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Investigating *Semecarpus anacardium* leaves extract towards augmenting growth and haematological indices in *Labeo rohita* fingerlings

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ABSTRACT

Semecarpus anacardium is an indigenous ethnomedicinal plant of Jharkhand, widely acclaimed for an array of pharmacological properties within the traditional system of medicine in India. The current study is aimed to valorize the therapeutic efficacy of *S. anacardium* leaves (SA(L)) extract *via* the assessment of its effect as a dietary supplement on growth performance and haematological responses of *Labeo rohita* (rohu) fingerlings. A 60-day feeding trial was conducted, with a total of 225 rohu fingerlings (13 ± 0.18 g), randomly distributed in five treatment groups in triplicates. Each group viz., control, T_1, T_2, T_3 and T_4 was fed with their respective diets formulated with graded levels (0, 0.5, 1, 1.5 and 2% SA(L)) respectively. The highest weight gain%, specific growth rate (SGR) and feed efficiency ratio (FER) while the lowest feed conversion ratio ($p < 0.05$) was recorded in the T_1 group, supplemented with the lowest level of SA(L). Incorporation of 0.5% of SA(L) also significantly elevated the total erythrocytic count (TEC), total leukocytic count (TLC) and haemoglobin (Hb) content, compared to the control. Overall, the current study signifies the beneficial role of *S. anacardium* leaves at 0.5% inclusion level on growth and haematological indices of *L. rohita*.

Figure : 00 References : 21 Tables : 02
KEY WORDS : Dietary supplementation, Growth, Haematology, *Labeo rohita, Semecarpus anacardium* leaves.

Introduction

Aquaculture, a vital sector in the Indian economy faces challenges due to intensified farming practices that lead to emergence of infectious diseases and economic losses[4,18]. Conventional prophylactic measures often have drawbacks such as immuno suppression and environmental concerns[3,14]. Parallel to this, freshwater fish (56.4%), especially carp species dominate global aquaculture, with *Labeo rohita* being prominent in India and contributing significantly to food security[8,10]. Hence towards this, feeding trials with herbal supplemented diet can be promising in contributing to a cost-effective, healthy, and high nutritive strategy to large-scale sustainable carp production, particularly *L. rohita*[11]. So far, numerous studies have been performed in *L. rohita* fingerlings employing crude extracts, solvent fractionates of different plant parts (roots, leaves, seeds, bulbs, kernel, rhizomes *etc.*) or isolated phytochemicals from medicinal plants[9]. However, much research has focused on the well-known medicinal plants, leaving behind a gap for potential indigenous ethnomedicinal plants, used traditionally by tribal communities.

Semecarpus anacardium, commonly known as “Bhallataka,” “Bhelwa,”, “marking nut tree” or ‘Oriental cashew’, is one such important ethnomedicinal plant of the state of Jharkhand[16]. The plant is widely utilized in the Indian medicinal system and is pharmacologically defined for properties like anti-inflammatory, antimicrobial, anti-cancer, and antioxidant activity, mainly attributed to the presence of phenolic compounds and

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flavonoids such as bhilawanol, semecarpol, tetrehydrobustaflavone tetrahydromentoflavone, semecarpuflavanone, gulluflavanone, jeediflavanone *etc.*[1]. Despite its wide acclaim in different mammalian models, its impact,particularly its leaves, on fish species remains unexplored. This study thus aims to pioneer research on the effect of *S. anacardium* on growth and hematology of *L. rohita* fingerlings through dietary supplementation of its leaves.

Materials and Methods

1. Experimental design

L. rohita fingerlings weighing (13 ± 0.18 g), length (12.55 ± 0.24 cm) were procured from a commercial fish farm near Bundu, Ranchi and acclimatized to laboratory conditions for 15 days. *L. rohita* fingerlings (n=225) were then distributed among five treatment groups in triplicates, using a completely randomized block design (CRD) in a 60-day feeding trial. Five different standard experimental diets containing graded levels viz 0, 0.5, 1,1.5 and 2% of SA(L) were prepared as uniform-sized pellets of diameter 2mm. The distribution was carried out in properly aerated, separate rectangular FRP tanks (300 L capacity) with 15 fish per tank, fed twice with their respective diets at 3% of their body weight. The uneaten feed was removed by siphoning to measure the feed intake and calculate the FCR. Regular exchange of tank water was carried out on alternate days. Throughout the culture period, water quality parameters were maintained within their normal range and the fishes were reared, as per the standard guidelines.

