

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*)

UNDER PEER REVIEW



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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.

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

22 **1. INTRODUCTION**

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

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

40 Fermentation is an economical and effective processing method that can improve the
41 nutritional quality of plant-based feed ingredients by reducing fiber and anti-nutritional
42 compounds while enhancing protein availability, amino acid profile, digestibility, and
43 palatability (Ndimele & Kumolu-Johnson, 2012; Flores-Miranda *et al.*, 2014; Zhang *et al.*,
44 2017). Fermented feed ingredients have been successfully used in livestock, poultry, and
45 aquaculture to improve growth performance, feed utilization, and disease resistance (Chiang
46 *et al.*, 2009; Ha *et al.*, 2019).

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

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51 **2. MATERIALS AND METHODS**

52 **Preparation of *Eichhornia* Fermented Silage.**

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

61 **Proximate Analysis**

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

65 **Growth Trial**

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

70 Five isonitrogenous and isolipidic diets were formulated by replacing groundnut oil cake
71 (GNOC) with Eichhornia-based fermented silage (EFS) at 0% (control), 25%, 50%, 75%, and
72 100%. Feed ingredients were weighed, mixed, ground, and dough was prepared with water,
73 then cooked in a vertical autoclave for 10–15 minutes. Vitamin–mineral premix and oil were
74 added before pelletization. Semi-sinking pellets were sun-dried, resized, and stored in
75 airtight containers.

76 The experiment used a completely randomized design (CRD) with five treatments and three
77 replicates each. Fish were stocked at 1 g/L and fed at 10% of their body weight for the first
78 30 days, then at 8%. Continuous aeration was provided, with daily siphoning to remove
79 feces and uneaten feed, and up to 30% water exchange to replace losses. Growth sampling
80 was done every two weeks, and growth, feed utilization, biometric indices (HSI and ISI), and
81 survival rates were calculated using standard formulas.

82 **Water Quality Parameters**

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

88 **Economics of Feed and Genotoxicity**

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

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

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

100 **Formulas for the calculation of growth parameters**

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

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

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

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

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

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

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

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

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

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

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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)

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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

128 the control ($921.78 \pm 69.33a$) to T4 ($555.21 \pm 31.62c$). In T1 ($919.75 \pm 73.67a$), it was not
 129 significantly different from the control ($p > 0.05$). The results for initial weight (INWT in g),
 130 final weight (FLWT in g), specific growth rate (SGR), feed conversion ratio (FCR), and feed
 131 conversion efficiency (FCE) are detailed in Table 5. The initial weight used in the experiment
 132 was 0.78 ± 0.04 g, while the final weight showed significant differences. The SGR was not
 133 significantly different between the control and T1, but showed decreasing trends in the
 134 remaining treatments. The control group had the best FCR, while T1 had the best FCE. PER
 135 and LER also exhibited decreasing trends from the control to T4. The hepatosomatic index
 136 (HSI) was not significantly different ($p > 0.05$) between the control and T3, while T4 showed
 137 a moderately lower value. The intact survival index (ISI) was not significantly different ($p >$
 138 0.05) among all treatments and the control. Fish survival remained unaffected throughout the
 139 experiment, indicating no negative impact from EFS (Fig. 1).

140 **Table 2: Ingredients incorporated in feed formulation and their proximate**
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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

143 (C(100%GNOC), T1(75%GNOC+25%Silage), T2(50%GNOC+50%Silage),
 144 T3(25%GNOC+75%Silage), T4(0%GNOC+100%Silage)); **GNOC Ground nut oil cake; *Nutritional
 145 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,
 146 Sulphur 7.2g, Copper 4.2g, Magnesium 6000mg, Iron 1500mg, Manganese 1500mg, Iodine 325mg,
 147 Cobalt 150mg, Potassium 100mg, Chromium 75mg, Selenium 7.5mg, Sodium 6mg, Calcium 191g,
 148 Phosphorous 95g. (CP = crude protein; CL = crude lipid; CF = crude fiber; DM= Dry matter *DB = Dry
 149 weight based; **WB = Wet weight based)
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151 **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

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

Treatments	Body Weight Gain (%)			
	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

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

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

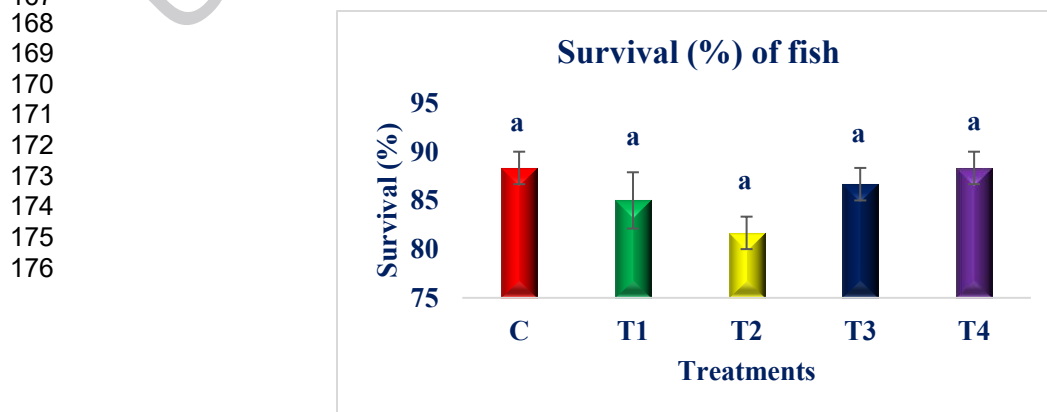
TREATMENTS	INWT	FLWT	SGR	FCR	FCE
C	0.77±0.05 ^a	8.88±0.04 ^a	3.87±0.11 ^a	2.42±0.05 ^c	0.41±0.01 ^{ab}
T1	0.75±0.05 ^a	8.63±0.11 ^a	3.86±0.12 ^a	2.46±0.02 ^{bc}	0.41±0.00 ^a
T2	0.80±0.01 ^a	8.20±0.26 ^a	3.67±0.02 ^{ab}	2.49±0.03 ^{bc}	0.40±0.00 ^{ab}
T3	0.84±0.01 ^a	7.10±0.29 ^b	3.37±0.06 ^{bc}	2.61±0.02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

