.50±1.83c
AWG (g)	10.13±0.86a	10.76±0.92b	11.66±0.87c	10.82±0.87b
SGR	1.23±0.019a	1.24±0.006b	2.19±0.015c	1.74±0.011b
FCR	2.04±0.075a	1.91±0.015b	1.82±0.041d	1.92±0.016c
C.F. (K)	1.31±0.014a	1.37±0.019b	1.57±0.072c	1.44±0.031d
ALG (mm)	28.19±2.04a	27.69±2.52b	45.21±3.15c	40.39±2.71b
FLG (mm)	21.87±2.23a	23.30±2.35b	24.88±3.02c	24.04±2.77c

The table exhibits how different dietary tryptophan concentrations affect initial body weight (IBW), final body weight (FBW) and average weight gain (AWG), with specific growth rate (SGR) and feed conversion ratio (FCR), condition factor (C.F.) and absolute length gain (ALG), and fork length gain (FLG) in *Catla catla*. T_0 served as the control against the three treatment groups T_1, T_2, and T_3. The statistical analysis utilizes P-values to determine the importance of group variations ($P < 0.05$ and $P < 0.01$).

Table 3. Effects of dietary tryptophan supplementation on growth performance and feed efficiency (mean ± SD) in *Catla catla* reared at 16°C

Parameters	T_0 (Control)	T_1	T_2	T_3
IBW (g)	7.47±0.35	7.68±0.44	7.58±0.41	7.68±0.39
FBW (g)	65.94±2.29a	67.60±2.27a	74.20±1.99c	70.50±1.53b
AWG (g)	9.80±0.93a	10.05±0.87a	11.15±0.87c	10.56±0.85b
SGR	1.12±0.011a	1.18±0.010a	1.98±0.008c	1.67±0.007b
FCR	2.14±0.076c	1.99±0.014b	1.86±0.065a	2.06±0.013bc
C.F. (K)	1.26±0.018a	1.32±0.021b	1.50±0.075d	1.39±0.030c
ALG (mm)	26.42±1.99b	25.91±2.51a	43.43±3.03d	39.12±2.91c
FLG (mm)	20.93±2.02a	21.82±1.96a	23.72±3.06c	23.08±2.73b

The table demonstrates the growth performance measurements, which show how *Catla catla* respondents at 16°C reacted to increasing dietary tryptophan concentrations through assessing IBW, FBW, AWG, SGR, FCR, C.F, ALG, and FLG. The T_0 control group received standard assessment, while T_1, T_2, and T_3 groups underwent different dietary procedures testing tryptophan.

Table 4. Effects of dietary tryptophan supplementation on growth performance and feed utilization (means ± SD) in *Cirrhinus mrigala* reared at 32°C

Parameters	T_0 (Control)	T_1	T_2	T_3
IBW (g)	7.82±0.34	8.00±0.39	7.96±0.45	7.90±0.41
FBW (g)	71.40±2.11a	74.20±2.94b	81.50±2.14d	75.50±3.93c
AWG (g)	10.65±0.77a	11.09±0.84b	12.30±0.82d	11.32±0.84c
SGR	1.33±0.08a	1.29±0.012a	2.32±0.008c	1.81±0.016b
FCR	1.97±0.03b	2.06±0.04c	1.96±0.04b	1.84±0.01a
C.F (K)	1.43±0.02a	1.51±0.045b	1.63±0.086d	1.56±0.062c
ALG (mm)	28.96±2.56a	28.70±3.69a	44.45±4.40b	48.26±3.78c
FLG (mm)	24.37±2.37a	26.46±3.43b	27.88±4.09c	26.89±3.58bc

This research utilizes a table to present data about how dietary tryptophan additions influence the growth characteristics and dietary consumption patterns of *Cirrhinus mrigala* when kept at 32°C. The analyzed parameters encompass initial body weight (IBW) and final body weight (FBW), average weight gain (AWG), specific growth rate (SGR), feed conversion ratio (FCR) and condition factor (C.F.), absolute length gain (ALG), and fork length gain (FLG). Research analysts used the control T_0 group to examine findings against T_1, T_2, and T_3 experimental groups.