2. Growth parameters

Fish were weighed for their initial and final weights using an electronic balance (Aczet CG 30). Growth parameters were then measured using the standard formulae:

$$\text{Weight gain \%} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial Weight}} \times 100$$

$$\text{Specific Growth Rate (SGR) (\%)} = \frac{(\text{In final weight} - \text{In initial weight})}{\text{Number of days}} \times 100$$

$$\text{Feed conversion ration (FCR)} = \frac{\text{Feed intake (g)}}{\text{Net weith gain (g)}} \quad ; \quad \text{Feed efficiency ration (FER)} = \frac{1}{\text{FCR}}$$

$$\text{Hepatosomatic index (HIS)} = \frac{\text{Weight of liver (g)}}{\text{Total weight (g)}}$$

$$\text{Survival percentage} = \frac{\text{Total no. of live fish at the end of trial}}{\text{Initial total number of live fishes}} \times 100$$

3. Haematological parameters

Blood was collected by caudal vein puncture from euthanized fishes (n=6) using 1.0 mL hypodermal syringe and 28-gauge syringe needles, priorly rinsed with 2.7% (w/v %) EDTA solution as an anti-coagulant. Total blood collected, was pooled yielding three replicates (each pool with six fish) per treatment.

Haematological parameters like haemoglobin (Hb), total erythrocyte count or RBC count and total leucocyte count or WBC count were measured using the standard method. RBC and WBC were counted in a haemocytometer (Improved Neubauer Weber Scientific Ltd) using the following formula-

$$\text{Number of cells per ml} = \frac{\text{Number of cells counted x dilution}}{\text{Area measured x depth of fluid}}$$

The values for TEC and TLC were expressed as number of cells per mm^3.

The haemoglobin level was estimated by cyanmethaemoglobin method using Drabkins fluid (Qualigens Diagnostics, Mumbai, India). The concentration of haemoglobin was measured as per the formula-

$$\text{Haemoglobin} = \left[\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}}\right] \times \frac{251}{1000} \times 60$$

TABLE-1 : Growth parameters of *L. rohita* fingerlings fed with graded levels of *S. anacardium* leaves extract

Groups	Weight gain %	SGR	FCR	FER	HSI	Survival percentage
Control	85.16 ± 2.37^{c}	1.03 ± 0.02^{c}	1.71 ± 0.014^{c}	0.47 ± 0.001^{c}	1.474 ± 0.05	97.78 ± 2.22^{a}
T_1	128.84 ± 4.19^{a}	1.38 ± 0.03^{a}	1.44± 0.012 ^{a}	0.55 ± 0.004^{a}	1.508 ± 0.03	100.00 ± 0.01^{a}
T_2	103.4 ± 2.03^{b}	1.18 ± 0.01^{b}	1.59 ± 0.017^{b}	0.50 ± 0.001^{b}	1.492 ± 0.02	97.78 ± 2.22^{a}
T_3	85.69 ± 1.03^{c}	1.03 ± 0.01^{c}	1.72 ± 0.005^{c}	0.46 ± 0.002^{d}	1.487 ± 0.01	93.33 ± 3.85^{ab}
T_4	82.05± 1.82^{c}	0.99 ± 0.01^{c}	1.73 ± 0.020^{c}	0.45± 0.003^{d}	1.481 ± 0.01	88.89 ± 2.22^{b}
P value	< 0.05	< 0.05	< 0.05	< 0.05	> 0.05	

Data are means of triplicate (n = 3). Means in a column are compared and analyzed by one-way ANOVA. Mean values with different superscripts (a, b, c) within the same column differ significantly ($p < 0.05$). Mean values without any superscript are non-significant ($p > 0.05$). SGR: Specific growth rate; FCR: Feed conversion Ratio; FER: Feed Efficiency Ratio; HSI: Hepatosomatic index

The values for Hb were expressed in unit g/dl.

