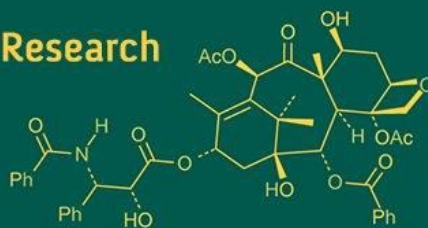


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Evaluating Eichhornia-based feeds: Nutritional and economic implications for *Labeo rohita* growth and health

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Abstract

The potential of Eichhornia-based leaf protein concentrate (ELPC) as an alternative protein source to groundnut oil cake (GNOC) in the formulated diet of rohu (*Labeo rohita*) was assessed. Five iso-nitrogenous diets (30% CP) were formulated, replacing GNOC with ELPC at different proportions in triplicate: Control (0% ELPC), T₁ (25% ELPC), T₂ (50% ELPC), T₃ (75% ELPC), and T₄ (100% ELPC). Feeding trials on rohu were conducted for a period of 60 days, maintaining stocking densities at 1g/L in a completely randomized design. Fish were fed at 10% of body weight for the first 30 days and 8% thereafter. Proximate analysis of raw ingredients, formulated feed, and carcass composition was conducted to assess the growth performance, water quality, genotoxicity, and feed economics at suitable intervals of time. The results revealed significant ($p < 0.05$) increases in BWG, SGR, FCE, PER, and LER with the incorporation of ELPC in the formulated feed, while there was a significant decrease ($p < 0.05$) in FCR. The HSI and ISI (%) showed non-significant ($p > 0.05$) differences throughout the experiment. Economically, there was a decreasing trend in the price/kg of formulated feed with ELPC incorporation, while the Economic Conversion Ratio (ECR) showed an increasing trend from control to T₄. The genotoxicity studies were conducted *via* The comet assay, to analyze the presence of anti-nutritional components in the formulated diet, however, the result revealed no significant DNA damage among the treatments.

Keywords: Eichhornia, leaf protein concentrate, groundnut oil cake, growth performance, genotoxicity, *Labeo rohita*

1. Introduction

In India, agriculture and fisheries have been vital for ensuring nutritional food security, generating employment, earning foreign revenue, and providing livelihoods for millions of people since ancient times. However, the burgeoning population, diminishing available resources such as land and water, and the effects of climate change present significant challenges to feeding the growing population. Fisheries and aquaculture hold immense potential in addressing crucial issues like nutrition, poverty, hunger, economic growth, and the sustainable utilization of natural resources. Indian fisheries make a substantial contribution to the global food supply, with carps being the primary species, accounting for over 85% of all aquaculture production in the country.

Labeo rohita, commonly known as rohu, is among the most cultured indigenous fish species in the Indian subcontinent and is highly regarded for its taste among carp varieties. In semi-intensive fish culture systems, the provision of supplemental feed plays a pivotal role, with approximately 60% of the production cost attributed solely to feed expenses. Achieving nutritional balance in feeds necessitates high-quality ingredients containing balanced amino acids, fatty acids, vitamins, and minerals. However, factors such as increasing stocking densities, indiscriminate fishing practices, and rising ingredient costs pose challenges to formulating feeds using conventional ingredients, making feed production progressively expensive. In light of the imperative to sustainably utilize resources and reduce the cost of

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formulated feeds, there is an urgent need to explore alternative or low-cost, non-conventional feed ingredients, particularly plant proteins, while ensuring feed quality. Non-conventional feed ingredients offer greater availability, sustainability, and cost effectiveness for aquaculture. Fish feed represents the most expensive input in aquaculture and is a major contributor to water quality deterioration during the culture period. *Eichhornia* spp. (water hyacinth) is an abundant aquatic weed found in many water bodies, exhibits rapid growth and high adaptability to varying environmental conditions. Its dense mats obstruct sunlight and water aeration, leading to oxygen depletion, reduced biodiversity, and competition with submerged vegetation. One acre of water hyacinth can reportedly pollute water equivalent to the sewage generated by 40 people (Dersseh *et al.*, 2019) [18]. With a productivity of 100-400 mt ha⁻¹ year⁻¹, it is considered one of the world's most problematic aquatic plants (Grodowitz, 1998) [21]. Despite its high protein content, balanced amino acid profile, and mineral richness, the utilization of raw *Eichhornia* leaves in fish feed is limited due to high fiber content and anti-nutritional factors (Stickney & Shumway, 1974; Buddington, 1980; Tucker & Debusk, 1981) [46, 10, 49]. Processing interventions, such as the production of *Eichhornia*-based leaf protein concentrate, can overcome these limitations and enable effective utilization of this abundant biomass (Ayanda *et al.*, 2020) [5]. Based on these considerations, the present study evaluates

the techno-economic feasibility and genotoxicity of formulated diets in which groundnut oil cake is partially replaced with *Eichhornia*-based leaf protein concentrate. The effects on growth performance, carcass composition, and water quality parameters of *Labeo rohita* are assessed to determine the sustainability and practicality of this alternative protein source for aquaculture feed formulations.