Table 5. Effects of dietary tryptophan supplementation on growth performance and feed utilization (means ± SD) in *Cirrhinus mrigala* reared at 16°C

Parameters	T_0 (Control)	T_1	T_2	T_3
IBW (g)	7.52±0.36	7.60±0.37	7.61±0.43	7.57±0.43
FBW (g)	67.34±3.13a	71.86±0.97b	79.47±2.2d	71.09±1.82c
AWG (g)	10.01±0.81a	10.79±0.78b	12.04±0.84d	10.67±0.82c
SGR	1.23±0.014b	1.21±0.004a	2.15±0.008d	1.76±0.008c
FCR	2.25±0.094d	2.14±0.045c	1.90±0.016a	1.98±0.012b
C.F. (K)	1.36±0.026a	1.44±0.044b	1.57±0.087d	1.50±0.061c
ALG (mm)	28.43±2.30b	27.69±3.84a	47.75±3.80d	44.70±3.29c
FLG (mm)	23.14±2.06a	25.02±3.79b	26.73±4.22c	27.12±3.73d

The table demonstrates the effects of different tryptophan levels in fish diets on *Cirrhinus mrigala* grown at 16°C regarding growth outcomes, together with feed conversion efficiency measurements. The assessment includes initial body weight (IBW) and final body weight (FBW) in addition to average weight gain (AWG) and specific growth rate (SGR). The analysis also measured feed conversion ratio (FCR), condition factor (C.F.), absolute length gain (ALG), and fork length gain (FLG). The study used T_0 as the control group to examine its variations with T_1, T_2, and T_3 treatment groups. The P-value set limits at $P < 0.05$ and $P < 0.01$ determine statistical significance.

Table 6. Effects of dietary tryptophan supplementation on the growth performance and feed efficiency (means ± SD) in *Labeo rohita* reared at 32°C

Parameters	T_0 (Control)	T_1	T_2	T_3
IBW (g)	7.83±0.38	8.00±0.37	7.96±0.42	7.90±0.44
FBW (g)	74.3±3.29a	78.40±1.90b	83.80±2.10d	79.40±2.63c
AWG (g)	11.12±0.69a	11.82±0.70b	12.71±0.75c	11.95±0.76b
SGR	1.49±0.01b	1.39±0.01a	2.42±0.01d	1.93±0.01c
FCR	2.09±0.09c	1.77±0.03b	1.60±0.02a	1.73±0.02ab
C.F. (K)	1.67±0.07a	1.79±0.08b	1.95±0.12d	1.86±0.09c
ALG (mm)	33.21±2.43a	38.32±2.96b	57.15±3.41d	53.79±3.10c
FLG (mm)	25.87±2.41a	27.24±2.86b	28.64±3.48c	28.06±3.05b

The data in this table shows how dietary tryptophan supplementation affects the growth performance, together with the feed efficiency of *Labeo rohita* at 32°C water temperature. The research parameters included initial body weight (IBW), final body weight (FBW), average weight gain (AWG), specific growth rate (SGR), feed conversion ratio (FCR), condition factor (C.F.), absolute length gain (ALG), and fork length gain (FLG). The T_0 control group received comparison from T_1 to the T_3 treatment groups. The FCR column contains different superscript letters to indicate treatments with meaningful variations ($P < 0.05$).

Table 7. Effects of dietary tryptophan supplementation on the growth performance and feed efficiency (means ± SD) in *Labeo rohita* reared at 16°C

Parameters	T_0 (Control)	T_1	T_2	T_3
IBW (g)	7.54±0.38	7.69±0.33	7.68±0.34	7.52±0.38
FBW (g)	70.29±2.35a	74.91±2.77b	82.86±1.25d	75.66±2.03c
AWG (g)	10.51±0.62a	11.26±0.67b	12.55±0.80d	11.38±0.62c
SGR	1.35±0.01a	1.29±0.011b	2.32±0.01d	1.94±0.01c
FCR	2.18±0.09c	1.88±0.026b	1.79±0.02a	1.65±0.03a
C.F. (K)	1.59±0.07a	1.71±0.082b	1.87±0.11d	1.80±0.09c
ALG (mm)	30.19±2.81a	36.94±2.76b	53.58±3.30d	50.29±2.83c
FLG (mm)	24.14±2.51a	26.74±2.64b	28.47±3.35c	26.84±3.12b