The percentage of the packed cell-volume or haematocrit was determined by the haematocrit tube reader.

5. Statistical analysis

All the data were statistically analyzed using SPSS version 22.0 (SPSS Inc., Chicago IL, USA). Results were presented as mean ± standard error (SE). Means were compared by One-way ANOVA and Duncan's test at 5% probability level (p d" 0.05)

Results

After feeding for 60 days with graded levels of SA(L) extract, the weight gain (WG) % and SGR were significantly increased in the T_1 group supplemented with the lowest level of SA(L) at 0.5%. The highest percent weight gain %, SGR and FER was recorded in the T_1 group followed by the T_2 group. Lower levels of FCR were however, achieved (Table-1). The hepatosomatic index of the treatment groups remained unchanged. In addition, the highest survival percentage was also obtained in the T_1 group. Parallel to the optimized results in the T_1 group, the changes in the higher supplemented groups were marginal and comparable to the control group, differing insignificantly. Thus, it can be inferred that SA(L) reflected contrasting results in the growth indices amidst the higher and lower supplemented treatment groups.

Apart from the growth performance, the changes in hematological parameters of SA(L) supplemented treatment groups were also recorded (Table-2). It was observed that the lower supplemented groups such as T_1 and T_2 showed increased total erythrocyte count (TEC) ($p < 0.05$), which decreased drastically in the higher supplemented groups, wrt control ($p < 0.05$). Similarly, linear increase in the TLC count towards the lower doses was noticed. Haemoglobin content increased in all treatment groups, with the highest in T_1. However, the haematocrit % recorded only a slight change among the groups, relative to the control.

Discussion

Prior investigations into dietary herbal supplementation in *L. rohita* have corroborated our findings of substantial improvement in growth parameters, encompassing absolute growth, percentage weight gain and specific growth rate. A significant gain in weight has also been reported[1] in emodin (30 mg kg), administered *L. rohita*[6]. Similar results of significantly higher weight gain % and SGR have been obtained with ethanolic extract of *Pedalium murex*, guava leaves (0.5%), dried ginger (*Zingiber officinale*) (0.8%) and blue lotus flower (0.4%) in *L. rohita* juveniles[8,12,17,19]. Further, lower values of FCR (Table-2) achieved in related study is indicative of better utilization of dietary nutrients on being fed with supplemental diets enriched with

TABLE-2 Hematological parameters of *L. rohita* fingerlings fed with supplemented diets for 60 days

Groups	TEC(× 10^6 mm^{-3})	TLC(× 10^3 mm^{-3})	Hb(g dl^{-1})	Hct(%)
Control	1.42 ± 0.01 [b]	13.25 ± 0.30[d]	6.32 ± 0.02[e]	17.40 ± 0.44[c]
T_1	1.76 ± 0.02 [a]	22.13 ± 0.34[a]	7.75 ± 0.01[a]	18.94 ± 0.14[a]
T_2	1.63± 0.01 [a]	19.41 ± 0.30[b]	7.61 ± 0.01[b]	18.62 ± 0.49[ab]
T_3	1.21 ± 0.02 [c]	15.35 ± 0.21[c]	6.85± 0.02[c]	18.14 ± 0.19[ab]
T_4	1.14 ± 0.09 [c]	14.49 ± 0.55[c]	6.70 ± 0.03[d]	17.79 ± 0.25[bc]
P value	< 0.05	< 0.05	< 0.05	d" 0.05

Data are means of triplicate (n = 3) Means in a column were compared and analyzed by one-way ANOVA. Mean values with different superscripts (a, b, c) within the same column differ significantly (p < 0.05). TLC: Total leukocyte count; TEC: Total erythrocyte count; Hb: Haemoglobin; Hct: Haematocrit

S. anacardium and *P. guajava* leaves, respectively, in *L. rohita*[8]. In addition, no significant change in HSI demonstrates that SA(L) did not effectively alter the size of the liver in supplemented *L. rohita* fingerlings. improved survival rates, similar to our findings were also noted in related studies on *L. rohita*, dealing with other herbal incorporated diets containing *Pedalium murex*[12] and peppermint (*Mentha piperita*)[13], respectively. The comparatively low growth performance at higher inclusion level could be due to the sensitivity of *L. rohita* to the antinutritional factors contained in the *S. anacardium* leaves[12].

