Original Research Article

Evaluation of Eichhornia-Based Fermented Silage as a Partial Protein Substitute for Groundnut Oil Cake in Growth and Water Quality of Rohu (Labeo rohita)

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ABSTRACT

Aims
This study evaluated the potential of Eichhornia-based fermented silage (EFS) as an alternative protein source to groundnut oil cake (GNOC) in the diet of rohu (*Labeo rohita*), focusing on growth performance, feed utilization, economics, water quality, and genotoxic safety.

Study Design
A completely randomized design with five dietary treatments, each with three replications.

Place and Duration of Study
The experiment was conducted under controlled laboratory conditions for 60 days.

Methodology
Five isonitrogenous (25% crude protein) and isolipidic (8% lipid) diets were formulated by replacing GNOC with EFS at 0% (C), 25% (T1), 50% (T2), 75% (T3), and 100% (T4). Rohu fingerlings (0.79 ± 0.04 g) were stocked at 1 g/L and fed at 10% body weight for the first 30 days and 8% thereafter. Growth performance, feed utilization indices, somatic indices, water quality, economic parameters, and genotoxicity (as assessed by the comet assay) were evaluated.

Results
EFS showed higher crude protein content than raw *Eichhornia*. Growth parameters (BWG, SGR, FCE, PER, and LER) declined with increasing EFS inclusion, while FCR increased. T1 showed no significant difference ($p > 0.05$) compared to the control. HSI and ISI were non-significant across treatments, except T1, which exhibited a lower HSI. Feed cost per kilogram and economic conversion ratio decreased with higher EFS inclusion. No DNA damage was observed in any treatment group.

Conclusion
EFS can replace GNOC up to 25% in rohu diets without adverse effects on growth or health, offering a safe and economically feasible alternative protein source.

Keywords: Labeo rohita, Eichhornia, Eichhornia-based Fermented silage, GNOC, growth performance, water quality, economics, genotoxicity

1. INTRODUCTION

Global food demand continues to rise, while food availability is declining, with nearly 193 million people facing acute food insecurity, an increase of about 40 million compared to 2020 (GRFC, 2022). The fisheries sector plays a vital role in addressing this challenge by providing affordable, nutrient-rich animal protein. Fish is a highly perishable yet widely consumed food commodity, and Indian major carps constitute a significant share of inland aquaculture production in the Indian subcontinent (Khan et al., 2003; Samal et al., 2022). Among them, rohu (Labeo rohita) is one of the most preferred freshwater fish due to its superior taste, rapid growth, and planktivorous feeding habit (Jhingran, 1991).

In semi-intensive and intensive aquaculture systems, feed cost accounts for nearly 60% of total production expenses, necessitating the exploration of low-cost alternative feed ingredients. Eichhornia spp. (water hyacinth) is a fast-growing aquatic weed producing large biomass and is widely available in freshwater bodies. It grows and reproduces very quickly with a high rate, yielding up to 100-400 mt/ha/year, and is included in the world's worst aquatic plants (Grodowitz, 1998). Despite its invasive nature, Eichhornia possesses potential as a feed resource; however, its high fibre content and presence of anti-nutritional factors such as tannins and phytic acid limit its direct utilization (Hajra et al., 2013).

Fermentation is an economical and effective processing method that can improve the nutritional quality of plant-based feed ingredients by reducing fiber and anti-nutritional compounds while enhancing protein availability, amino acid profile, digestibility, and palatability (Ndimele & Kumolu-Johnson, 2012; Flores-Miranda et al., 2014; Zhang et al., 2017). Fermented feed ingredients have been successfully used in livestock, poultry, and aquaculture to improve growth performance, feed utilization, and disease resistance (Chiang et al., 2009; Ha et al., 2019).

Therefore, the present study aimed to evaluate fermented Eichhornia as a low-cost, nutritious feed ingredient for Labeo rohita, to reduce feed cost while maintaining optimal growth and health performance.

2. MATERIALS AND METHODS

Preparation of Eichhornia Fermented Silage.

Eichhornia-based fermented silage (EFS) was prepared using Eichhornia spp. collected from Talod Village Lake, Himmatnagar, Gujarat, India. The plants were thoroughly washed, roots were removed, and only leaves and stems were used. The material was chopped into 1–3 cm pieces and sun-dried to achieve 65–70% moisture content. A total of 5 kg of chopped biomass was placed in an airtight plastic container in successive thin layers. Each layer was supplemented with rice bran (10% w/w), jaggery solution as a carbon source, and formic acid (15–20 mL per layer) to ensure rapid pH reduction. The container was sealed airtight and allowed to ferment for 45 days to obtain well-fermented silage.

Proximate Analysis

The proximate analysis of prepared Raw Eichhornia, EFS, and GNOC was conducted using Gerhardt semi-automated instruments and standard protocols. The parameters analyzed included Moisture, Dry matter, Crude protein, Crude lipid, Crude fiber, and Total ash.

Growth Trial

Healthy rohu (*Labeo rohita*) fingerlings were obtained from the Centre of Excellence, Ukai, Gujarat, India. Before acclimatization, the fish received a prophylactic bath in 0.05% potassium permanganate solution following standard protocols. They were acclimated for one month under laboratory conditions before the *in vivo* feeding trial.

Five isonitrogenous and isolipidic diets were formulated by replacing groundnut oil cake (GNOC) with Eichhornia-based fermented silage (EFS) at 0% (control), 25%, 50%, 75%, and 100%. Feed ingredients were weighed, mixed, ground, and dough was prepared with water, then cooked in a vertical autoclave for 10–15 minutes. Vitamin–mineral premix and oil were added before pelletization. Semi-sinking pellets were sun-dried, resized, and stored in airtight containers.

