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Effect of dietary *Houttuynia cordata* leaf meal and leaf extract on the growth performance, nutrient utilization and expression of IGF-I gene in *Labeo rohita*

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Abstract

A 60-day feeding trial was conducted to evaluate the effects of feeding *Houttuynia cordata* leaf meal and extract on the growth performance, nutrient utilization and expression of insulin-like growth factor-I (IGF-I) in *Labeo rohita* fingerlings. Two hundred seventy fingerlings were randomly distributed in six experimental groups in triplicates. Six isonitrogenous (350 g/kg CP) and isocaloric (17 MJ/kg DE) diets were prepared with graded levels of leaf meal (LM) and leaf extract (LE), namely C (control, without LE and LM), LE-0.25 (2.5 g/kg LE), LE-0.5 (5 g/kg LE), LE-1.0 (10 g/kg LE), LM-1.0 (10 g/kg LM) and LM-2.0 (20 g/kg LM). Significantly ($p < 0.05$) higher IGF-I expression, weight gain %, specific growth rate and lower feed conversion ratio were observed in LM-1.0 followed by LE-1.0 group compared to control group. Higher protease activity was observed in LE-1.0 compared to other experimental groups, while no significant changes were found for amylase and lipase activities. Compared to control, muscle alanine aminotransferase and aspartate aminotransferase activities were significantly higher in treatment groups except for LM-2.0. These results suggest that either LE-1.0 or LM-1.0 can be supplemented in the diet to improve the growth of *L. rohita*. However, the supplementation of 10 g *H. cordata* leaf meal/kg feed can be the better option considering the cost of extraction.

KEYWORDS
Growth promoter, *Houttuynia cordata*, IGF-I, *Labeo rohita*, leaf extract, leaf meal

1 | INTRODUCTION

Aquaculture has played a significant role in nutritional security, poverty reduction, as well as promoting the socioeconomic status of the poor people, especially in developing countries (Labh, Shakya, Gupta, Kumar, & Kayastha, 2017). The occurrence of diseases is a threat in fish production. However, feeding a proper diet improves the health condition and decreases the probability of diseases occurrence. There is a definite correlation between increased resistance to diseases, growth rate and survival (Fallahpour, Banaee, & Javadzade, 2014). Among many feed additives used to promote growth and immune system of fish, the herbal or natural product is always preferred due to their less residual effect and more consumer preferences (Harikrishnan, Balasundaram, & Heo, 2011). Abd El-Hakim, Ahmad, Azab, Lashien, and Baghdady (2010) observed an improved growth performance of fish with 10 g/kg inclusion of fennel (*Foeniculum vulgare*) seeds meal in the diet. Similar results were also observed in guppy, *Poecilia reticulate* (Cek, Turan, & Atik, 2007a); convict cichlid, *Cryptoheros nigrofasciatus*

(Cek, Turan, & Atik, 2007b); shrimps (Olmedo Sanchez, 2009); Nile tilapia, *Oreochromis niloticus* (Metwally, 2009); red seabream, *Pagrus major* (Ji et al., 2007); tilapia, *O. aureus* (Turan, 2006); rainbow trout, *Oncorhynchus mykiss* (Nya & Austin, 2009; Oskoii, Kohyani, Parseh, Salati, & Sadeghi, 2012) and zander, *Sander lucioperca* (Zakes, Kowalska, Demska-Zakes, Jeney, & Jeney, 2008), when fed with diets supplemented with various plants/plant extract.

Houttuynia cordata is a plant under the family Saururaceae and is native to the mountainous region of Eastern Asia, which has medicinal properties. It is distributed in Bhutan, China, Indonesia, Japan, Korea, Myanmar, Nepal, Thailand, Taiwan, Vietnam, North-West Himalayan region and North-East Region of India and is known by different local names, viz. Fish mint, Fish wort, Chameleon, Lizard's tail and fishy smell plant (Tutupalli & Chaubal, 1974). The herb is used as folk medicine as it has antiviral, antibacterial, immuno-stimulant, diuretic, anti-cancer and anti-inflammatory effects (Kumar, Prasad, & Hemalatha, 2014). This plant appears to have immense potential for the growth promoting properties in fish. The research on pig is proven that the *H. cordata* leaf meal and extract in the diet act as a growth promoter (Cho, Zhang, & Kim, 2012; Yan, Meng, & Kim, 2011). A diet containing the herb *H. cordata* fed to Pacific white-legged shrimp and cobia improved the growth and delayed the onset of any disease (Wu, Chen, Ueng, & Nan, 2016; Yun, Jian, & Qi, 2007). Liu, Zhang, Yang, Lu, and Ye (2003) observed that giving *H. cordata* in carp feed resulted in more intestinal favourable bacteria and thus eliminating the disease-causing non-prominent bacteria from the intestine.