2. Materials and Methods

2.1 Preparation of *Eichhornia*-leaf protein concentrate (ELPC)

Eichhornia-based leaf protein concentrate (ELPC) was prepared following the method of Virabalin *et al.* (1993) [50]. *Eichhornia* leaves were collected from Talod Village Lake, Himmatnagar, Gujarat, India, trimmed, and thoroughly washed to remove debris. The leaves were blended with water at a 1:3 (w/v) ratio to form a slurry, and the pH was adjusted to 8.5 using 0.1 M NaOH to enhance protein solubility. The slurry was filtered through cheesecloth, and the filtrate was acidified to pH 4 with 0.1 M HCl to induce protein coagulation. The coagulated filtrate was heated at 80-100 °C for 5 min to promote aggregation of protein clumps, which were subsequently collected and neutralized to pH 7 using 1 N NaOH. The coagulum was dried under sunlight or in a hot-air oven at 60 °C, then ground into a fine powder (Figure 1).



Fig 1: (a) Carefully collected apical leaves of *eichhornia* plant (b) Washed leaves carefully weigh (c) Cut into pieces and crushed with water in the blender (Solid to liquid ratio 1:3) (d) Add 1N, NaOH to get 8.5 pH (e) The slurry was filtered with cheesecloth and separated the liquid portion (f) the separated portion was acidified with 1.0 N HCL to up to pH 4.0 (g) the whole portion heated at 82 °C for 5 min (h) Allow to settled the mixture further supernatant was decant and the remaining matrix or LPC was carefully collected after precipitation (I) Repeated the process washed with water (K) Further, dried the precipitated LPC at 60 °C and crushed into LPC powder further stored in air tight container for proximate analysis.

2.2 Proximate analysis

The proximate composition of raw *eichhornia*, *Eichhornia*-based leaf protein concentrate (ELPC), and groundnut oil cake (GNOC) was determined using Gerhardt semi-automated instruments following standard protocols. Analyses included moisture, dry matter, crude protein, crude

lipid, crude fiber, and total ash contents. Before analysis, *eichhornia* leaves and stems were dried using combined sun and oven drying, with oven drying performed at 100 °C and moisture loss monitored at 30-minute intervals. This standardized procedure ensured accurate nutritional

evaluation and comparison among *Eichhornia*, ELPC, and GNOC.

2.3 Experimental animal

The fish were sourced from the Centre of Excellence in Ukai, Gujarat, India. Healthy and disease-free rohu fry were carefully chosen for prophylactic treatment, which involved bathing them in a 0.05% potassium permanganate solution before being stocked in a pre-cleaned tank. The fish were then reared for one month under controlled laboratory conditions to ensure proper acclimatization in optimal environments before being utilized for the *in vivo* experiment.

2.4 Formulation of experimental diets

Five iso-nitrogenous diets (~30% crude protein) were formulated by replacing groundnut oil cake (GNOC) with *Eichhornia*-based leaf protein concentrate (ELPC) at 0% (Control), 25% (T₁), 50% (T₂), 75% (T₃), and 100% (T₄). Feed ingredients were weighed, ground, and mixed with water to form a dough, which was autoclaved for 10-15 min. Vitamins, mineral premix, and oil were then added, followed by pelletization. Pellets were sun-dried, crushed, and stored in airtight containers until use.

2.5 Experimental design

The 60-day feeding trial followed a Completely Randomized Design (CRD) comprising four treatments and a control, each in triplicate. Fish were stocked at 1 g L⁻¹ and fed at 10% of body weight for the first 30 days and 8%

thereafter. Standard aeration, water exchange, and siphoning procedures were maintained throughout the experiment.

2.6 Growth and water quality parameters

Growth performance was monitored fortnightly by recording initial and final body weight, body weight gain (BWG), specific growth rate (SGR), feed conversion ratio (FCR), feed conversion efficiency (FCE), protein efficiency ratio (PER), lipid efficiency ratio (LER), hepatosomatic index (HSI), intestinal somatic index (ISI), and survival. Water quality parameters, including alkalinity, dissolved oxygen, and hardness, were analyzed following APHA (2005). pH, total dissolved solids (TDS), conductivity, and nitrogenous wastes (TAN, ammonia, nitrite, and nitrate) were measured using a HANNA multiparameter bench meter.