This research examines how dietary tryptophan supplementation affects the growth performance and feed efficiency of *Labeo rohita* when held at 16°C. The analyzed parameters consist of initial body weight (IBW) and final body weight (FBW) for calculating average weight gain (AWG), specific growth rate (SGR) in conjunction with feed conversion ratio (FCR) while measuring condition factor (C.F) and capturing absolute length gain (ALG) and fork length gain (FLG). The researchers compared T_0 against the three treatment groups (T_1, T_2, and T_3). The outcomes show significance based on P-values ($P < 0.05$, $P < 0.01$). FCR shows significant treatment variations ($P < 0.05$) when superscript letters appear in the particular column. AWG of 11.15±0.87 g, and FCR of 2.06±0.06, indicating that higher TRP concentrations improved growth and feed efficiency, particularly at higher temperatures.

Comparative analysis revealed significant species-specific responses to dietary TRP supplementation under thermal stress. *L. rohita* exhibited the strongest growth response (P<0.05), particularly at elevated temperatures (32°C), followed by *C. mrigala.* In contrast, *C. catla* showed minimal improvement in both growth performance and feed conversion efficiency. These findings demonstrate that tryptophan efficacy as a dietary supplement varies considerably among carp species, with *L. rohita* deriving the greatest physiological benefits.

Physicochemical Parameters:

Table 8. Effects of dietary tryptophan supplementation on water physicochemical parameters in major carp (*L. rohita, C. mrigala, C. catla*) under thermal stress conditions.

Parameters	Units	Means	Analysis Method
Dissolved Oxygen	mg/L	4.49±0.08	Oxygen meter
pH	_	7.29±0.10	pH meter
Ammonia	mg/L	0.02±0.00	Titrimetric method
Total Hardness	mg/L	225.14±2.63	Titration method
Electrical Conductivity	µSiemens/cm	3.09±0.05	Spectrophotometry
Calcium	mg/L	25.31±0.44	Spectrophotometry
Magnesium	mg/L	45.87±0.75	Spectrophotometry

DISCUSSION

A well-balanced dietary regimen containing essential amino acids is fundamental for optimal growth and protein accretion in fish. As demonstrated by (Machado *et al.,* 2019), amino acids serve as critical substrates for multiple physiological processes, including protein biosynthesis, enzymatic reactions, and neuromodulation. However, the specific amino acid requirements may vary significantly depending on the interplay of physiological factors such as species, developmental stage, and environmental conditions. Tryptophan is a precursor of serotonin, also known as 5-HT. A precursor that often enhances the fish's capacity to cope with Stress may be induced by increasing the amount of 5-HT in the brain (Morandini *et al.,* 2015).

Generally, temperature is the crucial factor that directly impacts fish's physiological processes and metabolic rates. Researchers are actively working on ways to reduce heat stress in freshwater aquaculture by investigating the supplementation of different feeds (Subasinghe *et al.,* 2009). The present experiment aimed to determine the optimum dietary tryptophan needs for fingerlings of *L. rohita, C. mrigala,* and *C. catla* at different temperatures.

The results of the present study showed that the concentration of L-tryptophan and the temperature of the diet have a profound effect on the blood composition and growth rate of these fish, and that increased TRP concentration is generally associated with RBC, WBC is associated with increased hemoglobin levels. In our study with *C. catla* in T_2, the RBC count was highest (1.227×106/µL) and lowest in T_0 (0.727×106/µL), indicating a positive effect of TRP concentration and increased gain on red cell production, and also on T_0 (5.80g/Dl) was active. Then, in T_2 (10.20g/dL) hemoglobin levels increased significantly, indicating an improved oxygen-carrying capacity of tryptophan. MCV and MCHC values also showed considerable variation in TRP levels, with T_2 showing the highest levels (115.57 fl for MCV and 41.52 g for MCHC).