Labeo rohita is the most popular and widely cultured carp in Southeast Asia and command high demand among the consumer. It contributes approximately 35%–40% of total aquaculture production in India, and the total production is 1.5 million tons in 2012 in Southeast Asia (FAO, 2014). In polyculture system, rohu usually stocked at relatively higher levels than the other Indian major carps due to its broader feeding niche, which extends from column to bottom.

Based on several types of research which show herbs have growth promoting effect in fish, the present study was aimed to delineate the effect of dietary *Houttuynia cordata* leaf meal and leaf extract on the growth performance, nutrient utilization and expression of IGF-I gene in *Labeo rohita*.

2 | MATERIALS AND METHODS

2.1 | Preparation of leaf meal and crude leaf extract

Houttuynia cordata (HC) leaves were collected from the Raha village (26.2468°N, 92.5223°E), Nagaon district, Assam, India. The collected HC leaves were washed with tap water to remove debris and air dried under shade. After complete drying, the HC leaf was powdered using a warring blender (Bajaj Electricals Ltd, Mumbai, India). For crude leaf extract preparation, 50 g of dried leaf powder was dissolved in 500 ml of 85% ethanol in 1 L beaker, and the ethanol-leaf powder mixtures were kept for 24 hr on an orbital shaker (Orbitek®—Scigenics Biotech, Chennai, India) for 24 hr. Then, the extract was filtered using a muslin cloth to exclude the leaf powder residue. The filtrate was centrifuged at 11,180 *g* for 5 min, and then, the supernatant was collected and concentrated in a rotary evaporator (IKA® RV10 Basic—Shanghai, China) at 150 rpm in 50°C and lyophilized. The resulting crude extract was collected and stored under refrigerated (4°C) condition until use.

2.2 | Procuring and acclimation of experimental animal

Fingerlings of *Labeo rohita* (rohu) were procured from Nisarga Aquaculture Pvt Ltd, Palghar, Thane District (Maharashtra), India and transported to the wet laboratory of the ICAR-Central Institute of Fisheries Education, Mumbai, India. The fishes were given a salt treatment (20 g/L) for 2 min and left undisturbed throughout the night. The stock was acclimatized for 15 days in 2,000 L cemented tank under the aerated condition and fed with the control diet (350 g/kg crude protein) to satiation twice daily.

2.3 | Experimental design and diets

Six different isonitrogenous (350 g/kg crude protein) and isocaloric (17 MJ/kg digestible energy) experimental diets were prepared with the purified ingredients as control, C (without leaf extract or meal), LE-0.25 (2.5 g/kg leaf extract), LE-0.5 (5 g/kg leaf extract), LE-1.0 (10 g/kg leaf extract), LM-1.0 (10 g/kg leaf meal) and LM-2.0 (20 g/kg leaf meal; Table 1). All the ingredients were weighed according to the formulation and mixed thoroughly to form a homogenous blend, and then, water was added to form dough. The dough was steam cooked in an autoclave for 10 min and then kept sometimes for cooling. Cod liver oil and sunflower oil, butylated hydroxytoluene and vitamin–mineral mixture were added along with the leaf extract/leaf meal, and then, the dough was pressed through a pelletizer (S.B. Panchal and Company, Mumbai, India) to get uniform sized pellets (2 mm diameter). The pellets were oven dried at 45°C for 12 hr. The dried feed pellets were kept in an air-sealed container and stored in a cool, dry place for further use. The proximate compositions of the experimental diets were carried out following the standard methods of AOAC (1995).