2.7 Economics of feed and genotoxicity studies

Feed production costs were calculated to determine the cost-benefit ratio using local market prices of ingredients from Himmatnagar, Gujarat (2022). The economic conversion ratio (ECR) was estimated based on ingredient inclusion levels. Genotoxic effects of the experimental diets were assessed using the alkaline single-cell gel electrophoresis (comet assay) method following Klaude *et al.* (1996) [30], with minor modifications. Blood samples were collected non-lethally from the caudal peduncle, smeared on slides containing 2% EDTA, and immediately subjected to comet analysis.

Formulas for the calculation of growth parameters

$$\% \text{ BWG} = \frac{(\text{Final body weight} - \text{Initial body weight})}{\text{Initial body weight}} * 100 \quad (1)$$

$$\% \text{ SGR} = \frac{(\text{Ln average Final weight} - \text{Ln average Initial weight})}{\text{Number of days}} * 100 \quad (2)$$

$$\text{Survival rate (\%)} = \frac{\text{Number of fish at the end of the expt}}{\text{Number of fish at the beginning of the expt.}} * 100 \quad (3)$$

$$\text{FCR} = \frac{\text{Feed intake}}{\text{Weight gain}} \quad (4)$$

$$\text{FCE} = \frac{\text{Weight gain}}{\text{Feed intake}} \quad (5)$$

$$\text{PER} = \frac{\text{Wet weight gain}}{\text{Protein intake on dry matter basis}} \quad (6)$$

$$\text{LER} = \frac{\text{Wet weight gain}}{\text{Lipid intake on dry matter basis}} \quad (7)$$

$$\text{HSI\%} = \frac{\text{Wet weight of liver}}{\text{Wet weight of whole fish}} * 100 \quad (8)$$

$$\text{ISI\%} = \frac{\text{Wet weight of intestine}}{\text{Wet weight of whole fish}} * 100 \quad (9)$$

$$\text{ECR} = \text{Cost of diet} \times \text{Feed Conversion Ratio (FCR)} \quad (10)$$

2.8 Statistical analysis

Statistical analysis was conducted using the statistical package SPSS version 26. The data obtained were subjected to one-way analysis of variance (ANOVA) at a significance level of $p < 0.05$. Significant differences between treatments were further evaluated using the Duncan multiple range test.

3. Results

3.1 Proximate and carcass analysis and estimated *eichhornia* biomass efficiency

For the preparation of *Eichhornia*-based leaf protein concentrate (ELPC), the efficiency of raw *eichhornia* was calculated. It was observed that approximately 20 kg of raw

eichhornia was required to extract 500 g of ELPC, resulting in an efficiency of 2.55%. The detailed proximate analysis of both raw ingredients and finished ELPC is presented in Table 1. The proximate analysis of ELPC reveals an increase in protein content and a decrease in crude fiber content. A total of five iso-nitrogenous diets (C, T₁, T₂, T₃,

and T₄) were formulated using the finished ELPC by substituting groundnut oil cake (GNOC) at varying levels of substitution. The proximate analysis of the formulated feeds and carcass composition is depicted in Tables 2 and 3, respectively.

Table 1: Proximate analysis of raw eichhornia, LPC and other ingredients

Components	Proximate values (in %)					
	Moisture	DM	CP	CL	CF	Ash
ELPC (% DB*)	9.75	90.25	39.88	4.69	2.65	15.5
GNOC	7.05	92.95	40	7.37	10.96	5.5
Eichhornia leaves (%WB**)	86	14	18.5	1.76	19.64	15.3
Eichhornia stems (% WB)	87.5	12.5	6	0.82	30.23	16.46

(CP = crude protein; CL = crude lipid; CF = crude fiber; DM= Dry matter*DB = Dry weight based; **WB = Wet weight based)

Table 2: Ingredients incorporated in feed formulation and its proximate

Ingredients	Grams of ingredients per 100 gras of feed				
	C	T ₁	T ₂	T ₃	T ₄
GNOC	30	22.5	15	7.5	0
LPC	0	7.5	15	22.5	30
Wheat Flour	15	15	15	15	15
Corn	12	12	12	12	12
Soyabean Meal	32	32	32	32	32
*Premix Vitamin	1	1	1	1	1
*Premix Mineral	1	1	1	1	1
Tapioca	5	5	5	5	5
Oil	4	4	4	4	4
Proximate data of Formulated feed (30% CP) replacing GNOC with ELPC					
Moisture	6	6	6.1	6	6
Dry matter	94	94	93.9	94	94
CP	30.01	29.97	29.93	29.9	29.86
CL	8.97	8.7	8.5	8.43	7.87
CF	5.82	5.93	5.7	5.5	5.23
Ash	10	10	10.9	11.2	10.8

(C(100%GNOC), T₁(75%GNOC+25%Silage), T₂(50%GNOC+50%Silage), T₃(25%GNOC+75%Silage), T₄(0%GNOC+100%Silage));

**GNOC Ground nut oil cake; *Nutritional value per kg, Vita. A 7, 00,000 I.U., Vit D3 70,000 I.U., vit. E 250 mg, Niacinamide 1g, Zinc 9.6g, Sulphur 7.2g, Copper 4.2g, Magnesium 6000mg, Iron 1500mg, Manganese 1500mg, Iodine 325mg, Cobalt 150mg, Potassium 100mg, Chromium 75mg, Selenium 7.5mg, Sodium 6mg, Calcium 191g, Phosphorous 95g.

