EFFECT OF DIETARY SUPPLEMENT OF *EUCALYPTUS GRANDIS* LEAF POWDER ON GROWTH PERFORMANCE AND HAEMATOLOGICAL PARAMETERS OF *LABEO ROHITA*

ABSTRACT

This study evaluated the impact of dietary *Eucalyptus grandis* leaf powder supplementation on growth performance and haematological parameters of *Labeo rohita* (rohu) fingerlings. One hundred and forty-four uniform rohu were randomly distributed into four experimental groups in triplicates. Iso-nitrogenous (30% crude protein) diets were prepared with graded levels of *E. grandis* leaf powder with 0% (C), 5% (T_1), 10% (T_2) and 15% (T_3) and fed for 45 days. Water quality was monitored fortnightly. Growth performance and haematological indices were assessed. Results showed diets with 10% leaf powder significantly enhanced weight gain, feed conversion ratio, and specific growth rate compared to the control ($p<0.05$). The 10% group also exhibited elevated red blood cell counts, haemoglobin levels and white blood cell counts indicative of improved immune function. This research demonstrates that short-term dietary supplementation with 10% *E. grandis* leaf powder promotes growth performance and haematological health of rohu fingerlings. Further studies are warranted to elucidate the bioactive compounds responsible and evaluate long-term effects on production.

Keywords: *Eucalyptus grandis*, growth performance, haematological parameters, *Labeo rohita*, Iso-nitrogenous diets

1. INTRODUCTION

Aquaculture is one of the rapidly growing animal food production sectors in the world. As the demand for fish and fishery products increases globally, aquaculture plays a vital role in meeting this demand through sustainable practices. Rohu (*Labeo rohita*), an economically important freshwater fish species, is commonly cultured in India using polyculture systems [1]. However, intensive aquaculture practices can negatively impact fish health due to increased biotic and abiotic stresses.

Traditionally, aquaculture relied on the use of synthetic growth promoters and antibiotics to improve production. However, indiscriminate use of chemicals has led to the development of antibiotic resistance in pathogens and accumulation of residue in cultured products and environment [2]. Hence, there is a growing need to replace chemical additives with safer alternatives. Herbal plants are recognized worldwide for their medicinal properties and minimal side effects. *Eucalyptus grandis* is one such plant widely used in traditional medicine systems.

E. grandis is extensively grown and utilized across various global regions due to its numerous therapeutic attributes. The leaves contain phytochemicals like terpenoids, flavonoids and phenolic compounds known to exhibit antioxidant, antimicrobial and immune-modulatory effects. Previous studies have reported the beneficial effects of *E. grandis* essential oil on growth, immunity and disease resistance in various fish species [3]. In India, *Labeo rohita* is the most popular and extensively cultivated carp and it is in great demand among consumers. According to the Handbook of Fisheries Statistics [4], freshwater aquaculture accounts for over 85% of India's total aquaculture production, of which *Labeo rohita* (rohu) provides roughly 35–40%. While numerous researchers have explored the significance of *Eucalyptus grandis* in a wide range of fields, there is a notable gap in studies concerning the impact of *E. grandis* extract in the diets of *Labeo rohita*. Therefore, the present study aims to evaluate the impact of *E. grandis* leaf powder supplementation on growth performance and haematological parameters of rohu.

2. METHODOLOGY

2.1 Experimental Design

The present experiment was conducted in twelve FRP tanks of 70L capacity for 45 days. The uniform sized fingerlings of rohu were procured from fish farm, College of Fishery Science, NDVSU, Jabalpur. Prior to the start of the experiment, fish stock was acclimatized for next fifteen days under aerated conditions. During this period fish were fed with a commercial diet. One hundred and forty-four healthy fingerlings of uniform size (9.44±0.16 g) and age (70 days) were randomly distributed in four experimental groups each with three replicates following a complete randomized design.

2.2 Collection of eucalyptus plant leaves

Fresh leaves of *Eucalyptus grandis* were collected from the campus of College of Fishery Science, Jabalpur. The leaves were washed and treated with 2 ppm potassium permanganate solution to prevent fungal growth. Then, the leaves were dried under shade for 7-10 days at room temperature. The dried leaves were ground into a fine powder and sieved through a 20mm mesh to ensure uniform size. The powder was stored in air-tight plastic bags at room temperature until initiation of experiment [5].