In our study, a higher red blood cell (RBC) count suggests increased metabolic performance, enhanced cellular immunity, and improved oxygen transport from RBC surfaces to tissues[5], as also reported in *L. rohita* fingerlings fed with *Mangifera indica*[15]. The concerted elevation in the haemoglobin content along with the RBC count is in line with the results of another study in the past[2]. Similarly elevated WBC count was also seen in *L. rohita* with the administration of *Hybanthus ennespermus* leaves (3g kg^{-1})[7] as well as with the ethanolic extract of *Pedalium murex*[12] and *Rauvolfia tetraphylla* (5g kg^{-1})[21]. However, in contrast to our results, no significant effect was noticed in the haemoglobin and haematocrit content on supplementation with *Hybanthus ennespermus* leaves in *L. rohita*[7]. Overall, the enhancement of blood parameters in the current study suggests potential immuno stimulating abilities of SA(L) in inducing haemopoietic stimulation in *L. rohita*[20].

Conclusion

Thus, our study recommends the incorporation of 0.5% of *S. anacardium* leaves in the diets of *L. rohita* fingerlings based on the improved growth parameters such as weight gain %, SGR and FCR and haematological parameters such as TEC, TLC count and hemoglobin content, exhibited by the extract *in vivo*. The study also suggest the beneficial effect of SA(L) at lower concentrations of 0.5% with simultaneous immuno suppressive effects at the higher concentrations.

References

1. Abdulla S, Pavithra AE, Santhy KS. Effects of Semecarpus anacardium L.f. ethanol extract on serum biochemical parameters and antioxidant activity of DAL induced mice. *Plant Sci Today*. 2022; **9**(3) : pp. 658-663.

2. Das R, Raman RP, Saha H, Singh R. Effect of *Ocimum sanctum* Linn. (Tulsi) extract on the immunity and survival of *Labeo rohita* (Hamilton) infected with *Aeromonas hydrophila. Aquac Res.* 2015; **46**(5) : 1111-1121.

3. Dawood MAO, Koshio S. Vitamin C supplementation to optimize growth, health and stress resistance in aquatic animals. *Rev Aquac*. 2018; **10**(2) : 334-350.

4. Erkinharju T, Dalmo RA, Hansen M, Seternes T. Cleaner fish in aquaculture: review on diseases and vaccination. *Rev Aquac*. 2021; **13**(1) : 189-237.

5. Esmaeili M. Blood performance: a new formula for fish growth and health. *Biology (Basel)*. 2021; **10**(12): 1-17.

6. Giri SS, Jai Suda S, Sukumaran V, Park SC. Dietary emodin affects the growth performance, immune responses, and disease resistance of *Labeo rohita* against *Aeromonas hydrophila. Aquac Int*. 2016; **24**(1): 85-99.

7. Giri SS, Jun JW, Sukumaran V, Park SC. Evaluation of dietary *Hybanthus enneaspermus* (Linn F. Muell.) as a growth and haemato-immunological modulator in *Labeo rohita. Fish Shellfish Immunol*. 2017; **68**: 310-317.

8. Giri SS, Sen SS, Chi C, Kim JHJ, Yun S, Park SC, Sukumaran V. Effect of guava leaves on the growth performance and cytokine gene expression of *Labeo rohita* and its susceptibility to *Aeromonas hydrophila* infection. *Fish Shellfish Immunol*. 2015; **46**(2): 217-224.