The experiment used a completely randomized design (CRD) with five treatments and three replicates each. Fish were stocked at 1 g/L and fed at 10% of their body weight for the first 30 days, then at 8%. Continuous aeration was provided, with daily siphoning to remove feces and uneaten feed, and up to 30% water exchange to replace losses. Growth sampling was done every two weeks, and growth, feed utilization, biometric indices (HSI and ISI), and survival rates were calculated using standard formulas.

Water Quality Parameters

Water quality parameters were checked regularly. Alkalinity, dissolved oxygen, and hardness were measured following APHA (2005). Temperature, pH, total dissolved solids, and conductivity were recorded with a multiparameter bench meter, while total ammonium nitrogen, ammonia-N, and nitrite-N were analyzed using a multiparameter photometer at appropriate intervals and wavelengths.

Economics of Feed and Genotoxicity

The production cost of the experimental diets was calculated to assess economic efficiency. Retail prices of raw ingredients (average market prices of 2022) were multiplied by their respective inclusion levels to estimate the cost of each diet. The economic conversion ratio (ECR) and cost per kilogram of feed were calculated based on total feed consumption during the experimental period using standard formulas.

Genotoxicity was evaluated using the alkaline single-cell gel electrophoresis (SCGE) or comet assay following Singh et al. (1988), with minor modifications as described by Klaude et al. (1996).

The data were analyzed using one-way ANOVA (analysis of variance), with descriptive values mean ± standard error, and homogeneity of subsets by using Tukey's method for different superscripts

Formulas for the calculation of growth parameters

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

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

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

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

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

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

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

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

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

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

3. RESULTS

The oven-dried raw eichhornia yielded 0.250 kg from 2 kg of raw leaves (12.5%), and moisture was reported at 86% in leaves and 87.5% in stems. The EFS was prepared as per the above-mentioned protocol, and after the process yield of silage was 2.2kg from 5 kg of raw eichhornia (leaves+stems), which was 44% of the total used green eichhornia leaves. The proximate analysis of raw eichhornia leaves & stems, GNOC, and EFS is illustrated in Table 1. The proximate analysis showed an increment in the percentage of protein and a decrease in crude fibre after preparing EFS. On the wet weight-based raw eichhornia leaves, 18.5% protein was reported, and it was increased to 25% in EFS, whereas the crude fibre was reduced to 12.81 from 19.64 in leaves and 30.23 in stems.

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

Components	**Proximate values (in %)**					
	Moisture	**DM**	**CP**	**CL**	**CF**	**Ash**
EFS (% DB*)	8.01	91.98	**25**	**10.95**	**12.81**	12.3
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)

The feed was prepared by replacing GNOC with EFS at different proportions. Ingredients used to make 100g of feed are listed in Table 2. The formulation was based on a 25% crude protein (CP) content. The proximate analysis of the prepared diets is shown in Table 2. Carcass composition of the fish body shown in Table 3. Data regarding growth performance are presented in Tables 4, 5, and 6. The growth performance of the fish showed a decreasing trend compared to the control diets as GNOC was replaced with EFS. The fortnightly observed body weight gain (BWG) is shown in Table 4. The body weight gain during the first 15 days was not significantly different, but after 15 days, it decreased from the control (921.78 ± 69.33a) to T4 (555.21 ± 31.62c). In T1 (919.75 ± 73.67a), it was not significantly different from the control ($p > 0.05$). The results for initial weight (INWT in g), final weight (FLWT in g), specific growth rate (SGR), feed conversion ratio (FCR), and feed conversion efficiency (FCE) are detailed in Table 5. The initial weight used in the experiment was 0.78 ± 0.04 g, while the final weight showed significant differences. The SGR was not significantly different between the control and T1, but showed decreasing trends in the remaining treatments. The control group had the best FCR, while T1 had the best FCE. PER and LER also exhibited decreasing trends from the control to T4. The hepatosomatic index (HSI) was not significantly different ($p > 0.05$) between the control and T3, while T4 showed a moderately lower value. The intact survival index (ISI) was not significantly different ($p > 0.05$) among all treatments and the control. Fish survival remained unaffected throughout the experiment, indicating no negative impact from EFS (Fig. 1).

Table 2: Ingredients incorporated in feed formulation and their proximate

Ingredients	**Grams of ingredients per 100 gras of feed**				
	Control	**T1**	**T2**	**T3**	**T4**
GNOC	17	12.75	8.5	4.25	0
Silage	0	6	12	18	24
Wheat Flour	10	10	10	10	10
Corn	14	14	14	14	14
Soybean Meal	34	34	34	34	34
Premix Vitamin	1	1	1	1	1
Premix Mineral	1	1	1	1	1
Oil	6	6	6	6	6
Tapioca	10	10	10	10	10
Filler	7	5.25	3.5	1.75	0
Total	100	100	100	100	100
Proximate data of formulated feed (25% CP) replacing GNOC with FS.					
	Control (C)	**T1**	**T2**	**T3**	**T4**
Moisture	6	6	6.4	6.4	6.4
DM	94	94	93.6	93.6	93.6
CP	25.22	25.02	24.82	24.62	24.42
CL	8.79	8.86	8.9	8.95	8.97
CF	5.05	5.75	6.58	6.78	6.85
Ash	10.1	10.67	11.32	12	12