Table 3: Carcass composition of fish body

Treatments	Moisture (% WB)	CP (% DB)
C	77.76	76.97
T ₁	79.36	78.51
T ₂	79.03	82.63
T ₃	78.64	79.28
T ₄	78.41	82.22

(CP = crude protein; *DB = Dry weight based; **WB = Wet weight based)

3.2 Growth performance of *Labeo rohita*

The growth performance parameters, including body weight gain (BWG%), initial and final weights (gm), specific growth rate (SGR%), feed conversion ratio (FCR), feed conversion efficiency (FCE), survival percentage, protein

efficiency ratio (PER), lipid efficiency ratio (LER), hepatosomatic index (HSI%), and intestinal somatic index (ISI%), are presented in Tables 4, 5, and 6, respectively. Throughout the experimental period, BWG (%) and SGR showed increasing trends, while FCR exhibited a decreasing trend with the incorporation of ELPC in the formulated diets from the control to T₄. The protein efficiency ratio (PER) and lipid efficiency ratio (LER) demonstrated increasing trends with the inclusion of ELPC in the fish diet. Conversely, the values of HSI (%) and ISI (%) showed fluctuating trends with the incorporation of ELPC in the feed. The survival percentage was found to be non-significant ($P>0.05$) and ranged between 95-100%, as depicted in Figure 2.

Table 4: Body weight gain of fishes at fortnightly during 60 days of experiment

Treatment	Body weight gain (%)			
	BWG15	BWG30	BWG45	BWG60
C	55.48±1.45 ^c	93.95±3.81 ^d	145.08±1.42 ^d	217.84±4.53 ^d
T ₁	63.41±2.69 ^c	129.06±5.50 ^c	186.80±6.53 ^c	252.33±3.24 ^c
T ₂	84.06±8.49 ^b	145.23±3.55 ^{bc}	203.62±3.43 ^c	284.11±8.93 ^b
T ₃	100.85±2.49 ^{ab}	162.13±3.38 ^{ab}	233.37±2.20 ^b	306.18±3.51 ^{ab}
T ₄	115.72±2.24 ^a	186.13±8.43 ^a	263.78±10.16 ^a	335.49±8.87 ^a

Note: All the values are presented in (mean±SE) n = 3. Mean values with different superscripts in the same column differ significantly ($p<0.05$)

Table 5: Growth parameters of *Labeo rohita* after 60 days of feeding trial

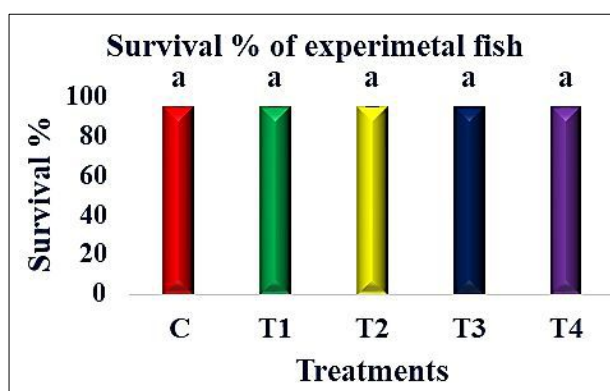
Treatments	INWT (g)	FLWT (g)	SGR (%)	FCR	FCE
C	2.34±0.02 ^a	7.44±0.06 ^d	1.93±0.02 ^d	2.84±0.02 ^a	0.35±0.00 ^b
T ₁	2.25±0.01 ^a	7.94±0.01 ^c	2.10±0.01 ^c	2.77±0.01 ^{ab}	0.36±0.00 ^{ab}
T ₂	2.24±0.07 ^a	8.61±0.12 ^b	2.24±0.03 ^b	2.65±0.08 ^{ab}	0.38±0.01 ^{ab}
T ₃	2.28±0.02 ^a	9.27±0.07 ^a	2.34±0.01 ^{ab}	2.64±0.03 ^{ab}	0.38±0.00 ^{ab}
T ₄	2.29±0.02 ^a	9.99±0.12 ^a	2.45±0.03 ^a	2.62±0.03 ^b	0.38±0.00 ^a