Similar results were observed in *C. mrigala* and *L. rohita*, with increasing TRP increasing RBC count, hemoglobin concentration, and MCV. For example, *C. mrigala* increased RBC content at T_2 (1.173×106/µL) and hemoglobin content at T_2 (8.37g/dL). L. Rohita showed the highest RBC count and hemoglobin at T_2 (1.027×106/µL and 8.07g/dL, respectively). Significant ($p<0.05$) differences among treatments reveal that dietary TRP has a remarkable effect on these blood parameters, improving both blood health and oxygen transport in carp species.

Because of its role as a precursor of two important neurotransmitters, namely serotonin and melatonin (meaning also stress response and immunological functions control in fish), affecting growth and hematological parameters under temperature stress. TRP is involved in other physiological and metabolic processes, including how these neurotransmitters affect levels of cortisol, a major part of the stress response. Under temperature stress, increased serotonin synthesis helps stabilize cortisol levels, hematological homeostasis, and attenuates stress-induced damage.

Several trials have shown dietary TRP supplementation to mitigate the degenerative effects of environmental stresses on fish. For example, TRP administration enhanced immunological responses to temperature and salt stress, reinstated hematological parameters, and improved growth performance in *Labeo rohita* (Akhtar *et al.,* 2013). Furthermore, it was observed that when 1.44% TRP was added to supplement *L. rohita* exposed to extended temperature increasing, immunohematological parameters had improved, and major stress indicators reduced (Kumar *et al.,* 2018). Importantly, these results demonstrate the importance of TRP for fish physiological homeostasis and increased fish resilience to environmental stress, thereby making TRP a vital nutrient for improving fish welfare in aquaculture methods.

Our findings have practical application in terms of the impacts of temperature stress on fish farms, where dietary supplementation of TRP (1.43%) (T_2) may have significant benefits in improving fish resistance. Our findings indicate that fish were able to grow, to maintain feed efficiency, and maintain hematological stability even during periods of extreme heat when this TRP dosage was used. For example, at 32 °C, all the species had the highest final body weight, increase, and feed conversion ratio. Such research also has important practical ramifications for aquaculture methods, because adding 1.43% TRP to fish feed may be an economical way to mitigate the detrimental effects of temperature swings and to ensure sustainable fish production. Supplementation of TRP at this dose may reduce mortality rates and improve feed conversion and production in fish farms by maintaining improved growth performance and immunological responses under stress.

Likewise, dietary tryptophan positively affects growth performance and feed utilization in *L. rohita*. At 32°C, higher TRP levels, especially T_2 level, indicating better feed efficiency as compared to other levels, led to the greatest final body weight and average weight gain, SGR, and the most efficient FCR. Condition factor and length gain (TL, FL) were also improved at T_2. At 16°C, although growth metrics were lower compared to 32°C. *C. mrigala* also showed strong performance, particularly in specific growth rate and feed conversion efficiency. *C. catla* demonstrated notable improvements with TRP but generally had lower growth performance and feed efficiency compared to the other species. These results demonstrate that dietary TRP enhances growth and feed efficiency in Rohu, with the most significant benefits observed at higher TRP levels, and helps to minimize stress.

We show that effective dietary tryptophan elevates hemoglobin concentration and oxygen transport capacity in fish exposed to elevated temperature. Such is the case as the results of Ahmed (2012) show that carp species fed with tryptophan under comparable stress conditions exhibit good hematological markers, such as stated hemoglobin and hematocrit levels.

Dietary tryptophan supplementation has shown beneficial effects in mitigating thermal and salinity stress in fish, particularly in *L. rohita* and *Morone saxatilis*. Studies have demonstrated that tryptophan supplementation can improve growth performance, restore hemato-immunological parameters, and reduce stress responses in fish exposed to elevated temperatures and salinity (Kumar *et al.,* 2018; Cabanillas-Gamez *et al.,* 2022). (Docan *et al.,* 2010) Also, describe that increases in RBC, hematocrit, and hemoglobin in the HD group were indicators of stress and higher energy expenditure, resulting in growth suppression.