9. Gupta A, Gupta SK, Priyam M, Siddik MAB, Kumar N, Mishra PK, Gupta KK, Sarkar B, Sharma TR, Pattnayak A. Immunomodulation by dietary supplements/ : A preventive health strategy for sustainable aquaculture of tropical. *Rev Aquac*. 2021; (May): 1-31.

10. Mishra A, Shah BR, Roy K, Abdelsalam EEE, Pia¡ckov´ V, Shaik HA, Dvo¡r´ak P, Velí¡sek J, Agbeko KFK, Mr´az J. Andrographolide loaded Pickering emulsion/ : A bioactive component for improved growth, digestibility, and haematological properties in cultured common carp *Cyprinus carpio.* 2023; **562** (August 2022): 1-9.

11. Mondal H, Chandrasekaran N, Mukherjee A, Thomas J. Viral Infections in Cultured Fish and Shrimps: Current Status and Treatment Methods. *Aquaculture International*. 2022; **30**: 227–262.

12. Ojha ML, Chadha NK, Saini VP, Damroy S, Gupta CP, Savant PB. Effect of Ethanolic Extract of *Pedalium murex* on Growth and Haemato-Immunological Parameters of *Labeo rohita. Proc Natl Acad Sci India Sect B - Biol Sci*. 2014; **84**(4): 997-1003.

13. Padala D, Marakini GN, Kokkam Valappil A, Prabhakaran PL, Muhammad Abdullah Al M, Kavalagiriyanahalli Srinivasiah R. Effect of dietary peppermint *(Mentha piperita)* on growth, survival, disease resistance and haematology on fingerlings of rohu *(Labeo rohita). Aquac Res*. 2021; **52**(6): 2697-2705.

14. Rico A, Phu TM, Satapornvanit K, Min J, Shahabuddin AM, Henriksson PJG, Murray FJ, Little DC, Dalsgaard A, Brink PJV. Use of veterinary medicines, feed additives and probiotics in four major internationally traded aquaculture species farmed in Asia. *Aquaculture*. 2013; 412-413: 231-243.

15. Sahu S, Das BK, Pradhan J, Mohapatra BC, Mishra BK, Sarangi N. Effect of *Magnifera indica* kernel as a feed additive on immunity and resistance to *Aeromonas hydrophila* in *Labeo rohita* fingerlings. *Fish Shellfish Immunol*. 2007; **23**(1): 109-118.

16. Semalty M, Semalty A, Badola A, Joshi G, Rawat MSM. *Semecarpus anacardium* Linn.: A review. *Pharmacogn Rev*. 2010; **4**(7): 88-94.

17. Singha S, Kumar S, Kumar S, Kumar P, Banik A, Prakash P. Blue lotus *(Nymphaea nouchali)* flower and leaf supplements enhance growth and hemato-immunological parameters in Indian major carp *(Labeo rohita)* juveniles. *Anim Feed Sci Technol*. 2023; **304**(May): 115741.

18. Stachnik M, Matras M, Borzym E, Maj-Paluch J, Reichert M. Emerging viral pathogens in sturgeon aquaculture in Poland: Focus on herpesviruses and mimivirus detection. *Viruses*. 2021; **13**(8): 1496-1506.

19. Sukumaran V, Park SC, Giri SS. Role of dietary ginger *(Zingiber officinale)* in improving growth performances and immune functions of *Labeo rohita* fingerlings. *Fish Shellfish Immunol*. 2016; **57**: 362-370.

20. Sula E, Aliko V, Barceló D, Faggio C. Combined effects of moderate hypoxia, pesticides and PCBs upon crucian carp fish, *Carassius carassius,* from a freshwater lake- in situ ecophysiological approach. *Aquat Toxicol*. 2020; **228**: 105644.

21. Yogeshwari G, Jagruthi C, Anbazahan SM, Mari LSS, Selvanathan J, Avockiaraj J, Dhayanithi NB, Ajith Kumar TT, Balasundaram C, Ramasamy H. Herbal supplementation diet on immune response in *Labeo rohita* against *Aphanomyces invadans. Aquaculture*. 2015; **437**: 351-359.