Two hundred and seventy healthy fingerlings of rohu with the initial average weight of 3.37 ± 0.23 g were randomly distributed in six distinct experimental groups in triplicates with 75/m^2 stocking density following a completely randomized design. The experiment was conducted in 18 rectangular plastic tubs (length 57 cm, breadth 36 cm, depth 47 cm, capacity 96 L) by maintaining a constant water depth of 37 cm for 60 days at wet laboratory of ICAR-CIFE, Mumbai. The experimental tubs were syphoned regularly to remove the faeces, and a constant water volume (75 L) was maintained in each tub throughout the feeding trial. Each tub was provided with aeration throughout the experimental period. Water quality parameters viz. temperature, dissolved oxygen, pH, total hardness, ammonia, nitrite and nitrate were estimated in every alternate day following the

TABLE 1 Ingredient and nutrient composition of the experimental diets fed to *Labeo rohita* fingerlings

Ingredients	Composition (g/kg)					
	Control	LE-0.25	LE-0.5	LE-1.0	LM-1.0	LM-2.0
Casein[a]	322	322	322	322	322	322
Gelatin[a]	73	73	73	73	73	73
Starch[a]	225.5	225.5	225.5	225.5	225.5	225.5
Dextrin[a]	175	175	175	175	175	175
Cellulose[a]	100	97.5	95	90	90	80
Cod liver oil[b]	30	30	30	30	30	30
Sunflower oil[b]	30	30	30	30	30	30
Vitamin-Mineral premix[c]	20	20	20	20	20	20
Carboxy methyl cellulose[a]	15	15	15	15	15	15
Betaine[a]	9	9	9	9	9	9
Butylated hydroxytoluene[a]	0.5	0.5	0.5	0.5	0.5	0.5
H. cordata	0	2.5	5	10	10	20
Total	1000	1000	1000	1000	1000	1000
Proximate composition (g/kg; on dry matter basis; mean of triplicates)						
Dry matter (DM)	890.5	889.4	894.1	890.6	888.9	891.9
Crude protein (CP)	358.6	357.2	361.2	355.3	348.9	353.9
Ether extract (EE)	60.8	58.6	62.7	59.1	56.5	58.3
Crude fiber (CF)	20.9	20.1	26.2	22.4	20.2	26.6
Nitrogen free extract (NFE)[d]	529.6	532.8	516.7	536.7	545.8	531.2
Ash	30.1	31.2	33.2	26.4	28.6	30.0
Digestible energy (MJ/kg)[e]	17.15	17.10	17.05	17.16	17.10	17.01

[a]Himedia Laboratories, Mumbai, India. [b]Universal Medicare Pvt Ltd, Mumbai, India. [c]Composition of vitamin–mineral mix (PRE-EMIX PLUS) (quantity/kg) Vitamin A, 5,500,000 IU; Vitamin D3, 1,100,000 IU; Vitamin B2, 2,000 mg; Vitamin E, 750 mg; Vitamin K, 1,000 mg; Vitamin B6, 1,000 mg; Vitamin B12, 6 mg; 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, 2,000 mg; Co, 450 l-lysine, 10 g; dl-Methionine, 10 g; Selenium 125 mg; Vitamin C,2,500 mg. [d]NFE = 1000 − (g/kg CP + g/kg EE + g/kg CF + g/kg ash). [e]Calculated digestible energy (DE) (MJ/kg) = 16.74 × CP (g/kg) + 37.66 × EE (g/kg) + 16.74 × NFE (g/kg) Halver (1976).

method of APHA (1998). The fishes were fed twice daily to the satiation level at 0900 and 1700 hr.

