

## Evaluation of *Nymphaea nouchali* leaf meal based diets on growth, digestive enzymes and haematological parameters of *Labeo catla* fingerlings (Hamilton, 1822)

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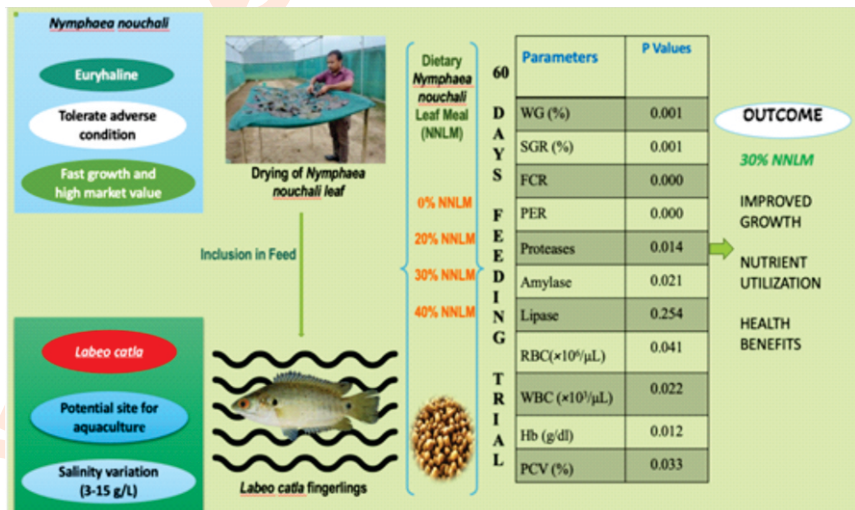
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### Abstract

**Aim:** This study was conducted to evaluate the nutraceutical traits of *Nymphaea nouchali* herbal plant ingredients on the growth performance, digestive enzymes and health status of *Labeo catla* fingerlings owing to the abundance of phytoconstituents.

**Methodology:** Four isonitrogenous and isocaloric diet with different inclusion levels of *Nymphaea nouchali* leaf meal (NNLM) i.e., 0%, 20%, 30% and 40% were formulated and designated as T-0, T-I, T-II and T-III treatment groups. A total of 144 fingerlings of *L. catla* were randomly distributed into the experimental cisterns in triplicates following completely randomized design.

**Results:** At the end of the experiment, the percent weight gain and specific growth rate (SGR) were significantly higher ( $P < 0.05$ ) with significantly lower ( $P < 0.05$ ) feed conversion ratio (FCR) in T-II group as compared to the other experimental groups. Significantly lower ( $P < 0.05$ ) percent weight gain and SGR with significantly higher ( $P < 0.05$ ) FCR were recorded in T-0 and T-III. The protease and amylase activities were found to be significantly higher ( $P < 0.05$ ) in the T-II with lower ( $P < 0.05$ ) values recorded in T-0 and T-III groups. The red blood cell count and haemoglobin level were found to be significantly higher ( $P < 0.05$ ) in the T-I and T-II groups as compared to T-0 and T-III, respectively.



**Interpretation:** The present study demonstrated that dietary inclusion of *Nymphaea nouchali* leaf meal with 30% improved growth, nutrient utilization and better health benefits of *L. catla* fingerlings in the present culture condition.

**Key words:** Digestive enzymes, Herbal plant, *Labeo catla*, Leaf meal, *Nymphaea nouchali*

## Introduction

Aquaculture plays a major role in accomplishing nutritional security for the growing population and substantiates the economic benefits to the rural people. Aquaculture is the most growing sector in the animal based food production system and continuously upscaling to meet the global fish demand of 109 million tonnes by 2030 (FAO, 2022). However, the global food fish demand required additionally around 46.4 million tonnes of fish by 2050 to feed the growing population (Nazeemashahul et al., 2020; FAO, 2022). This demand could only be possible through feed-based intensive aquaculture where feed contributes 60-70% of the total input cost (Talukdar et al., 2020, 2021). Although, feed based intensive culture system often results in stress condition which leads to outbreak, mortality and lower growth rate of the cultured fish (Fallahpour et al., 2014). To cope with the growing food demand, the availability of sustainable animal protein from fish must be supported by feeding proper nutritional aquafeed that must have nutraceutical benefits in promoting growth and health of the cultured fish. Feeding nutraceutical feed attributes for having health benefits along with better growth, simultaneously minimizing the chances of disease incidence (Khan et al., 2022).