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

Table 3: Carcass composition of the fish body

Treatments	Moisture (%)	CP (%)
C	77.76	77.51
T1	79.36	81.55
T2	79.03	78.4
T3	78.64	76.37
T4	78.41	83.86

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

	Body Weight Gain (%)			
Treatments	**BWG15**	**BWG30**	**BWG45**	**BWG60**
C	120.89 ± 11.10^{a}	344.93 ± 22.20^{a}	595.66 ± 44.58^{a}	921.78 ± 69.33^{a}
T1	133.67 ± 19.72^{a}	341.01 ± 17.08^{ab}	566.23 ± 23.32^{a}	919.75 ± 73.67^{a}
T2	101.18 ± 7.95^{a}	271.46 ± 18.15^{bc}	500.24 ± 17.10^{ab}	803.21 ± 10.57^{ab}
T3	83.18 ± 2.67^{a}	236.75 ± 1.28^{c}	433.74 ± 6.11^{bc}	657.83 ± 27.15^{bc}
T4	100.69 ± 5.32^{a}	221.19 ± 10.94^{c}	343.93 ± 18.66^{c}	555.21 ± 31.62^{c}

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	FLWT	SGR	FCR	FCE
C	0.77 ± 0.05^{a}	8.88 ± 0.04^{a}	3.87 ± 0.11^{a}	2.42 ± 0.05^{c}	0.41 ± 0.01^{ab}
T1	0.75 ± 0.05^{a}	8.63 ± 0.11^{a}	3.86 ± 0.12^{a}	2.46 ± 0.02^{bc}	0.41 ± 0.00^{a}
T2	0.80 ± 0.01^{a}	8.20 ± 0.26^{a}	3.67 ± 0.02^{ab}	2.49 ± 0.03^{bc}	0.40 ± 0.00^{ab}
T3	0.84 ± 0.01^{a}	7.10 ± 0.29^{b}	3.37 ± 0.06^{bc}	2.61 ± 0.02^{b}	0.38 ± 0.00^{bc}
T4	0.80 ± 0.04^{a}	5.81 ± 0.09^{c}	3.13 ± 0.08^{c}	2.79 ± 0.02^{a}	0.36 ± 0.00^{c}

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

Treatments	PER	LER	HSI	ISI
C	1.66 ± 0.03^{a}	5.92 ± 0.13^{a}	0.34 ± 0.0^{a}	3.33 ± 0.52^{a}
T1	1.63 ± 0.01^{ab}	5.82 ± 0.05^{ab}	0.35 ± 0.4^{a}	3.35 ± 0.46^{a}
T2	1.61 ± 0.01^{ab}	5.75 ± 0.06^{ab}	0.36 ± 0.0^{a}	3.49 ± 0.27^{a}
T3	1.53 ± 0.01^{bc}	5.47 ± 0.05^{bc}	0.35 ± 0.2^{a}	3.24 ± 1.83^{a}
T4	1.44 ± 0.01^{b}	5.13 ± 0.04^{b}	0.33 ± 0.0^{b}	3.12 ± 0.27^{a}

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

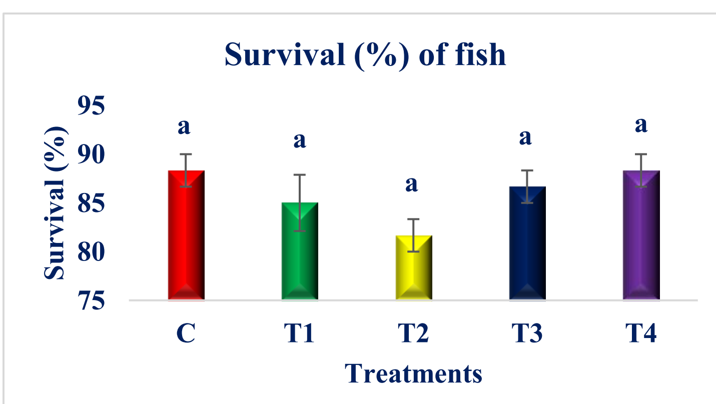

Fig. 1: Survival (%) of fish at the end of the experiment

The water quality was observed non-significant among all treatments. The range of water quality was found well within the optimum and acceptable range for the fish culture throughout the experiment. The water quality range is given in table 7.

Table 7: Physicochemical water quality parameters range observed during the experiment

Water quality parameters	Range
Temperature (◦C)	23.50-32.53
pH	7.29-8.61
Dissolved Oxygen (mg/L)	4.43-6.09
Total alkalinity (mg/L)	253.33-398.67
Conductivity (µS/cm)	360.33-467.00
Hardness (mg/L)	232.00-358.67
Total Dissolved Solids (mg/L)	420.67-531.90
Total Ammonium Nitrogen (mg/L)	0.19-0.65
Ammonia (mg/L)	0.17-0.01
Nitrite (mg/L)	0.006-0.091

The Economics of feed was measured by calculating feed rate and Economic conversion ratio (ECR) as per the current prices (2022) of ingredients and feed used during the experiment. The calculated rate of diets utilized during the experimental period and rate of per kg prepared diets were observed to decrease with the incorporation of EFS also the economic conversion ratio (ECR) observed decreased with the use of EFS. The cost of feed utilized in the study was found in the range from 15.82±0.357d to 23.27±0.433a, whereas, the price (Rs/kg) of the formulated feed of the experiment was calculated in the range from 52.38 (T4) to 55.78 (control). The results of feed rate and ECR of used feed are given in table 8. Whereas the results of feed rate and ECR per kg of feed are presented in fig. 2.