2.4 | Growth indices

All fishes from each tub were weighed at every 15 days interval to assess the increase in body weight of the experimental fishes. Fishes were starved for 24 hr before taking the body weight. The growth performance indices were calculated according to the formulae given below:

$$\text{Weight gain percentage (WG \%)} = [(\text{Final weight} - \text{Initial weight})/\text{Initial weight}] \times 100$$

$$\text{Specific growth rate (SGR)} = [(\text{Log}_e\text{Final weight} - \text{Log}_e\text{ Initial weight})/\text{Experiment duration in days}] \times 100$$

$$\text{Feed conversion ratio (FCR)} = \text{Feed intake (dry weight in g)}/\text{Body weight gain (wet weight in g)}$$

$$\text{Protein efficiency ratio (PER)} = \text{Total wet weight gain (g)}/\text{Protein fed (g)}$$

2.5 | Fish sampling for enzyme assay

After 60 days feeding trial, three fishes from each replicate were anaesthetized with clove oil (50 µl/L). The fishes were dissected out, and tissue samples (liver, muscle and intestine) were collected followed by homogenization in chilled sucrose solution (0.25 M) using a mechanical tissue homogeniser (MICCRA D-9, Germany). Immediately after homogenization, tissue homogenate was centrifuged (Heraeus Megafuge 8R, Thermo Fisher Scientific, USA) at 2,800 *g* for 10 min at 4°C, and the supernatants were collected and stored at −20°C until further use.

2.6 | Digestive enzyme assay

The intestinal protease enzyme activity was determined by the casein digestion method (Drapeau, 1976). One unit of enzyme activity was defined as the amount of enzyme needed to release acid-soluble fragments equivalent to Δ0.001 at 280 nm/min at 37°C and pH 7.8. Amylase activity was assayed following the method of Rick and Stegbauer (1974) as reducing sugars produced by the action of glucoamylase and α-amylase on carbohydrate in the presence of dinitrosalicylic-acid (DNS). Amylase activity was expressed in micromole maltose released/min/mg protein at 37°C. The lipase activity was estimated by following the method of Cherry and Crandall (1932). Units of intestinal lipase activity per gram tissue were measured by subtracting the volume of N/20 NaOH required for control tube from experimental tube and expressed in units/hr/mg protein at 37°C.

2.7 | Metabolic enzyme assay

The muscle and liver aspartate aminotransferase (AST) activities were assayed according to the method as described by Wooten (1964). The substrate for AST was comprised of DL-aspartic acid and α-ketoglutarate in phosphate buffer (pH 7.4). The AST activity was expressed as nanomoles oxaloacetate formed/mg protein/min at 37°C. The methods adopted for the estimation of alanine aminotransferase (ALT) activity was same as that for AST activity estimation except that the substrate comprised of DL-alanine instead of aspartic acid. The ALT activity expressed as nanomoles pyruvate formed/mg protein/min at 37°C.

Lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) enzyme activities were assayed in the muscle following the method of Wroblewski and Ladue (1955), and Ochoa (1955), respectively. The enzyme activity expressed as units/mg protein/min at 37°C, where 1 unit was equal to Δ0.01 OD/min.

2.8 | RNA extraction and cDNA synthesis

Relative gene expression was analysed in liver tissue from nine fish per treatment using real-time PCR. The collected liver tissue sample was placed in a sterile and DNase-/RNase-free tubes, and the total RNA was extracted using *Trizol* reagent (Invitrogen Bioservices, India) following the manufacturer's guidelines. The quality and quantity of extracted RNA samples were examined and verified with nanodrop spectrophotometer (Thermo Scientific, USA) at 260 and 280 nm. The RNA was treated with RNase-free DNase I (Thermo Scientific) to remove the genomic DNA contamination and again the quality and quantity of the treated RNA were measured. Then, the mRNA pool was converted into its complementary DNA using the first-strand cDNA synthesis Kit (Thermo Scientific) as per the protocol provided by the manufacturers. The synthesized first-strand cDNA was directly used for RT-PCR to amplify IGF-I and Beta-actin gene using primers designed in Gene Runner 3.0 software based on available sequences in GenBank (https://www.ncbi.nlm.nih.gov; Table 2).