Therefore, nutritional manipulation is a reliable option for sustainable performance of the aquaculture sector within the changing environments and disease incidences. Aquatic and terrestrial macrophytes have been frequently used as non-conventional feed ingredients in aqua feed (Wee et al., 1991). Incorporation of plant based ingredients are less expensive than animal ingredients but the former contain anti-nutritional factor that limit its application in animal feed (Song et al., 2010). Although, several studies have evaluated the positive effect of aquatic plants such as *Lemna* sp., *Azolla* sp. *Ceratophyllum demersum*, *Hydrilla*, *Verticillata*, *Salvinia cucullata*, *Trapanatans*, *Lemna minor* and *Ipomoea reptans* on the growth of fish (Murthy et al., 1991; Munglue 2016; Rahman et al., 2019; Naseem et al., 2021). Herbal plants' having functional traits are considered to be the backbone of traditional medicines for humans in rural areas throughout the world (Dash et al., 2013; Nirmala and Kanchana, 2018). Recently, plants having functional bioactive components are gaining importance in animal nutrition due to their nutraceutical benefits (Doan et al., 2020).

Currently, herbal plants are being inherited in aquaculture as a safe and eco-friendly feed ingredient to enhance growth and immunity in fish (Tadase et al., 2021). Previous studies have reported the positive effect of herbs such as *Toona sensis* (Wu et al., 2010), *Psidium guajava* (Gobi et al., 2016), *Ocimum gratissimum* (Abdel-Tawwab et al., 2018), *Eriobotrya japonica* (Hoseinifar et al., 2018), *Rosemarinus officinalis* (Yousefi et al., 2019) and *Quercus castaneifolia* (Doan et al., 2020) on appetite, growth and immunity in fish. *Nymphaea nouchali* is a common emergent aquatic plant that can grow freely over the water surface or over marshy ground widely available in the North-eastern states of India (Devi et al., 2015). It is considered to be an

herbal plant having nutraceutical traits and mentioned as prominent herb in Ayurveda and Siddha system of medicines with multiple medicinal properties (Kiranmai et al., 2023; Sigha et al., 2023). *N. nouchali* is an important herbal plant with a wide range of biologically active phytoconstituents such as alkaloids, glucoside, essential oils, steroids, terpenoids, flavonoids and novel phenolic compounds that justifies its medicinal value (Sikder et al., 2012; Singh and Jain, 2017; Mishra et al., 2018).

Thus, identification of such novel plants having positive effects on the growth and immunity of fish is noteworthy in the aquaculture venture. Evaluation of *Nymphaea nouchali* leaf as a herbal plant ingredient in fish diet for improving growth and health needs more demonstration in different cultivable species. Previous study on feeding dietary *Nymphaea nouchali* leaf and flower significantly improves the growth and haematological parameters of *Labeo rohita* (Singha et al., 2023). This suggests the positive effects of *Nymphaea nouchali* in augmenting the growth of fish and opens up new avenues to explore in other cultivable fish species. Besides, the non-conventional alternative plant ingredients with low cost and high availability appear to be the key to cost effective aqua feed production. Therefore, the present study aimed to evaluate the effect of *Nymphaea nouchali* leaf meal (NNLM) based diet on the growth performance, digestive enzyme activities and health status of *Labeo catla* fingerlings in the present culture condition.

## Materials and Methods

**Experimental fish and design:** The experiment was conducted at farm site of Kamargaon, Dhemaji district of Assam, India from October, 2022 to December, 2022. Fingerlings of *L. catla* were procured from the local fish farm of Lakhimpur district, Assam, India. The fingerlings of *L. catla* were randomly distributed into five circular tanks (1000 l capacity) and kept for 15 days for acclimatization to the experimental condition. During the acclimatization period, fish were fed with commercial diet twice a day to satiation level. The experiment was conducted for a period of 90 days in twelve cemented cisterns (2.0m x 2.0m x 1.5m, 6000 l volume) designated as T0, T-I, T-II and T-III groups in triplicates with 0%, 20%, 30% and 40% inclusion level of NNLM, respectively. About 144 numbers of *L. catla* fingerlings (initial body weight  $10.74 \pm 0.10$ g) were randomly stocked into triplicate cisterns for each treatment group following a completely randomized design (CRD) with 12 fish for each cistern (stocking density 2 nos  $m^{-2}$ ). Before commencing the experiment, the bottom of the cisterns was provided with 6-inch soil bed and lime was applied @ 200 gm  $ha^{-1}$ .