The present results suggest that after administering TRP at a T_2 level of 1.43%, the final body weights (FBW) at 32°C were 77.50 ± 3.64 g for Catla, 81.50 ± 2.14 g for Mori, and 83.80 ± 2.10 g for Rohu. At 16°C, Catla, Mori, and Rohu had somewhat lower FBW values of 74.20 ± 1.99 g, 79.47 ± 2.20 g, and 82.86 ± 1.25 g, respectively. The fish's health and hematological indicators increased dramatically ($p<0.05$) by 1.43%. Similar results were reported by (Kumar *et al.,* 2018; Akhtar *et al.,* 2013) that dietary (L-TRP) supplementation can mitigate the effects of temperature and salinity stress in fish, particularly in *Labeo rohita*. TRP supplementation has been found to improve growth performance, reduce stress markers like cortisol and glucose, and enhance immune-hematological parameters under elevated temperature conditions. Similar stress-mitigating effects of TRP have been observed in rainbow trout (*Oncorhynchus mykiss*), where elevated dietary TRP intake counteracted stress-induced plasma cortisol elevation (Lepage *et al.,* 2002). These findings suggest that TRP supplementation could be an effective strategy for improving fish welfare under stressful conditions.

In line with these findings, Yousefi *et al.* (2016) saw no substantial disparity in growth performance but did not have an elevated FCR in rainbow trout when stocked at densities of 10 or 30 kg/m3. The addition of TRP in this study enhanced the growth performance and feed efficiency of the fish. However, it was not able to prevent or reduce the negative effects on growth performance and feed efficiency caused by the high-density circumstances. There is limited data about the impact of adding TRP to the diet on the growth performance of rainbow trout when they are exposed to stressful conditions. Tejpal *et al.* (2009) discovered that adding TRP to the diet of Indian major carp, *C. mrigala*, at levels of 1.94% and 3.88% of the dietary protein, resulted in a considerable improvement in the fish's growth performance, regardless of whether they were under normal or high-density settings. The impact of dietary TRP on the development performance of fish is influenced by factors such as the species of fish, the quantity of TRP in their diet, and the specific type of stress they experience (Hoseini *et al.,* 2019).

The efficacy of TRP supplementation demonstrated in this study suggests practical applications for improving aquaculture productivity in controlled environments. Future research should prioritize two key areas: first, longitudinal assessment of TRP effects under realistic, multifactorial stress conditions (combining temperature, salinity and dissolved oxygen variations); second, systematic evaluation of TRP's interactions with emerging feed supplements like microbial probiotics and plant-derived immunostimulants (Herrera *et al.,* 2019). Addressing these knowledge gaps will enable the development of precision feeding regimens that maximize the stress-adaptation benefits of TRP while ensuring nutritional and economic efficiency.

Conclusion: In the present experiment, it was shown that the nominal supplementation of tryptophan significantly ($p < 0.05$) improved the growth and also the feeding performance of *C. catla, C. mrigala,* and *L. rohita*, particularly under increasing temperature of 32 °C. C. We observed the highest final body weights (FBW) (77.50 ± 3.64 g Catla; 81.50 ± 2.14 g Mori; 83.80 ± 2.10 g Rohu) with a 1.43% tryptophan-supplemented diet. These findings need to be confirmed further in other studies, and their long-term impacts need to be investigated. Incorporating the optimal TRP level of (1.43%) in fish feeds, however, is economically and ecologically beneficial, especially in regions prone to heating and cooling. Economically, it can improve growth, feed efficiency, and productivity, reducing feed costs and enhancing profitability. Ecologically, TRP supplementation boosts fish resilience to stress, lowering mortality rates and promoting sustainable aquaculture practices.

Credit Authorship Contribution Statement: The contributions to the experimental research were as follows: K.S. was responsible for conceptualization, writing, methodology, and data curation. S.A. oversaw supervision and investigation. S.P. conducted review and editing, while M.S. handled formal analysis.

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