2.9 | Quantitative RT-PCR

The expression of IGF-I and Beta-actin mRNA were studied by real-time PCR (AriaMx Real-Time PCR, Agilent, US) using maxima R SYBR green qPCR master mix (Thermo Scientific). Standard reaction mixture (10 µl) contained 5 µl of SYBR green qPCR master mix (2×), 1 µl of forward and reverse primers (10 picomole), 1 µl of template cDNA and rest with nuclease-free water. Each sample was normalized with gene-specific beta-actin primer. Beta-actin gene expression was used as an internal control gene to normalize any error in the initial RNA concentration. The ΔΔct values of each sample were determined by subtracting the average Δct value of the control from the Δct of the sample. The fold change in expression compared to the control was expressed as $2^{(-\Delta\Delta ct)}$ (Livak & Schmittgen, 2001).

2.10 | Statistical analysis

In the present study, comparison among mean values was made at 5% level of significance ($p < 0.05$) through one-way analysis of variance (ANOVA), and Duncan's multiple range test (DMRT) was used for post hoc comparisons using SPSS 16.0 for windows.

3 | RESULTS

3.1 | Water quality parameters

Different water quality parameters such as temperature, dissolved oxygen, pH, total hardness, ammonia, nitrite and nitrate were found to be in the range from 27 to 29.5°C, 6 to 7.9 mg/L, 7.2 to 8.5, 230 to 246 mg/L, 0.03 to 0.1 mg/L, 0.05 to 0.09 mg/L and 0.5 to 1.1 mg/L, respectively, throughout the experiment.

TABLE 2 Primers used for expression study in real-time PCR

Primer	Sequence 5′→3′	Accession no.	Amplicon size (bp)	Tm value (°C)
IGF-1 forward	GCAAACCGACAGGCTATGGGC	KX455871	166	57
IGF-1 reverse	GTGTCTGTGTGCCGTTCCGC			
Beta-actin forward	CACTGCTGCTTCCTCCTCCTCC	EU184877	138	58
Beta-actin reverse	GATACCGCAAGACTCCATACCCAAG			

3.2 | Growth performance and nutrient utilization

The effects of *H. cordata* leaf meal and leaf extract on the growth performance of *L. rohita* are shown in Table 3. The specific trend of fish body weight gain with an interval of 15 days was shown in Figure 1. The weight gain percentage and SGR was significantly ($p < 0.05$) higher in LM-1.0 and LE-1.0 group than LE-0.25, LM-2.0 and control group except LE-0.5. Though FCR values in fish of control were significantly ($p < 0.05$) higher than LE-0.5, LE-1.0 and LM-1.0 group, the fish of LE-0.25 and LM-2.0 group exhibited similar FCR values with the control group. Whereas the fish group fed with LM-1.0 and LE-1.0 was showed significantly ($p < 0.05$) higher PER than control, LE-0.25 and LM-2.0 fed groups.

3.3 | Digestive enzyme activities

The leaf extract fed groups (LE-0.25, LE-0.5 and LE-1.0) showed increasing trends of the intestinal protease activity, whereas LE-1.0 group showed significantly ($p < 0.05$) higher protease activity than leaf meal fed groups (LM-1.0 and LM-2.0) and control (Table 4). There was no significant variation ($p > 0.05$) in amylase and lipase activities among the different experimental groups.

3.4 | Metabolic enzyme activity

There was no significant ($p > 0.05$) difference in muscle ALT activity in both leaf extract and leaf meal fed groups in compared with control diet. However, LM-2.0 showed significantly ($p < 0.05$) lower value than other treatment groups. The most elevated muscle AST activity was recorded in LM-1.0 group compared to other experimental groups (control, LE-0.25 and LM-2.0) except LE-0.5 and LE-1.0. In case of the liver ALT and AST activity, the experimental groups were not differed significantly ($p > 0.05$).

The muscle LDH activity was significantly ($p < 0.05$) higher in control group compared to the treatment groups, and LE-1.0 group showed significantly lower muscle LDH activity than other experimental groups except LE-0.5 fed group. However, there was a reduction in LDH activity with the increasing dietary level of both leaf extract and leaf meal. Similarly, the control group was showing significantly higher muscle MDH activity compared to treatment groups (Table 5).

3.5 | Expression of IGF-I

The group fed with 10 g leaf meal per kg diet (LM-1.0) showed significantly ($p < 0.05$) higher IGF-I expression among the experimental groups, while the control and LE-0.25 were exhibited significantly lower expression compared to other treatment groups (Figure 2).