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

Treatments	FEED RATE	ECR
C	23.27 ± 0.43^{a}	56.20 ± 0.69^{a}
T1	21.36 ± 0.64^{ab}	52.50 ± 2.04^{ab}
T2	19.35 ± 0.63^{bc}	48.11 ± 1.19^{bc}
T3	18.33 ± 0.49^{c}	47.87 ± 1.47^{bc}
T4	15.82 ± 0.36^{d}	44.09 ± 1.40^{c}

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

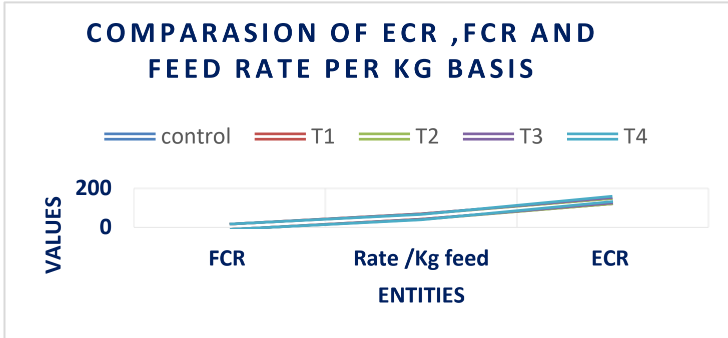

Fig. 2: Comparison of ECR, FCR, and feed rate per kg basis

The genotoxicity study was carried out during the experiment and the presence or absence of a “tail” was observed, from the observation presence or absence of a “tail” and the “tail length” indicates the (%) DNA damage. In the present study, the absence of “comet tail” indicates no damage at the DNA level in experimental fish when fed with EFS-based formulated diets. From the observation, the results can be interpreted that even if, the presence of anti-nutritional factors and acid residue present in the formulated feed they, are unable to express themselves and are hindered to damage the DNA of the cell in particular. The result of genotoxicity is given in figures 3 and 4.

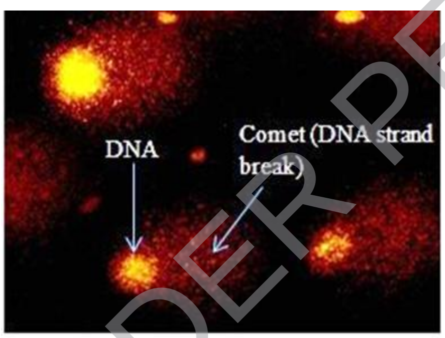

Fig. 3: Reference image of a comet assay

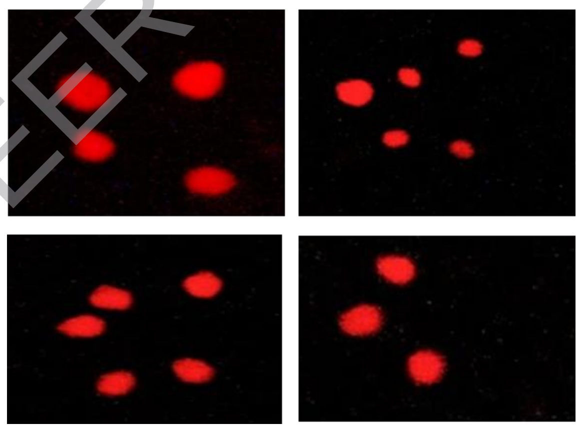

Fig. 4: Observed slides from different treatments

4. DISCUSSION

The present study evaluated the suitability of Eichhornia fermented silage (EFS) as a replacement for groundnut oil cake (GNOC) in the diet of Labeo rohita. The results indicate that increasing levels of EFS incorporation led to a progressive decline in growth performance, suggesting that EFS can be utilized only at limited inclusion levels without compromising fish growth.

Raw Eichhornia crassipes is characterized by moderate crude protein and high fiber content, which limits its direct use as a feed ingredient. In the present study, raw Eichhornia leaves and stems showed crude protein contents of 18.5% and 6%, respectively, with high fiber levels. Similar proximate compositions have been reported by Dairo (1997) and Arayana et al. (1984). Fermentation significantly improved the nutritional quality of Eichhornia by increasing crude protein to 25% and reducing fiber content to 12.84%, supporting earlier findings that microbial fermentation enhances nutrient availability and reduces anti-nutritional factors in plant-based feed ingredients (Wee, 1991; Bairagi et al., 2002, 2004; Saha & Ray, 2011; Ali & Kaviraj, 2018).

Despite nutritional improvement through fermentation, growth parameters such as final weight, body weight gain, and specific growth rate showed decreasing trends with increasing EFS inclusion. This reduction may be attributed to the relatively higher fiber content of EFS compared to GNOC and the acidic nature of fermented silage, which can impair feed palatability, digestibility, and nutrient assimilation (Stickney & Shumway, 1974; Hardy et al., 1984; Siddique, 2009). High dietary fiber is known to reduce digestibility and growth in fish by limiting nutrient absorption (Hastings, 1964; De Silva et al., 1990; Ramachandran & Ray, 2007). Similar growth depression with silage-based feeds has been reported by Hardy et al. (1984), although contrasting observations exist depending on species and silage type (Ramasubharayan et al., 2013; Tanuja et al., 2016).