The stocking of the experimental fish was done after one week of lime application. Based on the initial observation of feed consumption, all groups of fish were hand fed twice a day (09:00 and 18:00 h) to satiation level for 90 days. Water was exchanged at weekly basis during the experimental period to remove excreta and the leftover feed and replenished by equal volume of fresh water. Water quality parameters including water temperature,

**Table 1:** Ingredients and proximate composition of experimental diets

	Experimental diets			
	T-0	T-I	T-II	T-III
Ingredients (%)				
NNLM	0	20	30	40
MOC <sup>1</sup>	35	25	22	20
Fish meal <sup>1</sup>	13.5	13.5	13.5	13.5
Rice bran <sup>1</sup>	27	22	17	13
Wheat flour <sup>1</sup>	18	13	11	7
Fish oil: Veg oil (1:1) <sup>1</sup>	5	5	5	5
Vit-Min mix <sup>2</sup>	1	1	1	1
BHT <sup>3</sup>	0.1	0.1	0.1	0.1
CMC <sup>3</sup>	0.4	0.4	0.4	0.4
Total	100	100	100	100
Proximate composition of the diet (%; on dry matter basis, in triplicates)				
Moisture	6.87	6.68	6.76	6.83
Crude protein	35.33	35.26	35.26	35.21
Crude lipid	6.46	6.53	6.66	6.34
Crude fiber	4.31	5.49	5.46	5.46
Total ash	10.01	10.06	9.86	8.86
NFE	43.89	42.66	42.76	44.13
DE (MJ kg <sup>-1</sup> )	15.85	15.66	15.72	15.83

1 Procured from local market, India; 3 Procured from Himedia, India. NNLM; *Nymphaea nouchali* leaf meal, MOC: Mustard oil cake; 2 Composition of vitamin mineral mix (Premix Plus) (quantity kg<sup>-1</sup>) and vitamin A, 55,00,000 IU; vitamin D<sub>3</sub>, 11,00,000 IU; vitamin B<sub>2</sub>, 2000 mg; vitamin E, 750 mg; vitamin K, 1,000 mg; vitamin B<sub>6</sub>, 1,000 mg; vitamin B<sub>12</sub>, 6 mcg; calcium pantothenate, 2,500 mg; nicotinamide, 10 g; choline chloride, 150 g; Mn, 27,000 mg; I, 1,000 mg; Fe, 7,500 mg; Zn, 5,000 mg; Cu, 2000 mg; Co, 450 mg; L-lysine, 10 g; DL-methionine, 10 g; selenium, 125 mg; vitamin C, 2500 mg. Nitrogen free extract (NFE)=100–(CP+ EE + CF + TA), Digestible energy (MJ kg<sup>-1</sup>) = {(CP × 17) + (EE × 37) + (NFE × 17)} / 100 (Halver, 1976).

dissolved oxygen concentration, pH (Systronics, pH system 361), total alkalinity and ammonia were recorded at weekly interval following the standard methods of APHA (2017).

**Diet preparation:** *N. nouchali* was collected from the wetlands of Kamargaon village adjoining the KrishiVigyan Kendra, AAU, Dhemaji, Assam, India. After complete drying, the leaf was grounded into a fine powder with a grinder and sieved to obtain a homogeneous particle size. Four iso-caloric (15 MJ kg<sup>-1</sup>) and iso-nitrogenous (35% crude protein) diets were prepared having different level of *Nymphaea nouchali* leaf meal comprising other practical ingredients (Table 1). All the practical ingredients were properly mixed with required amount of water to make a dough and steam-cooked in a pressure cooker for 30 min. Oil and vitamins mineral mixture (Premix Plus, India) were added into the dough after cooling and the dough was pressed through a mechanical pelletizer (Uniextrude, S.B. Panchal and Company, India) of 2 mm diameter to prepare the pellets. The feed pellets were air-dried followed by drying in a hot air oven at 50 °C (Talukdar et al., 2020) until the moisture level was below 10%, labeled according to the treatments and stored at 4 °C until further use.

**Proximate Composition:** The proximate composition of the experimental diets and fish was determined following the

standard methods of AOAC (1995) for moisture, crude protein, ether extract, crude fiber and total ash. Moisture content was determined by drying samples in an oven at 102 °C till constant weight was obtained. Crude protein content was determined using an automated micro Kjeldahl assembly. Ether extract was determined by the solvent extraction method using the Soxhlet apparatus (Model SD2, 1045, PELICAN, India). Total ash content was determined by burning the samples in a muffle furnace at 550 °C for 6 hr. The crude protein was determined by Fibre tech.