4 | DISCUSSION

The studied water quality parameters such as temperature, dissolved oxygen, pH, total hardness, ammonia, nitrite and nitrate were found to be in the optimum range of fish rearing (Banerjea, 1967; Debnath et al., 2007; Kumar et al., 2010; Mohapatra et al., 2012). The inclusion of *H. cordata* leaf meal or extract significantly affected the growth performance of *L. rohita* fingerlings. The improvement in the growth performance and feed efficiency in *H. cordata* fed groups could be due to the capability of the bioactive compounds in stimulating the secretion of digestive enzymes, thus resulting in nutrient utilization and muscle growth. Lee, Ra, Song, Sung, and Kim (2012) reported that garlic extract in the diet of Starlet sturgeon exhibited better FCR and PER. Similarly, Kaleeswaran, Ilavenil, and Ravikumar (2011) also reported that *Catla catla* fed with dietary *Cynodon dactylon* leaf meal showed an increase in weight gain, SGR and better FCR than the control group. The existence of anti-nutritional factors (ANFs) in fish diet beyond the tolerant limit could inhibit nutrient absorption and causes reduction in growth (Francis, Makkar, & Becker, 2001). The fish fed with 20 g/kg leaf meal has significantly reduced the growth rate compared to its 10 g/kg inclusion counterpart. These suggest that the level of ANFs in 20 g/kg leaf meal was higher, and thereby, it may cause growth impairment. This assertion is further supported by the lower PER value recorded in LM-2.0 fed group. Similar result was also reported by Wu et al. (2016) who observed that dietary 10 g/kg *H. cordata* meal in cobia increases the weight gain % and lowers FCR compared to the 20 g/kg fed group.

TABLE 3 Growth performances of *Labeo rohita* fingerlings fed with different diets during 60 days of experimental period

Treatments	Initial wt. (g)	Final wt. (g)	Wt. gain %	SGR	FCR	PER
Control	3.50 ± 0.13	7.46 ± 0.15^{a}	113.76 ± 7.00^{a}	1.26 ± 0.05^{a}	2.49 ± 0.09^{c}	1.12 ± 0.06^{a}
LE-0.25	3.31 ± 0.12	7.52 ± 0.21^{a}	127.51 ± 4.89^{ab}	1.37 ± 0.04^{ab}	2.32 ± 0.06^{bc}	1.21 ± 0.03^{ab}
LE-0.5	3.39 ± 0.07	8.11 ± 0.38^{ab}	139.46 ± 5.31^{bc}	1.45 ± 0.04^{bc}	2.19 ± 0.05^{ab}	1.26 ± 0.03^{bc}
LE-1.0	3.37 ± 0.17	8.60 ± 0.38^{b}	155.34 ± 8.40^{c}	1.56 ± 0.05^{c}	2.07 ± 0.06^{a}	1.37 ± 0.04^{cd}
LM-1.0	3.36 ± 0.29	8.73 ± 0.44^{b}	161.65 ± 8.94^{c}	1.60 ± 0.06^{c}	2.03 ± 0.06^{a}	1.42 ± 0.04^{d}
LM-2.0	3.28 ± 0.16	7.54 ± 0.30^{a}	129.97 ± 7.82^{ab}	1.39 ± 0.06^{ab}	2.30 ± 0.09^{bc}	1.23 ± 0.05^{ab}
p-Value	0.959	0.032	0.005	0.004	0.005	0.002

Notes. FCR, feed conversion ratio; PER, protein efficiency ratio; SGR, specific growth rate.
All values are expressed as mean ± *SE*, $n = 3$. Mean values in the same column with different superscript differ significantly ($p < 0.05$).