Feed conversion ratio increased with higher EFS incorporation, indicating reduced feed utilization efficiency. This trend is consistent with the observed decline in growth and may be linked to lower digestibility and possible residual acidity of the diets. Protein efficiency ratio (PER) and lipid efficiency ratio (LER) also declined with increasing EFS levels, reflecting reduced efficiency of nutrient utilization at higher inclusion rates. Survival rate was not affected by EFS-based diets, indicating that fermented Eichhornia did not exert any toxic or adverse physiological effects on L. rohita. Similar findings have been reported for E. crassipes inclusion in fish diets by Sotolu and Sule (2011), Tanuja et al. (2016), and Serrano et al. (2017). Hepatosomatic index (HSI) remained statistically non-significant among treatments, suggesting no severe metabolic stress, although a slight reduction at the highest EFS level may indicate altered energy metabolism.

Water quality parameters throughout the experimental period remained within acceptable ranges for carp culture, confirming that environmental factors did not confound dietary effects. A slight reduction in pH with increased EFS inclusion was observed but remained within optimal limits for fish growth (Bhatnagar & Singh, 2010; Kumari et al., 2017). Economically, the use of EFS reduced feed cost by approximately 8%, improving economic conversion ratio and supporting the potential of plant-based ingredients for cost-effective aquaculture feeds (Hardy & Tacon, 2002; Sarker et al., 2020). Additionally, the utilization of Eichhornia contributes to environmental management by valorizing an invasive aquatic weed.

Genotoxicity assessment revealed no DNA damage in fish fed EFS-based diets, as evidenced by the absence of comet tails, indicating that fermentation effectively mitigated potential anti-nutritional or toxic effects associated with plant-based ingredients (Wu & Sun, 2011).

5. CONCLUSION

In conclusion, fermented Eichhornia silage can be used as a partial replacement for GNOC in *Labeo rohita* diets at lower inclusion levels. However, higher incorporation adversely affects growth and feed efficiency, emphasizing the need for optimized inclusion levels to balance nutritional performance and economic benefits.

REFERENCES

Ali, S., & Kaviraj, A. (2018). Aquatic weed Ipomoea aquatica as feed ingredient for rearing Rohu, Labeo rohita (Hamilton). The Egyptian Journal of Aquatic Research, 44(4), 321-325. https://doi.org/10.1016/j.ejar.2018.09.004

Arayana, G. L., Rao, K. S., Pantulu, A. J., & Thyagarajan, G. J. A. B. (1984). Composition of lipids in roots, stalks, leaves and flowers of Eichhornia crassipes (Mart.) Solms. Aquatic botany, 20(3-4), 219-227. https://doi.org/10.1016/0304-3770(84)90088-3

Atack, T.H., Jauncey, K. and Matty, A.J. (1979). The utilization of some single cell proteins by fingerlings minor carp (Cyprinus carpio). Aquaculture, 18: 337- 348. https://doi.org/10.1016/0044-8486(79)90037-1

Bairagi, A., Sarkar Ghosh, K., Sen, S., & Ray, A. (2002). Duckweed (Lemna polyrhiza) leaf meal as a source of feedstuff in formulated diets for rohu (Labeo rohita Ham.) fingerlings after fermentation with a fish intestinal bacterium. Bioresource Technology, 85(1), 17–24. https://doi.org/10.1016/s0960-8524(02)00067-6

Bairagi, A., Sarkar Ghosh, K., Sen, S. K., & Ray, A. K. (2004). Evaluation of the nutritive value of Leucaena leucocephala leaf meal, inoculated with fish intestinal bacteria Bacillus subtilis and Bacillus circulans in formulated diets for rohu, Labeo rohita (Hamilton) fingerlings. Aquaculture Research, 35(5), 436–446. https://doi.org/10.1111/j.1365-2109.2004.01028.x

Bhatnagar, A., & Devi, P. (2013). Water quality guidelines for the management of pond fish culture. International journal of environmental sciences, 3(6), 1980-2009. https://doi.org/10.6088/ijes.2013030600019

Bhatnagar, A., & Singh, G. (2010). Culture fisheries in village ponds: a multi-location study in Haryana, India. Agriculture and Biology Journal of North America, 1(5), 961–968. https://doi.org/10.5251/abjna.2010.1.5.961.968

Buddington, R.K. (1980) Hydrolysis-resistant organic matter as a reference for measurement of fish digestion efficiency. Transactions of the American Fisheries Society 109, 653–656. https://doi.org/10.1577/1548-8659(1980)109<653:HOMAAR>2.0.CO;2

Chiang, G., Lu, W. Q., Piao, X. S., Hu, J. K., Gong, L. M., & Thacker, P. A. (2009). Effects of Feeding Solid-state Fermented Rapeseed Meal on Performance, Nutrient Digestibility, Intestinal Ecology and Intestinal Morphology of Broiler Chickens. Asian-Australasian Journal of Animal Sciences, 23(2), 263–271. https://doi.org/10.5713/ajas.2010.90145

Dada SA (2002). The utilization of water hyacinth (Eichhornia crassipes) by West African dwarf (wad) growing goats. African Journal of Biomedical Research, 4: 147-149. 10.4314/ajbr.v4i3.53904

Dairo, F. A. S. (1997). Evaluation of water hyacinth (eichornia crassipes) as feed ingredient and yolk colouring agent in layers diets. Nigerian Journal of Animal Production, 24(1), 43-45. https://doi.org/10.51791/njap.v24i1.2349

Dawood, M. A., Koshio, S., & Esteban, M. N. (2017). Beneficial roles of feed additives as immunostimulants in aquaculture: a review. Reviews in Aquaculture, 10(4), 950–974. https://doi.org/10.1111/raq.12209

De Silva, S. S., Shim, K. F., & Khim Ong, A. (1990). An evaluation of the method used in digestibility estimations of a dietary ingredient and comparisons on external and internal markers, and time of faeces collection in digestibility studies in the fish Oreochromis aureus (Steindachner). Reproduction, nutrition, development, 30(2), 215–226. https://doi.org/10.1051/rnd:19900207

Emshaw, Y., Getahun, A., & Geremew, A. (2021). The effect of Aspergillus niger on the nutritive value of water hyacinth as fish feed using solid state fermentation. Ethiopian Journal of Biological Sciences, 20(2), 143-154.