**Sampling and sample preparation:** Three fish from each tank were anesthetized with clove oil (50 µl l<sup>-1</sup>) for collecting of blood following the guidelines of the Ethical Approval Committee, EAC at the College of Fisheries, Assam Agricultural University Assam. Blood samples were drawn from the caudal vein with a 1 ml hypodermic syringe and transferred immediately into Eppendorf tubes containing EDTA, gently shaken and preserved for the analysis of haematological parameters. Dissected tissues of intestine were collected and immediately homogenized with ice-cold 0.25 M sucrose solution in a glass tube using Teflon-coated mechanical tissue homogenizer (MICCRA D-9, ART Prozess and Labortechnik, Germany) to prepare a 5% tissue homogenate. The homogenate samples were centrifuged (5,000 rpm for 10 min at 4 °C) in a refrigerated-centrifuge (Thermo Scientific, USA) and the

**Table 2:** Growth performance, nutrient utilization and survival (%) of *Labeo catla* fingerlings fed diets containing different NNLM levels for 90 days

Parameters	Treatments				SEM	P values
	T-0	T-I	T-II	T-III		
Initial weight (g)	10.71	10.76	10.94	10.87	0.09	0.122
Initial length (cm)	11.39	11.47	11.49	11.38	0.12	0.104
Final weight (g)	125.83 <sup>a</sup>	142.23 <sup>b</sup>	158.63 <sup>c</sup>	126.85 <sup>ab</sup>	2.14	0.002
Final length (cm)	121.82 <sup>a</sup>	138.50 <sup>b</sup>	151.52 <sup>c</sup>	122.50 <sup>b</sup>	0.35	0.000
Final length (cm)	124.05 <sup>a</sup>	140.43 <sup>b</sup>	155.65 <sup>c</sup>	125.39 <sup>ab</sup>	2.25	0.001
Specific growth rate (%)	0.89 <sup>a</sup>	0.97 <sup>b</sup>	1.04 <sup>c</sup>	0.90 <sup>ab</sup>	0.02	0.001
Feed conversion ratio	3.18 <sup>c</sup>	3.01 <sup>b</sup>	2.75 <sup>a</sup>	3.23 <sup>b</sup>	0.23	0.000
Protein efficiency ratio	0.89 <sup>a</sup>	0.94 <sup>b</sup>	1.03 <sup>c</sup>	0.88 <sup>a</sup>	0.02	0.000
Survival (%)	100	100	100	100	-	-

Data expressed as mean values, n=3; Mean values in same row with different superscripts differ significantly (P<0.05). SEM: Standard error means.

supernatant was collected in sample vials and stored at -20°C until further use (Talukdar *et al.*, 2020). Quantification of protein in different tissues was estimated by the method of Lowry *et al.* (1951).

**Growth parameters, nutrient utilization and survival:** Fish were starved for 24 hr before taking the final body weight and were randomly collected at an interval of 15 days to determine the growth, length, feed utilization and survival. The parameters related to growth and nutrient utilization were calculated following the standard formulae of NRC (2011).

**Estimation of digestive enzyme:** The protease activity was assayed following the method of Drapeau (1974) using casein as substrate. Amylase activity was assayed by dinitro-salicylic-acid method of Rick and Stegbauer (1974). Lipase activity was estimated by Cherry and Crandall (1932) method and the reaction mixture was titrated against 0.05 N NaOH until it showed a permanent pink colour.

**Haematological parameters:** The red blood cell (RBC) count was done using Neubauer's counting chamber of a haemocytometer as described by Hendrick (1952). The white blood cells (WBC) count was performed following the method of Shaw (1930). The haemoglobin (Hb) content was estimated following the cyanmethemoglobin method as described by Van Kampen and Zijlstra (1961) using Drabkins Fluid (Qualigens, GlaxoSmithKline Pharmaceutical Ltd., India). The optical density (OD) was read at 540 nm, and the final concentration was calculated.

**Statistical analyses:** The data statistically analyzed using the software program SPSS 22.0 (IBM Inc., Chicago, USA) for Windows 10 and expressed as mean values and standard error mean (SEM). All data were subjected to One-way ANOVA and post-hoc analysis was carried out by Duncan's Multiple Range Test to compare the means for finding the significant difference at 5% probability level (P<0.05).