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

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163 **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

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



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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

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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

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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)

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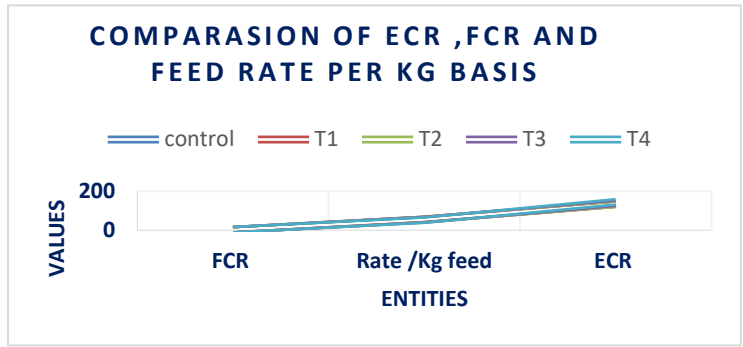


Fig. 2: Comparison of ECR, FCR, and feed rate per kg basis

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.

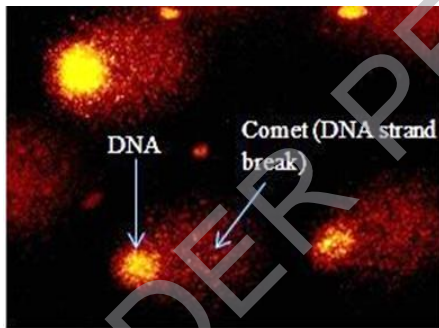


Fig. 3: Reference image of a comet assay

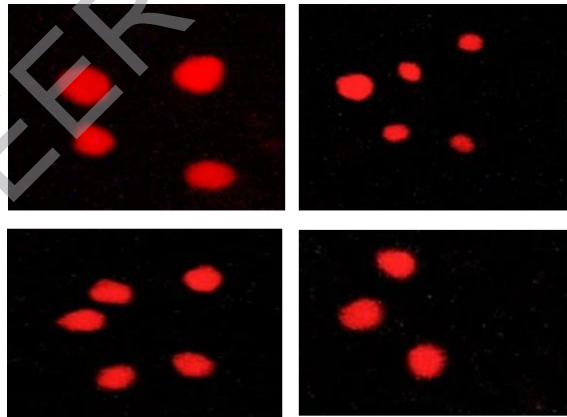


Fig. 4: Observed slides from different treatments

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249 **4. DISCUSSION**

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

256 Raw *Eichhornia crassipes* is characterized by moderate crude protein and high fiber content,
257 which limits its direct use as a feed ingredient. In the present study, raw *Eichhornia* leaves
258 and stems showed crude protein contents of 18.5% and 6%, respectively, with high fiber
259 levels. Similar proximate compositions have been reported by Dairo (1997) and Arayana et
260 al. (1984). Fermentation significantly improved the nutritional quality of *Eichhornia* by
261 increasing crude protein to 25% and reducing fiber content to 12.84%, supporting earlier
262 findings that microbial fermentation enhances nutrient availability and reduces anti-nutritional
263 factors in plant-based feed ingredients (Wee, 1991; Bairagi et al., 2002, 2004; Saha & Ray,
264 2011; Ali & Kaviraj, 2018).

265 Despite nutritional improvement through fermentation, growth parameters such as final
266 weight, body weight gain, and specific growth rate showed decreasing trends with increasing
267 EFS inclusion. This reduction may be attributed to the relatively higher fiber content of EFS
268 compared to GNOC and the acidic nature of fermented silage, which can impair feed
269 palatability, digestibility, and nutrient assimilation (Stickney & Shumway, 1974; Hardy et al.,
270 1984; Siddique, 2009). High dietary fiber is known to reduce digestibility and growth in fish
271 by limiting nutrient absorption (Hastings, 1964; De Silva et al., 1990; Ramachandran & Ray,
272 2007). Similar growth depression with silage-based feeds has been reported by Hardy et al.
273 (1984), although contrasting observations exist depending on species and silage type
274 (Ramasubharayan et al., 2013; Tanuja et al., 2016).

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

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

295 Genotoxicity assessment revealed no DNA damage in fish fed EFS-based diets, as
296 evidenced by the absence of comet tails, indicating that fermentation effectively mitigated

297 potential anti-nutritional or toxic effects associated with plant-based ingredients (Wu & Sun,
298 2011).

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300 5. CONCLUSION

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

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539
 540 **APPENDIX**
 541

- 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 ₃ BO ₃ | 40gm/L |
| 0.1N HCL | 8.3ml/L |
| Conc. H ₂ SO ₄ | 20 ml/1gm sample |
| K ₂ SO 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 ₂ SO ₄	
	NaOH	
3.1	Dissolved oxygen	
	Sodium thiosulphate	1.25gm/L
	Starch solution	1gm/100mL
	Conc. H ₂ SO 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 ₂ CO ₃	3-5gm/L
	0.1N HCL	2.8ml/L
2.3	Hardness	
	Stock solution	
	NH ₄ CL	16.9gm/143 conc. NH ₄ OH
	Magnesium salt	250mg/100ml sample
	Eriochrome Black T	0.5gm/100ml triethanolamine