2.3 Proximate analysis

The proximate analysis of Eucalyptus leaves was conducted following the standardized method outlined by the AOAC [6]. The proximate analysis covers the determination of various components as shown in Table 1 including moisture content, crude protein, ether extract, crude fibre, total ash and nitrogen-free extract.

Table 1. Proximate composition of *Eucalyptus grandis* leaf

Proximate Composition	***Eucalyptus grandis* Leaf**
Moisture (%)	71.29±0.19
Dry matter (%)	28.71±0.19
Crude protein (%)	13.92±0.13
Total ash (%)	7.63±0.2
Ether extract (%)	2.67±0.17
Crude fibre (%)	10.51±0.21
Nitrogen free extract (%)	65.27±0.31

2.4 Feed formulation and experimental diet

Feed formulation was done using Pearson's Square Method [7] and 30% crude protein diet was formulated using various ingredients listed in Table 2. The ingredients were pulverized, mixed thoroughly and moistened with water to form a dough. The dough was then autoclaved for 15 minutes at 121 °C and a pressure of 15 lbs/cm^2. After cooling, different amounts of eucalyptus leaf powder (ELP) were added to the basal diet at 0% (C), 5% (T_1), 10% (T_2) and 15% (T_3). The vitamin-mineral mixture dissolved in oil was incorporated into the diet and mixed well. The dough was then pressed through a hand pelletizer to obtain uniformly sized pellets with a diameter of 2 mm. The pellets were air

dried for 72 hours until the moisture content was less than 10%. Finally, the pellets were packed in airtight bags and labelled.

Table 2. Formulation of different experimental diets

S. No	Ingredient (%)	C (0%)	T_1 (1%)	T_2 (2%)	T_3 (4%)
1.	Fish meal	5	5	5	5
2.	Soyabean meal	25	25	25	25
3.	Groundnut Oil Cake	24.32	24.32	24.32	24.32
4.	Deoiled- rice bran	22.56	17.56	12.56	7.56
5.	Wheat flour	10.7	10.7	10.7	10.7
6.	Corn flour	6.22	6.22	6.22	6.22
7.	Vitamin and mineral mixture	2	2	2	2
8.	Oil (Sunflower oil+ Cod liver oil)	4.2	4.2	4.2	4.2
9.	Eucalyptus leaf powder	0	5	10	15
	Total	**100**	**100**	**100**	**100**

2.5 Growth Parameters

The weight of the fish was initially recorded. The fish were sampled every fifteen days to analyze the growth parameters. A total of four samplings over the forty-five days of the experimental period were done. The fish were fed according to their body weight. An electronic weighing balance was used to record the weight. The following parameters of growth were recorded as per the method given by Halver [8]:

- Body Weight Gain (g) = Final body weight (g) – Initial body weight (g)
- Weight Gain Percentage (%) = [(Final weight (g) – Initial weight (g) / Initial weight (g)] x 100
- Feed Conversion Ratio = Feed intake (g) / Weight gain (g)
- Specific Growth Rate (%/day) = [ln of final body weight (g) – ln of initial body weight (g)] / Experimental period (days)
- Survival Rate (%) = (total number of experimental fish harvested / Number of experimental fish stocked) x 100

2.6 Feeding trial and maintenance of experimental fish

The experimental tanks were thoroughly cleaned on alternate days through manual siphoning to remove any residual feed pellets and faecal matter. Water samples were collected fortnightly for water quality analysis. Water quality parameters including temperature, pH, dissolved oxygen, free carbon dioxide, total alkalinity, total hardness and ammonia were recorded in the present study as per standard methods of APHA [9]. The feeding rate for fingerlings was 5% of their body weight and subsequent adjustments were made based on weight gain [10]. The daily ration was divided into two equal parts and fed at 0800 and 1700 hr.

2.7 Haematological analysis

After completion of experiment, the fish were anesthetized with clove oil (50 µL of clove oil per litre of water) followed by the collection of blood samples from fish of each treatment group [11]. The collected blood was immediately transfer to Eppendorf tubes coated with EDTA and shaken well to prevent blood clotting. The blood parameters such as erythrocyte count (TEC), leucocyte count (TLC), packed cell volume (PCV), haemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin

(MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were estimated by following standard methods [12].

2.8 Statistical analysis

In the current investigation, a comparison was conducted among the average values at a significance level of 5% ($P < 0.05$) utilizing one-way analysis of variance (ANOVA). Furthermore, for post hoc comparisons, Duncan's multiple range test (DMRT) was employed, with SPSS 16.0 for windows.