Flores-Miranda, M. D. C., Luna-González, A., Cortés-Espinosa, D. V., Álvarez-Ruiz, P., Cortés-Jacinto, E., Valdez-González, F. J., Escamilla-Montes, R., & González-Ocampo, H. A. (2014). Effects of diets with fermented duckweed (Lemna sp.) on growth performance and gene expression in the Pacific white shrimp, Litopenaeus vannamei. Aquaculture International, 23(2), 547–561. https://doi.org/10.1007/s10499-014-9835-x

Global Report on Food Crises - 2022 **|** World Food Programme (wfp.org)

Grodowitz, M. J. (1998). An active approach to the use of insect biological control for the management of non-native aquatic plants. Journal of aquatic plant management, 36, 57-61. https://www.researchgate.net/publication/299205040

Gullu, K., Acar, U., Tezel, R. and Yozukmaz, A. (2014). Replacement of Fish Meal with Fish Processing By-Product Silage in diets for the Rainbow Trout, Oncorhynchus mykiss. Pakistan J. Zool., 46:1697-1703. Microsoft Word - 1697-1703 (29) PJZ-1960-14 23-9-14 Revised_Version (zsp.com.pk)

Ha, N., Jesus, G. F. A., Gonçalves, A. F. N., de Oliveira, N. S., Sugai, J. K., Pessatti, M. L., Mouriño, J. L. P., & el Hadi Perez Fabregat, T. (2019). Sardine (Sardinella spp.) protein hydrolysate as growth promoter in South American catfish (Rhamdia quelen) feeding: Productive performance, digestive enzymes activity, morphometry and intestinal microbiology. Aquaculture, 500, 99–106. https://doi.org/10.1016/j.aquaculture.2018.10.004

Hajra A, Mazumder A, Verma A, Ganguly DP, Mohanty BP, Sharma AP (2013) Antinutritional factors in plant origin fish feed ingredients: the problems and probable remedies. Advances in Fish Research 5: 193–202.

Hardy, R. W., & Tacon, A. G. (2002). Fish meal: Historical uses, production trends and future outlook for sustainable supplies. In R. R. Stickney & J. P. McVey (Eds.), Responsible Marine Aquaculture (311– 325). Wallingford, UK: CABI Publishing. 10.1079/9780851996042.0311

Hardy, R. W., Shearer, K. D., & Spinelli, J. (1984). The nutritional properties of co-dried fish silage in rainbow trout (Salmo gairdneri) dry diets. Aquaculture, 38(1), 35–44. https://doi.org/10.1016/0044-8486(84)90135-2

Hassaan, M., Goda, A. S., & Kumar, V. (2016). Evaluation of nutritive value of fermented de-oiled physic nut, Jatropha curcas, seed meal for Nile tilapia Oreochromis niloticus fingerlings. Aquaculture Nutrition, 23(3), 571–584. https://doi.org/10.1111/anu.12424

Hastings, W.H. 1964. Fish feed processing research. Feedstuffs, 36: 13-21.

Hlophe, S., Moyo, N., & Sara, J. (2011). Use of Kikuyu Grass as a Fishmeal Substitute in Practical Diets for Tilapia rendalli. Asian Journal of Animal and Veterinary Advances, 6(11), 1076–1083. https://doi.org/10.3923/ajava.2011.1076.1083

Hossain, M. E., Sikder, H., Kabir, M. H., & Sarma, S. M. (2015). Nutritive value of water hyacinth (Eichhornia crassipes). Online J. Anim. Feed Res, 5(2), 40-44. http://www.science-line.com/index/; http://www.ojafr.ir

Jackson, A., Capper, B., & Matty, A. (1982). Evaluation of some plant proteins in complete diets for the tilapia Sarotherodon mossambicus. Aquaculture, 27(2), 97–109. https://doi.org/10.1016/0044-8486(82)90129-6

Jannathulla, R., Dayal, J. S., Vasanthakumar, D., Ambasankar, K., Panigrahi, A., & Muralidhar, M. (2019). Apparent digestibility coefficients of fungal fermented plant proteins in two different penaeid shrimps—A comparative study. Aquaculture Research, 50(5), 1491–1500. https://doi.org/10.1111/are.14024

Jhingran, V. G. (1991). Fish and Fisheries of India, Hindustan Publishing Corp. India) New Delhi 727p.