## Results and Discussion

The water temperature, dissolved oxygen concentration, pH, total alkalinity and ammonia ranged from 27-30 °C, 5.5-6.3 mg l<sup>-1</sup>, 7.9-8.4, 148-160 mg l<sup>-1</sup>, 0.012 - 0.034 mg l<sup>-1</sup>, respectively, throughout the experimental period as recommended for warm water fish culture (Boyd, 1992). The final length, final weight, weight gain, SGR and PER were significantly higher (P<0.05) in the T-II treatment group with significantly lower (P<0.05) values in the T-0 and T-III groups (Table 2). The FCR value was significantly higher (P<0.05) in the T-0 and T-III treatment groups whereas significantly lower (P<0.05) values were observed in T-II group followed by T-I treatment group (Table 2).

The improvement in the growth performance and feed efficiency in T-II group fed with diet containing 30 % NNLM could be due to the potentiality of the bioactive constituent's of *Nymphaea nouchali* in stimulating the secretion of digestive enzymes, thus facilitating better nutrient utilization and growth. Further, herbal plants are reported to be a prominent appetite stimulator that result in better feed and nutrient utilization (Citarasu, 2010; Xu *et al.*, 2020; Tadese *et al.*, 2021). The feed conversion ratio was lower in 30% NNLM fed group indicating better utilization of available nutrients by *L. catla* fingerlings, which correspond to the better growth observed in the same group. Garg *et al.* (2019) reported that supplementation of *Houttuynia cordata* leaf meal in the diet of *L. rohita* exhibited better growth performance, feed conversion ratio and protein efficiency ratio. Similarly, Kaleeswaran *et al.* (2011) also reported that *L. catla* fed with dietary *Cynodon dactyl* leaf meal showed an increase in weight gain, SGR and better FCR than the control diet. It has been reported that antinutritional factors beyond the tolerable limit could inhibit nutrient absorption and lead to reduced growth (Francis *et al.*, 2001; Frankic *et al.*, 2009). This could be the reason for lower growth performance and SGR with higher FCR found in the T-III group (40% NNLM). Similar to our study, Saha *et al.* (2017) and Bairagi *et al.* (2004), respectively reported better growth response and nutrient

**Table 3:** Digestive enzymes activities of *Labeo catla* fingerlings fed diets containing different NNLM levels for 90 days

Parameters	Treatments					SEM	P values
	T-0	T-I	T-II	T-III			
Proteases	6.48 <sup>a</sup>	7.02 <sup>b</sup>	7.78 <sup>c</sup>	6.55 <sup>ab</sup>		0.08	0.014
Amylase	3.02 <sup>a</sup>	3.77 <sup>b</sup>	3.89 <sup>b</sup>	3.12 <sup>a</sup>		0.15	0.021
Lipase	0.68	0.70	0.64	0.67		0.05	0.254

Data expressed as mean values (n=3); Mean values in the same row with different superscripts differ significantly (P<0.05). SEM: Standard error means; Protease activity is expressed as millimole of tyrosine released min<sup>-1</sup> g<sup>-1</sup> protein. Amylase activity is expressed as the micromole of maltose released from starch min<sup>-1</sup> mg<sup>-1</sup> protein. Lipase activity is expressed as units<sup>-1</sup> mg<sup>-1</sup> protein.

**Table 4:** Haematological parameters of *Labeo catla* fingerlings fed diets containing different NNLM levels for 90 days

Parameters	Treatments					SEM	P values
	T-0	T-I	T-II	T-III			
RBC(×106 μl <sup>-1</sup> )	1.97 <sup>a</sup>	2.25 <sup>b</sup>	2.36 <sup>b</sup>	2.10 <sup>ab</sup>		0.11	0.041
WBC (×103 μl <sup>-1</sup> )	20.36 <sup>a</sup>	25.92 <sup>b</sup>	26.80 <sup>bc</sup>	21.23 <sup>a</sup>		0.21	0.022
Hb (g d l <sup>-1</sup> )	8.93 <sup>a</sup>	12.40 <sup>b</sup>	13.70 <sup>c</sup>	9.77 <sup>a</sup>		0.24	0.012
PCV (%)	41.30 <sup>a</sup>	48.03 <sup>b</sup>	49.95 <sup>b</sup>	43.11 <sup>a</sup>		2.16	0.033

Data expressed as mean values (n=3); Mean values in the same row with different superscripts differ significantly (P<0.05). SEM: Standard error means; RBC: Red blood cell, Hb: Haemoglobin, WBC: White blood cell, PCV: Packed cell volume

utilization in *L. rohita* fingerlings fed with 30% *Eichhornia crassipes* and 30% *Leucaena leucocephala* leaf meal. Lower FCR with higher weight gain (%), SGR and PER in the T-II group could attribute towards better feed and nutrient utilization prompt by the optimal level of NNLM (30%), beyond which it negatively affects growth and nutrient utilization in *L. catla* fingerlings. Sivani *et al.* (2013) also reported that 30% to 40% inclusion level of *Nymphaea* leaf is optimum for better growth and health status of *L. rohita*.