3. RESULTS AND DISCUSSION

3.1 Physico-chemical parameters of water

Different physico-chemical parameters of water such as temperature (°C), pH, dissolved oxygen (mg/l), total alkalinity (mg/l), and ammonia-N (mg/l) were found to be in range of 19.54 to 19.68 °C, 7.23 to 8.64, 7.23 to 8.00 mg/l, 94.66 to 128.35 mg/l and 0.01 – 0.07 mg/l, respectively, throughout the experiment and were found to be in the optimum range [13]; [14]; [15].

3.2 Effects of *E. grandis* on growth parameters and feed utilization

The present investigation recorded significant difference in growth parameters measures (final weight, weight gain, daily weight gain, percentage weight gain, and specific growth rate) among the different treatments presented in table 3. Similar findings were reported by Goda [16] and Bahi et al. [17] who observed a significant improvement in growth when incorporating herbal plants into diets. In contrast, Motamedi-Tehrani et al. [18] did not find significant effects of plant extracts on growth. These discrepancies in the impact of plant extracts on growth performance could be attributed to several factors, including the specific plant species used, the type of fish species studied, and variations in the composition of the extract solutions (Motamedi-Tehrani et al. [19].

The potential of aquaculture feeds can be assessed by evaluating growth performance and feed conversion ratio, as mentioned by Hoseinifar et al. [20] and Rashidian et al. [21]. The highest growth performance and best feed conversion ratio was observed in the group fed a diet supplemented with 10% (T_2 group) *E. grandis*. This suggests 10% *E. grandis* in the diets of *L. rohita* could stimulate gut secretions, thereby improving protein synthesis and higher growth performance [22]. This supports the findings reported by Nurudeen et al. [5] that applying *E. globulus* leaf extract meal in tilapia up to 10% stimulated appetites and increased growth rate over 60 days. The group fed over 10% of *E. grandis* experienced reduced growth performance as well as poor feed utilization. This could be due to the high fiber content of medicinal plants (herbs), which tends to decrease feed efficiency and growth performance [23].

The use of medicinal plants has been found to enhance appetite and improve feed conversion ratio (FCR) in cultured fish, thereby promoting better growth performance [24]. In this study, the groups fed diets T_1 and T_2 displayed the most favorable FCR, which aligns with previous research indicating improved FCR in fish receiving plant extracts [25]. Conversely, the groups fed diets containing higher levels of *E. grandis* leaves (T_3) exhibited poorer FCR. A similar outcome was observed in a study on *O. niloticus* where diets supplemented with elephant's foot (*Elephanto pusscaber*) extract resulted in inferior FCR [26].

Table 3. Growth performance of rohu (*Labeo rohita*) fingerlings fed with different experimental diets for period of 45 days

Treatments	IW (g)	FW (g)	BWG(g)	WGP(%)	FCR	SGR	SR(%)
C (0%)	9.23 ± 0.02^{a}	18.56 ± 0.16^{c}	9.3 ± 0.11^{c}	100.44 ± 0.8^{a}	2.24 ± 0.02^{c}	1.55 ± 0.01^{a}	100±0

T_1 (5%)	9.25±0.02[a]	19.26±0.06[b]	10.12±0.06[b]	110.85±0.77[b]	2.03±0.01[b]	1.66±0.01[b]	100±0
T_2 (10%)	9.24±0.02[a]	20.66±0.22[d]	11.44±0.14[d]	124.01±0.95[c]	1.81±0.01[a]	1.79±0.01[c]	100±0
T_3 (15%)	9.22±0.02[a]	17.72±0.12[a]	8.76±0.15[a]	97.85±1.98[a]	2.3±0.05[c]	1.52±0.02[a]	100±0
P-value	P > 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P > 0.05

Note:- Data is presented as Mean± SE (n=3);
C, Control group with no inclusion of *E. grandis* leaf powder (ELP); T1, inclusion of 5 % ELP; T2, inclusion of 10% ELP; T3, inclusion of 15% ELP;
IW, Initial Weight; FW, Final Weight; BWG, Body Weight Gain; WGP, Weight Gain Percentage; FCR, Feed Conversion Ratio; SGR, Specific Growth Rate; SR, Survival Rate

3.3 Effects of *E. grandis* on haematological parameters

Haemato-biochemical parameters namely, total erythrocyte count (TEC), total leucocyte count (TLC), haemoglobin (Hb), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), packed cell volume (PCV) and mean corpuscular volume (MCV) were assessed at the end of experimental trial as shown in table 4. Haemato-biochemical response is an important aspect to determine evaluation of the physiological status and cellular response to stress of the fish.