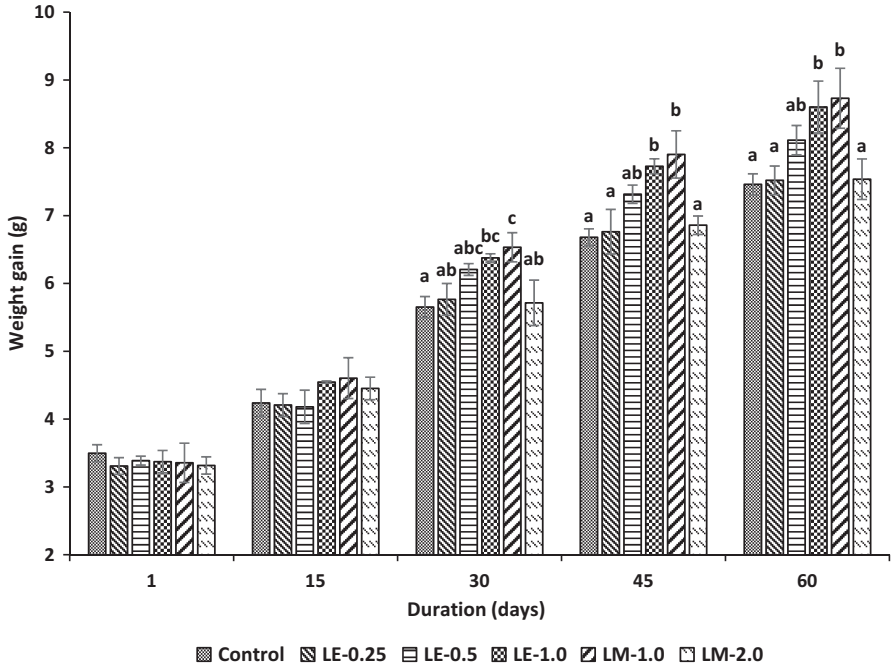


FIGURE 1 Body weight gain (g) of *Labeo rohita* fingerlings fed with different diets with duration (days). Bars with different superscript differ significantly ($p < 0.05$) between the experimental groups within the same duration (days). Values are expressed as mean ± *SE*, $n = 3$

TABLE 4 Digestive enzyme activities in the intestine of *Labeo rohita* fingerlings fed with different diets at the end of 60 days of experimental period

Treatments	Protease[a]	Amylase[b]	Lipase[c]
Control	47.54 ± 2.80[a]	13.24 ± 1.20	6.08 ± 0.27
LE-0.25	50.33 ± 1.21[a]	14.05 ± 0.76	6.44 ± 0.07
LE-0.5	68.35 ± 2.32[b]	14.37 ± 1.29	6.04 ± 0.23
LE-1.0	78.30 ± 2.68[c]	13.82 ± 0.88	7.51 ± 0.55
LM-1.0	68.35 ± 1.55[b]	15.63 ± 0.61	7.12 ± 0.24
LM-2.0	69.77 ± 1.36[b]	11.23 ± 0.39	6.67 ± 0.43
p-Value	0.000	0.085	0.054

Note. All values are expressed as mean ± *SE*, $n = 3$. Mean values in the same column with different superscript differ significantly ($p < 0.05$). [a]Protease activity is expressed in nanomole of tyrosine released/min/mg protein at 37°C. [b]Amylase activity is expressed in micromole maltose released/min/mg protein at 37°C. [c]Lipase activity is expressed in units/hr/mg protein at 37°C.

The highest protease activity was shown by the 10 g/kg leaf extract fed group and leaf meal-based groups showed lower activity. This result suggests that the intestinal protease activity might have reduced by the presence of some ANFs such as polyphenolic compounds (Chiow, Phoon, Putti, Tan, & Chow, 2016; Kumar et al., 2014; Yan et al., 2011). Amylase and lipase activities were not affected due to the feeding of *H. Cordata* irrespective of its inclusion level either in the form of leaf or extract. Ojha, Chadha, Saini, Damroy, and Chandraprakash (2014) had reported similar results in *L. rohita* fed with *Mucuna pruriens* leaf extract. Lee et al. (2012) also stated that there was a significant difference in the protease activity of Starlet sturgeon regardless of inclusion level of dietary garlic extract. Kaleeswaran et al. (2011) reported elevated levels of protease activity in the *Catla catla* fed with herbal supplementation *Cynodon dactylon* leaf meal at 5 - 50 g/kg in the diet.