Note: All the values are presented in (mean±SE) n = 3. Mean values with different superscripts in the same column differ significantly ($p < 0.05$), INWT= initial weight, FLWT= final weight, SGR= specific growth rate, FCR= feed conversion ratio, FCE= feed conversion efficiency

Table 6: Growth parameters of *Labeo rohita* after 60 days of feeding trial

Treatments	PER	LER	HIS (%)	ISI (%)
C	1.17±0.01 ^b	4.39±0.04 ^b	0.44±0.09 ^a	4.60±0.52 ^a
T ₁	1.20±0.00 ^{ab}	4.51±0.02 ^{ab}	0.44±0.08 ^a	4.48±0.83 ^a
T ₂	1.26±0.04 ^{ab}	4.73±0.15 ^{ab}	0.45±0.01 ^a	4.35±0.31 ^a
T ₃	1.26±0.01 ^{ab}	4.74±0.05 ^{ab}	0.45±0.06 ^a	4.54±0.83 ^a
T ₄	1.27±0.01 ^a	4.78±0.06 ^a	0.34±0.14 ^a	4.97±0.62 ^a

Note: All the values are presented in (mean±SE) n = 3. Mean values with different superscripts in the same column differ significantly ($p < 0.05$), PER= protein efficiency ratio, LER= lipid efficiency ratio, HSI= hepatosomatic index, ISI= intestinal somatic index

**Fig 2:** Survival (%) of experimental fish of different treatments

3.3 Physicochemical water quality parameters during the experiment

The physico-chemical water quality parameters remained well within the optimum range across all treatments

throughout the experiment, and their variations were found to be non-significant ($p > 0.05$) during the entire experimental period. The detailed range of recorded water quality parameters is presented in Table 7.

Table 7: Physico- chemical water quality parameters range observed during experiment

Water quality parameters	Range
Temperature (°C)	23.5-30.5
pH	7.32-8.62
Dissolved Oxygen (mg/L)	4.82-6.48
Total alkalinity (mg/L)	204.33-330.67
Conductivity (µS/cm)	357.00-462.67
Hardness (mg/L)	227.33-350.67
Total Dissolved Solids (mg/L)	481.88-631.90
Total Ammonium Nitrogen (mg/L)	0.20-0.90
Ammonia (mg/L)	0.01-0.23
Nitrite (mg/L)	0.00-0.28

3.4 Techno-economic feasibility of formulated feed

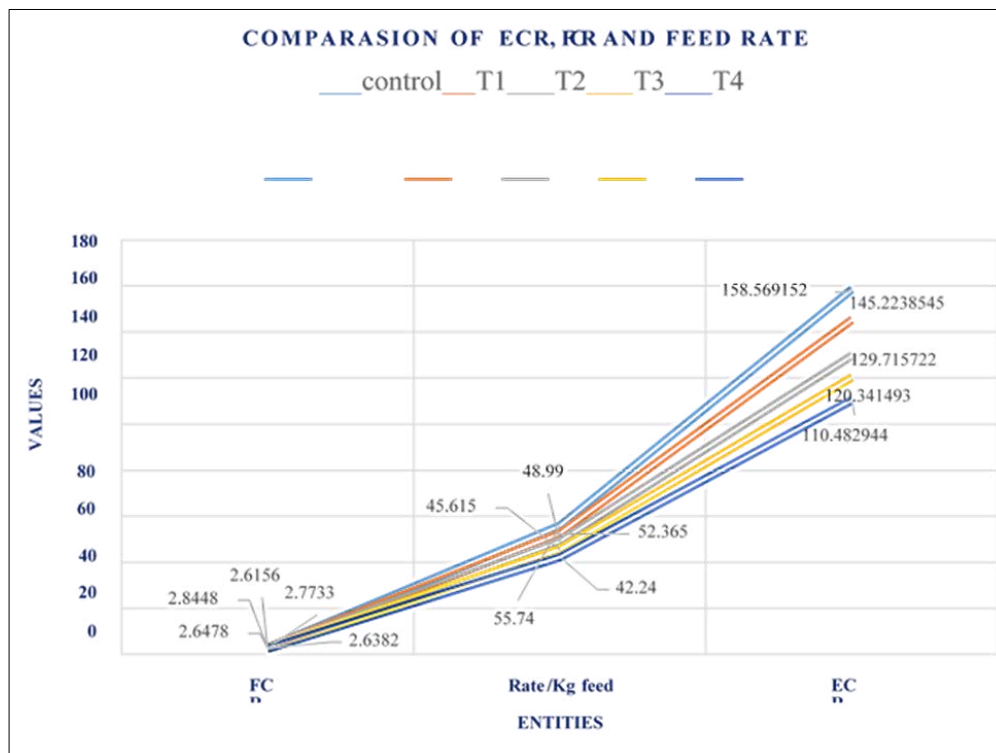
The techno-economic analysis of the formulated feed was conducted by calculating the price per kilogram (kg) of formulated feed and the Economic Conversion Ratio (ECR) based on the current price rates of raw ingredients in Gujarat. It was observed that both the calculated price per kg of formulated feed and the ECR exhibited a decreasing