The highest TEC was recorded in T_2 group (1.71±0.01) while the lowest value was observed in C group (1.33±0.01). The highest mean value of TLC was observed in T_2 group (74.99±0.01) and the lowest was observed in C group (63.91±0.02). The haemoglobin concentration in T_2 group exhibited highest value (7.77±0.12), whereas the lowest concentration was observed in C group (6.7±0.11). The maximum mean corpuscular hemoglobin (MCH) was recorded in the C group (50.38±0.75), whereas the minimum MCH was noted in the T_3 group (42.44±1.36). The highest mean corpuscular hemoglobin concentration (MCHC) was found in the T_2 group (32.42±0.46), while the lowest was observed in the T_1 group (28.55±0.44). The highest PCV was observed in T_1 group (25.57±0.03) and lowest was observed in C group (20.66±0.02). Furthermore, the highest MCV was observed in T_1 group (168.24±1.75) and lowest was observed in T_3 group (131.8±0.86).

In this investigation, there was a significant increase in white blood cells potentially caused by a noteworthy rise in lymphocyte levels. Previous findings support this study, as they also observed an elevation in white blood cells in *Clarias gariepinus* fish that were given diets containing varying levels of *Vernonia amygladina* concentrations. In contrast, Aderolu [27] found a decrease in white blood cells when young African catfish (*C. gariepinus*) were fed diets enhanced with extracts from *Gongronema latifolia* (benth). The increase in white blood cells suggests an increased ability to protect against harmful pathogens.

The concentration of Haemoglobin (Hb) exhibited an upward trend as the levels of *E. grandis* extract increased, up to T_2 group (10%). However, there was a subsequent decline in the concentration of Hb at T_3 group (15%). This observation suggests that the inclusion of higher amounts of *E. grandis* extract in the diets of *L. rohita* positively influences the oxidation process of the ingested feed and facilitates the efficient removal of carbon dioxide from the fish. It is worth noting that haemoglobin serves the crucial physiological role of transporting oxygen to the various tissues within the fish's body, thereby facilitating respiration and overall metabolic functions [28].

Table 4. Haematological parameters of *L. rohita* fingerlings fed with different experimental diets for period of 45 days

Treatments	[1]TEC	[2]TLC	[3]Hb	[4]MCH	[5]MCHC	[6]PCV	[7]MCV
C (0%)	1.33±0.01[a]	63.91±0.02[a]	6.7±0.11[a]	50.38±0.75[c]	31.15±0.55[b]	20.66±0.02[a]	155.38±1.18[c]
T_1 (5%)	1.52±0.02[b]	66.61±0.01[b]	7.3±0.11[b]	48.03±0.68[bc]	28.55±0.44[a]	25.57±0.03[d]	168.24±1.75[d]
T_2 (10%)	1.71±0.01[d]	74.99±0.01[d]	7.77±0.12[c]	45.34±1.00[ab]	32.42±0.46[b]	24.93±0.03[c]	145.55±1.38[b]

T_3 (15%)	1.63±0.01[c]	70.07±0.03[c]	6.9±0.17[ab]	42.44±1.36[a]	32.19±0.83[b]	21.44±0.02[b]	131.8±0.86[a]
P-value	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

[1]TEC= Total Erythrocyte count (10^6 cells/µl); [2]TLC=Total Leucocyte Count (10^3 cells/µl); [3]Hb= Haemoglobin (g/dl); [4]MCH= Mean Corpuscular Haemoglobin (pg); [5]MCHC= Mean Corpuscular Haemoglobin Concentration (mg/dl); [6]PCV= Packed Cell Volume (%); [7]MCV= Mean Corpuscular Volume (fl)

4. CONCLUSION

In conclusion, the present investigation substantiated the fact that the application of a 10% *E. grandis* leaf powder in the feed for a duration of 45 days exhibited a positive impact on the growth performance and overall health status of *L. rohita* fingerlings. The incorporation of this naturally occurring supplement in the diet of rohu, primarily owing to its easy accessibility, is highly recommended for the purpose of enhancing its sustainable production. Further experimentation is required under field conditions to validate the optimum dosage of 10% *E. grandis* leaf powder in the feed of *L. rohita*. This will enable the recommendation of *E. grandis* for widespread implementation within commercial aquaculture practices.

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