Khan, M. A., Jafri, A. K., Chadha, N. K., & Usmani, N. (2003). Growth and body composition of rohu (Labeo rohita) fed diets containing oilseed meals: partial or total replacement of fish meal with soybean meal. Aquaculture Nutrition, 9(6), 391–396. https://doi.org/10.1046/j.1365-2095.2003.00268.x

Klaude, M., Eriksson, S., Nygren, J., & Ahnström, G. (1996). The comet assay: mechanisms and technical considerations. Mutation Research/DNA Repair, 363(2), 89–96. https://doi.org/10.1016/0921-8777(95)00063-1

Kumari, R., Ojha, M. L., Saini, V. P., & Sharma, S. K. (2017). Effect of Azolla supplementation on proximate composition and digestibility of Labeo rohita (Ham.) fingerlings. Journal of Entomology and Zoology, 5, 715-718. https://www.researchgate.net/profile/V-P-Saini/publication/319980201

Mohamed, H. M., Emeish, W. F., Braeuning, A., & Hammad, S. (2017). Detection of aflatoxin-producing fungi isolated from Nile tilapia and fish feed. EXCLI journal, 16, 1308. http://dx.doi.org/10.17179/excli2017-960

Narayan, S. P., Anitha, K., & Prasad, S. N. (2007). Water hyacinth (Eichhornia crassipes) management of an invasive weed, the Indian scenario. Sarovar Saurabh-Envis newsletter wetland ecosyst, 3(4), 3-7. Sarovar_Saurabh_VOL_3_4-with-cover-page-v2.pdf (d1wqtxts1xzle7.cloudfront.net)

Ndimele, P., & Kumolu-Joh, C. (2012). Some Aspects of the Physicochemistry and Heavy Metal Content of Water, Sediment and Cynothrissa mento (Regan, 1917) from Badagry Creek, Lagos, Nigeria. Trends in Applied Sciences Research, 7(9), 724–736. https://doi.org/10.3923/tasr.2012.724.736

Pratiwi, D. Y., & Pratiwy, F. M. (2021). Effect of fermented water hyacinth (Eichhornia crassipes) on growth performance of fish. International Journal of Fisheries and Aquatic Studies, 9(4), 139–141. https://doi.org/10.22271/fish.2021.v9.i4b.2533

Ramachandran, S., & Ray, A. K. (2004). Inclusion of extruded grass pea, Lathyrus sativus seed meal in compound diets for rohu, Labeo rohita (Hamilton, 1822) fingerlings. Acta Ichthyologica et Piscatoria, 34(2), 205–218. https://doi.org/10.3750/aip2004.34.2.08

Ramachandran, S., & Ray, A. K. (2007). Nutritional evaluation of fermented black gram (Phaseolus mungo) seed meal in compound diets for rohu, Labeo rohita (Hamilton), fingerlings. Journal of Applied Ichthyology, 23(1), 74–79. https://doi.org/10.1111/j.1439-0426.2006.00772.x

Ramasubharayan, R., Iyapparaj, P., Subhashini, K. J., Chandran, M. N., Palavesam, A., & Immanuel, G. (2013). Characterization and nutritional quality of formic acid silage developed from marine fishery waste and their potential utilization as feed stuff for common carp Cyprinus carpio fingerlings. Turkish Journal of Fisheries and Aquatic Sciences, 13(2). https://doi.org/10.4194/1303-2712-v13_2_10

Saha, S. & Ray, A. K. (2011). Evaluation of Nutritive Value of Water Hyacinth (Eichhornia crassipes) Leaf Meal in Compound Diets for Rohu, Labeo rohita (Hamilton, 1822) Fingerlings after Fermentation with Two Bacterial Strains Isolated from Fish Gut. Turkish Journal of Fisheries and Aquatic Sciences, 11 (2), 199-207. Retrieved from https://dergipark.org.tr/en/pub/trjfas-ayrildi/issue/13273/160339

Sahzadi, T., Salim, M., & Shahzad, K. (2006). Growth performance and feed conversion ratio (FCR) of hybrid fingerlings (Catla catla× Labeo rohita) fed on cottonseed meal, sunflower meal and bone meal. Pakistan Veterinary Journal, 26(4), 163-166. https://www.researchgate.net/publication/26520458

Samal, B., Kumar, S., & Singh, V. (2022). Constraints Related to Production and Marketing of Freshwater Fish Rohu (Labeo rohita) in Cuttack District of Odisha. Asian Journal of Agricultural Extension, Economics & Sociology, 81–85. https://doi.org/10.9734/ajaees/2022/v40i830939

Santhosh, B., & Singh, N. P. (2007). Guidelines for water quality management for fish culture in Tripura. ICAR Research Complex for NEH Region, Tripura Center, Publication, 29(10). https://www.researchgate.net/publication/320322935_Guidelines_for_water_quality_management_for_fish_culture_in_Tripura_Publication_No_27_Santhosh_B_and_Singh_NP_2007ICAR_Research_Complex_for_NEH_Region_Tripura_Centre_Lembucherra_West_Tripura_799210_In

Sarker, M. A. A., Nahar, K., Banu, H., & Nesa, T. (2020). Incorporation of Water Hyacinth, Eichhornia crassipes Meal in Aqua-feed and its Efficacy on Growth Performance of roho labeo, Labeo rohita (Hamilton, 1822) Reared in Cagev. International Journal of Aquaculture and Fishery Sciences, 6(2), 043–049. https://doi.org/10.17352/2455-8400.000055

Serrano, A. E., Declarador, R. S., Sedanza, M. G. C., & Tumbokon, B. L. M. (2017). Replacement of Soybean Meal with Rhizoclonium riparium Protein Concentrate in the Diet of Pacific White Shrimp Litopenaeus vannamei Postlarvae. Israeli Journal of Aquaculture - Bamidgeh, 69. https://doi.org/10.46989/001c.20871

Siddik, M. A., Howieson, J., & Fotedar, R. (2019). Beneficial effects of tuna hydrolysate in poultry by-product meal diets on growth, immune response, intestinal health and disease resistance to Vibrio harveyi in juvenile barramundi, Lates calcarifer. Fish & Shellfish Immunology, 89, 61–70. https://doi.org/10.1016/j.fsi.2019.03.042