trend with the percentage incorporation of ELPC in the formulated feed. The price per kg of formulated feed ranged from Rs. 42.24 to 55.74 in T₄ and the control group, respectively, while ECR values fluctuated between 110.48 to 158.56 in the control and T₄, respectively. The detailed data on Price per kg feed and ECR are illustrated in Figure 3 and presented in Table 8.

Table 8: Economical analysis of experimental diets in terms of feed rate and ECR

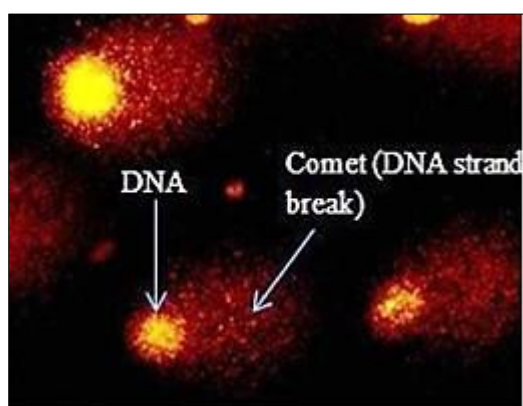
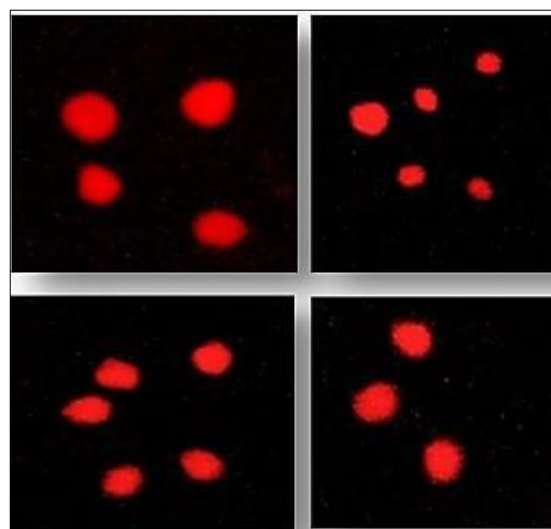
Treatments	Feed Rate	ECR
C	20.12±0.71 ^a	57.22±1.61 ^a
T ₁	20.52±0.68 ^a	56.89±1.85 ^a
T ₂	20.42±0.42 ^a	53.99±0.81 ^a
T ₃	21.89±0.66 ^a	57.78±2.39 ^a
T ₄	22.72±0.43 ^a	59.44±1.61 ^a

Note: All the values are presented in (mean±SE) n = 3. Mean values with different superscripts in the same column differ significantly ($p < 0.05$), ECR= economic conversion ratio

**Fig 3:** Comparison of ECR, FCR and feed rate per kg basis

3.5 Genotoxicity study via. Comet assay analysis

To assess the impact of anti-nutritional and anti-growth factors present in raw eichhornia and the finished formulated feed on growth performance, a comet assay was conducted. Cell damage was evaluated based on the presence or absence of a "comet tail" and the length of the tail, which indicates the percentage of DNA damage. In this study, the absence of a "comet tail" indicates no DNA damage at the experimental fish level when fed with ELPC-based formulated diets. Reference images of a comet tail and the experimental results are depicted in Figures 4 and 5, respectively.

**Fig 4:** Reference image of comet assay**Fig 5:** Comet Assay among different treatments

4. Discussion

In previous studies, eichhornia has been explored as feed for grass carp by Mahmood *et al.* (2016) [31], as well as for many other fishes (Pratiwi & Andhikawati, 2021) [37]. In our current investigation, we report the proximate values of crude protein (CP), crude lipid (CL), and fiber content (%) in raw eichhornia leaves as 18.5%, 1.76%, and 19.64%, respectively. Our findings corroborate those of Arayana *et*