The potency of digestive enzyme activities influences nutrient utilization and subsequently improves the growth performance of fish that depends on the availability of dietary nutrients in the intestine (Sagada *et al.*, 2017). Several herbal plants having bioactive compounds were considered potent stimulator of intestinal digestive enzymes to facilitate better nutrient utilization and assimilation in fish (Frankic *et al.*, 2009; Citarasu, 2010). In the present study, the protease activity was found to be significantly higher (P>0.05) in the T-II treatment group whereas significantly lower (P>0.05) value was observed in T-0 and T-III groups (Table 3). The amylase activity was found to be significantly higher (P>0.05) in T-I and T-II (P>0.05) groups whereas, significantly lower (P>0.05) activity was found with T-0 and T-III treatment groups (Table 3).

The higher activities of protease and amylase in NNLM fed groups upto 30% attributes towards optimal level or tolerable limit of ANF's in the diet which does not jeopardize the digestive

enzymes activity, thus enabling better nutrient utilization in the T-II group. The presence of higher ANF's in the diet significantly reduces the intestinal digestive enzyme activity as suggested by Chiow *et al.* (2016). Further, the results infer that fish try to maximize the utilization of available nutrients at most favorable level and further increase could not lead to the added benefits. Kurian *et al.* (2021), Talukdar *et al.* (2021) and Wang *et al.* (2018) reported that dietary *L. aspera* and Chinese herbal medicine mixture significantly enhanced the digestive enzyme activities in GIFT tilapia, *Rita rita* and Japanese seabass, respectively. The lipase was found to be similar which could be related to similar content of lipid in all the experimental diets.

Haematological parameters are considered to be potent marker of fish health and physiological status of fish in response to plant-based diet (Fawole *et al.*, 2017, 2020; NRC, 2011). In the present study, the WBC count, RBC count, Hb level and PCV were found to be significantly higher (P<0.05) in T-I and T-II groups which indicates the potency of *N. nouchali* to stimulate the non-specific immunity in *L. catla* fingerlings (Table 4). *N. nouchali* leaf is known to have a strong immunomodulatory effects due to the presence of several bioactive compounds such as quercetin, alkaloids, flavonoids and polyphenols (Kiranmai *et al.*, 2023). This may have facilitated the absorption of biologically potent nutrients from the intestine as supported by positive effect on growth and haematological parameters (Fawole *et al.*, 2020). Similar improvement in the haematological parameters by the dietary supplementation of leaf meal based diet or herbal

supplements were reported by Kurian *et al.* (2020) in *Nile tilapia* and Fawole *et al.* (2017) in *L. rohita* fingerlings. During study, the water quality parameters of the experimental cisterns were found to be within the desirable limits for optimal fish growth (water temperature-26.06-31.97°C, dissolve oxygen-5-15 mg l<sup>-1</sup>, pH-6.5-9, alkalinity->20 mg l<sup>-1</sup> and ammonia-<0.1 mg l<sup>-1</sup>) recommended for warm water fish culture (Boyd, 1998).

The present study concluded that NNLM beyond 30% in the diet negatively affect growth, nutrient utilization, digestive enzyme activities and health status of *L. catla* fingerlings. Therefore, this preliminary study demonstrated the potency of NNLM in augmenting the growth, better nutrient digestion and immunity in *L. catla* fingerlings. Therefore, the present study suggests that 30% NNLM based diet could be a potential alternative herbal feed for *L. catla* fingerlings to enhance its culture production and commercial application.

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**Ethical approval:** The experiment and subsequent handling and sampling of the experimental fish were carried out as per the Guidelines of College of Fisheries Science, Raha, Assam Agricultural University, Raha, Assam, India.

**Conflict of interest:** The authors declare that they have no sort of competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Data availability:** The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Consent to publish:** All authors agree to publish the paper in *Journal of Environmental Biology*.

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