Siddique Milad El-Ajnaf. (2009). Development of fermented fish silage derived from pelagic sardine and apple pomace by-product for inclusion in diets for the European seabass (Dicentrarchus labrax). Ph.D. Thesis submitted to the University of Plymouth. http://hdl.handle.net/10026.1/2720

Singh, N. P., McCoy, M. T., Tice, R. R., & Schneider, E. L. (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. Experimental Cell Research, 175(1), 184–191. https://doi.org/10.1016/0014-4827(88)90265-0

Sotolu AO, & Sule SO. (2011). Digestibility and performance of water hyacinth meal in the diets of African catfish Clarias gariepinus. Tropical and Subtropical Agroecosystems. 14, 245-250. http://www.redalyc.org/articulo.oa?id=93915703024

Sotolu, A. (2012). Management and Utilization of Weed: Water Hyacinth (Eichhornia crassipes) for Improved Aquatic Resources. Journal of Fisheries and Aquatic Science, 8(1), 1–8. https://doi.org/10.3923/jfas.2013.1.8

Stickney, R. R., & Shumway, S. E. (1974). Occurrence of cellulase activity in the stomachs of fishes. Journal of Fish Biology, 6(6), 779–790. https://doi.org/10.1111/j.1095-8649.1974.tb05120.x

Stone, N. M., & Thomforde, H. K. (2004). Understanding your fish pond water analysis report (pp. 1-4). Cooperative Extension Program, University of Arkansas at Pine Bluff, US Department of Agriculture and county governments cooperating. https://www.uaex.uada.edu/publications/PDF/FSA-9090.pdf

Su, W., Sun, Q., Xia, M., Wen, Z., & Yao, Z. (2018). The Resource Utilization of Water Hyacinth (Eichhornia crassipes [Mart.] Solms) and Its Challenges. Resources, 7(3), 46. https://doi.org/10.3390/resources7030046

Sun, H., Tang, J. W., Yao, X. H., Wu, Y. F., Wang, X., Liu, Y., & Lou, B. (2015). Partial substitution of fish meal with fermented cottonseed meal in juvenile black sea bream (Acanthopagrus schlegelii) diets. Aquaculture, 446, 30–36. https://doi.org/10.1016/j.aquaculture.2015.04.020

Tanuja, S., Kumar, A., & Nayak, S. (2016). Effect of dietary intake of acid ensiled fish waste on the growth, feed utilization, hematology and serum biochemistry of rohu Labeo rohita,(Hamilton, 1822) fingerlings. Indian Journal of Animal Research, 0f. https://doi.org/10.18805/ijar.v0i0f.3792

Tucker, C., & Debusk, T. (1981). Seasonal growth of Eichhornia crassipes (Mart.) solms: Relationship to protein, fiber, and available carbohydrate content. Aquatic Botany, 11, 137–141. https://doi.org/10.1016/0304-3770(81)90055-3

Wee, K.L. (1991). Use of non-conventional feedstuff of plant origin as fish feeds – is it practical and economically feasible? In: S.S. De Silva (Ed.), Fish Nutrition Research in Asia. Proceedings of the Fourth Asian Fish Nutrition Workshop. Asian Fisheries Society, Special Publication 5, Manila, Philippines: 13-32.

Wu, W., & Sun, Y. (2011). Dietary safety evaluation of water hyacinth leaf protein concentrate. Human & experimental toxicology, 30(10), 1514-1520. https://doi.org/10.1177/0960327110392085

Zhang, D., Zhang, Y., Liu, B., Jiang, Y., Zhou, Q., Wang, J., ... & Kuang, Q. (2017). Effect of replacing fish meal with fermented mushroom bran hydrolysate on the growth, digestive enzyme activity, and antioxidant capacity of allogyno genetic crucian carp (Carassius auratus gibelio). Turkish Journal of Fisheries and Aquatic Sciences, 17(5), 1039-1048. https://doi.org/10.4194/1303-2712-v17_5_20

APPENDIX

1.2 Fermented Silage

1N formic acid 120ml/5kg of eichhornia

During the preparation of fermented silage 120 ml of 1N formic acid to reduce pH during fermentation

2.1 Crude Protein

40% NaOH	400gm/L
4% H_3BO_3	40gm/L
0.1N HCL	8.3ml/L
Conc. H_2SO_4	20 ml/1gm sample
K_2SO_4	9gm/1gm sample
$CuSO_4$	1gm/1gm sample

Chemicals were prepared in double distilled water, by using the above concentrations, for every 1gm of the sample, the used amount of chemicals were as per above.

2.2 Crude Lipid

Petroleum ether 150 ml/1gm sample

2.3 Crude fiber

H_2SO_4

NaOH

3**.1 Dissolved oxygen**

Sodium thiosulphate	1.25gm/L
Starch solution	1gm/100mL
Conc. H_2SO_4	2 ml/125ml sample
Winkler A	
manganous sulphate	20gm/100ml

20gm of manganous sulphate, add distilled water to make final volume 100ml

Winkler B	
Sodium hydroxide	41gm/100ml
Potassium iodate	25 gm/100ml

41 gm of Sodium hydroxide and 25 gm of Potassium iodate to make the final volume 100ml.

3.2 Alkalinity

0.05N Na_2CO_3	3-5gm/L
0.1N HCL	2.8ml/L

2.3 Hardness

Stock solution

NH_4CL	16.9gm/143 conc. NH_4OH
Magnesium salt	250mg/100ml sample
Eriochrome Black T	0.5gm/100ml triethanolamine