al. (1984)^[3] and Dada (2002)^[14] concerning eichhornia. However, our observed values were slightly lower compared to those documented by Teye *et al.* (2020)^[48] for eichhornia leaf (20%) and stem (10%). Scientific literature suggests that crude protein percentage (CP%) can be enhanced through appropriate treatments, such as acid and alkali treatments. In our investigation, raw eichhornia leaves were utilized to produce Eichhornia-based leaf protein concentrate (ELPC), resulting in a significant increase in CP% from 18.5% (in the leaf) to 39.88% (in ELPC), accompanied by a substantial decrease in fiber content. These outcomes align with the findings of Abo Bakr *et al.* (1984)^[1], who observed an increase in CP from 27.75% (in raw eichhornia) to 49.60% (in ELPC), alongside a reduction in crude fiber from 21.5% (in raw eichhornia) to 1.70% (in ELPC). Adeyemi & Osabor (2016)^[2] similarly demonstrated that protein levels increased to 56.30% and crude fiber decreased to 1.02% with suitable treatments. The improvement in nutritive value, particularly in terms of CP%, may be attributed to the higher protein percentage in a single cell (El-Sayed & Tacon, 1997)^[20]. Carcass composition analysis of rohu conducted at the conclusion of the experiment revealed moisture (WWB%) and crude protein (DWB%) levels within the ranges of 77.76-79.36% and 76.97-82.63%, respectively.

The observations in our study are consistent with the findings of Khan *et al.* (2003)^[28], who reported moisture and protein levels ranging from 76.47±0.33 to 80.13±0.18 and 81.02±0.11 to 83.65±0.23, respectively, during their experimentation on *Labeo rohita*. In our investigation, we noted a significant increase in the crude protein (CP) level in fish carcasses, which may be attributed to the superior amino acid profile of Eichhornia-based leaf protein concentrate (ELPC) compared to other ingredients used in the feed formulation. *Eichhornia* spp. Possesses almost all essential amino acids (EAAs), particularly leucine, valine, arginine, phenylalanine, threonine, lysine, methionine, and tryptophan (Jubie *et al.*, 2015)^[25]. Amino acids play crucial roles in growth, feed utilization, digestive enzyme activity, feed intake, feed efficiency, and protein efficiency ratio. Additionally, amino acids contribute significantly to enzymatic activities, and there is evidence suggesting their involvement in antioxidant activities.

In the present experiment, the growth performance of rohu exhibited a significantly higher growth rate, with both the body weight gain (BWG%) and specific growth rate (SGR) showing an increasing trend with the rising substitution of Eichhornia-based leaf protein concentrate (ELPC) with groundnut oil cake (GNOC). This phenomenon may be attributed to the high protein content, superior amino acid profile compared to other ingredients, enhanced digestibility, lower fiber content, and increased palatability. Similar outcomes were observed by Ayyat *et al.* (2021)^[6] during the feeding of leaf protein concentrates from *Beta vulgaris* and *Daucus carota* as a substitute for soybean meal in *Oreochromis niloticus* fingerlings' diets. The increasing trends in final body weight gain, SGR, and overall final growth of fish in the experiment may be due to the lower fiber content in ELPC. High crude fiber levels can decrease feed digestibility, as supported by research from Kamarudin *et al.* (2018)^[26], Ramachandran *et al.* (2004)^[38], Bairagi *et al.* (2002, 2004)^[8, 7], De Silva *et al.* (1990)^[17], Jackson *et al.* (1982)^[24], and Atack *et al.* (1979)^[4], which revealed that high crude fiber content in plant-based ingredients in

formulated feed plays a crucial role in poor digestibility. Adeyemi & Osabor (2016)^[2] and Mahmood *et al.* (2016)^[31] also observed lower SGR due to high fiber content and low feed digestibility. Furthermore, the increasing trends in growth performance with ELPC also suggest the absence of toxic substances, contaminants, or anti-nutritional factors that could potentially cause acute toxicological effects and hinder fish growth. This finding is supported by Chavez *et al.* (2016)^[11]. The survival rate of fish did not show any significant difference ($P>0.05$) among the treatments, including the control, throughout the experiment period. These observed results are consistent with the findings of Sotolu and Sule (2011)^[44], Sotolu (2012)^[45], Serrano *et al.* (2017)^[42], and Ayyat *et al.* (2021)^[6]. These observed results highlight the robustness and stability of fish survival across all treatment groups, suggesting that the experimental diets, including ELPC, did not adversely affect the overall survival rates. The protein efficiency ratio (PER) and feed efficiency ratio (LER) values were found to be significant ($P<0.05$) and exhibited an increasing trend from the control group to T₄. This trend could be attributed to the higher protein content and improved feed utilization in the experimental diets. Moreover, the protein and lipid profiles of fish are closely linked to their growth, immune function, and stress response, as emphasized by Mohamed *et al.* (2017)^[34]. Similar results were also observed by Cowey (1978)^[13]. The hepato-pancreas in fish plays a crucial role in biotransformation and detoxification of metabolites (Mohamed *et al.*, 2017; El-Houseiny *et al.*, 2019)^[34, 19]. Therefore, the hepato-somatic index (HSI%), which reflects the relative size of the liver in relation to the overall body weight, can provide valuable insights into liver conditions and the overall well-being of the fish. In the present experiment, the HSI% was reported to be non-significant ($P>0.05$) among the treatment groups and the control, indicating the absence of harmful toxic substances such as heavy metals, contaminants, and anti-nutritional factors in the formulated feed. Similarly, the intestinal somatic index (ISI%), which indicates the relative size of the intestine, also showed marginal differences in values, but no significant difference ($P>0.05$) was observed among the treatment groups and the control. The condition of the intestine is crucial as it reflects the feed intake of the fish, with the size of the intestine being influenced by the fiber content of the feed. Kihara & Sakata (1997)^[29] suggested that highly fibrous feed leads to elongation of the intestine. Feed cost constitutes approximately 50-60% of the overall production cost in aquaculture. Among the various components of feed, protein stands out as the most expensive (Hardy and Tacon, 2002)^[22]. Hlophe *et al.* (2011)^[23] and Sarker *et al.* (2020)^[41] have suggested that utilizing plant-based ingredients in aquafeed formulations is a key strategy to reduce feed costs. Economic conversion ratio (ECR) serves as a valuable index for analyzing the economic efficiency of formulated feeds. Ogino *et al.* (1978)^[36], Chiesa & Gnansounou (2011)^[12], and Ayyat *et al.* (2021)^[6] have advocated for the inclusion of leaf protein concentrate (LPC) in aqua feeds due to its provision of high-quality protein from plant leaves at a lower cost.

The water quality records from the present study indicate optimal ranges conducive to rohu culture. The temperature remained consistent throughout the experimental period and was deemed non-significant ($P>0.05$). Das *et al.* (2005)^[16] suggested that the temperature range, 26-36 °C, is not fatal to *L. rohita* fry. The pH levels exhibited a decreasing trend

during the experiment, but were found to be non-significant ($P>0.05$). Das *et al.* (2006) [15] reported pH levels of 7.75-8.4 for optimal fish culture. The dissolved oxygen (DO) concentration is crucial for supporting good fish production, with an optimum level of more than 5 mg/l recommended by Bhatnagar and Singh (2010) [9]. The observed alkalinity range, ranging from 204.33-330.67, sufficient alkalinity for most aquaculture purposes, fell well within the acceptable range of 20-400 mg/l Munni *et al.*, 2013 [35], further supported by Saha & Ray (2011) [40] proposed alkalinity ranges for fish culture of 160-182 mg/L. Regarding hardness levels, the observed range of 227.33-350.67 aligns with the recommendations of Keremah *et al.* (2014) [27], who suggested overall hardness 50-400mg/l levels as a desirable range, and also, Tanuja *et al.* (2016) [47] reported that hardness ranging from 236 to 245 mg/L had no significant impact on fish growth. Bhatnagar and Singh (2010) [9] suggested a recommended level of less than 0.2 mg/l for suitable fish culture, and they found ammonia ranged from 0.29-3.44 mg/l in a managed pond. Similarly, the nitrite levels were found to fall within the acceptable range. Maximum acceptable levels for nitrite are species specific and contingent on pond circumstances, but, for freshwater aquatic life a value of less than 0.60 mgL-1 for freshwater aquatic life is recommended. Additionally, Rumpa *et al.* (2016) [39] identified levels above 1.0 ppm as lethal for many warm-water fishes.

The genotoxicity study was conducted using the comet assay to assess the potential impact of any toxicants or anti-nutritional factors on the growth of rohu fed plant-based *Eichhornia*-based leaf protein concentrate (ELPC) diets. Wu & Sun (2011) [51] suggested that plant ingredients may harbor anti-nutritional factors that could potentially damage cell DNA. In the present study, the absence of a "comet tail" in the comet assay indicates no DNA damage in the experimental fish. However, it's important to note that these results do not conclusively rule out the presence of harmful substances in the ELPC, warranting further detailed investigation. Based on the observations, it can be interpreted that even if anti-nutritional factors and acid residues are present in the formulated feed, they are unable to express themselves and are hindered from damaging the DNA of the cells.

5. Conclusion

In conclusion, this study underscores the potential of utilizing non-conventional feed ingredients, such as *Eichhornia*-based leaf protein concentrate (ELPC), in aquafeed formulations. While conventional feed ingredients may have limitations, our findings demonstrate that ELPC can serve as a viable alternative, with no observed DNA damage in experimental fish. This highlights the importance of exploring alternative sources to meet the growing demand for aquafeed, particularly with suitable technological interventions. Moving forward, further research and development are warranted to optimize the utilization of non-conventional feed ingredients for sustainable aquaculture practices.

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7. Statement Declaration

During the preparation of this work, the author(s) used the ChatGPT tool for paraphrasing purposes. After using this

tool, the author(s) reviewed and edited the content as needed, and take(s) full responsibility for the content of the publication.

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