Aspartate aminotransferase and alanine aminotransferase is the vital aminotransferase enzyme that redistributes amino nitrogen among amino acids. AST catalyses the inter-conversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate (Kumar et al., 2010; Walton & Cowey, 1982). ALT transfers the amino group from alanine to α-ketoglutarate and convert to glutamic acid forming pyruvate. The pyruvate and oxaloacetate thus formed are responsible for the synthesis of non-essential amino acids such as alanine, asparagine and glutamine, which in turn help in protein synthesis and enhance the growth of the animal (Knox & Greengard, 1965). Apart from this, the amino acids are deaminated to produce TCA cycle intermediates and also help in energy production in the form of ATP (De Silva & Anderson, 1995).

The ALT and AST activities in muscle were significant ($p < 0.05$) among the experimental groups. Abalaka, Esievo, and Shoyinka (2011) studied the effect of aqueous and ethanolic extract of *Parkia biglobasa* pods in *Clarias gariepinus* and reported a significant increase in ALT and AST activities in treatment groups. Our results corroborate the findings of Ojha (2014), who reported the increased muscle ALT and AST activity in *L. rohita* fed with *Pedalium murex* leaf extract. The AST and ALT activities of the liver were not affected due to the feeding of *H. cordata* leaf/extract.

Lactate dehydrogenase is the terminal enzyme of the glycolytic pathway. Lactate dehydrogenease converts lactate to pyruvate in the presence of coenzyme NADH, which converted to NAD^+. Thus, lactate dehydrogenase helps in maintaining the glycolysis cycle by supplying NAD^+. In the presence of enough oxygen, pyruvate enters the Krebs's cycle, but when there is an oxygen shortage in the tissue, pyruvate is converted to lactate (Murray, Granner, Mayes, & Rodwell, 2000). In our study, fish fed with leaf extract containing

TABLE 5 Activity of different metabolic enzymes in muscle and liver in *Labeo rohita* fingerlings fed with different diets at the end of 60 days of experimental period

	Alanine aminotransferase (ALT)[a]		Aspartate aminotransferase (AST)[b]		Lactate dehydrogenase (LDH)[c]	Malate dehydrogenase (MDH)[d]
Treatments	Muscle	Liver	Muscle	Liver	Muscle	Muscle
Control	0.54 ± 0.07^{ab}	0.40 ± 0.02	0.70 ± 0.11^{a}	0.60 ± 0.07	0.83 ± 0.02^{d}	0.83 ± 0.04^{c}
LE-0.25	0.64 ± 0.04^{b}	0.44 ± 0.05	1.11 ± 0.08^{b}	0.62 ± 0.01	0.57 ± 0.02^{b}	0.48 ± 0.06^{b}
LE-0.5	0.62 ± 0.03^{b}	0.46 ± 0.04	1.20 ± 0.01^{bc}	0.60 ± 0.04	0.52 ± 0.02^{ab}	0.26 ± 0.01^{a}
LE-1.0	0.65 ± 0.03^{b}	0.49 ± 0.02	1.14 ± 0.04^{bc}	0.69 ± 0.10	0.48 ± 0.01^{a}	0.22 ± 0.02^{a}
LM-1.0	0.66 ± 0.03^{b}	0.45 ± 0.02	1.52 ± 0.26^{c}	0.69 ± 0.05	0.68 ± 0.04^{c}	0.59 ± 0.02^{b}
LM-2.0	0.41 ± 0.05^{a}	0.40 ± 0.02	0.67 ± 0.02^{a}	0.62 ± 0.09	0.57 ± 0.05^{b}	0.51 ± 0.04^{b}
p-Value	0.015	0.403	0.003	0.828	0.000	0.000

Notes. All values are expressed as mean ± *SE*, *n* = 3; Mean values in the same column with different superscript differ significantly ($p < 0.05$).
[a]ALT: specific activities expressed as nanomoles of sodium pyruvate released/min/mg protein at 37°C. [b]AST: specific activities expressed as nanomoles of oxaloacetate released/min/mg protein at 37°C. [c]LDH activity expressed in Unit/mg protein/min at 37°C. [d]MDH activity expressed in Unit/mg protein/min at 37°C.

diets had lower LDH activity in muscle than fish fed the control diet. Increasing LDH activity may be due to the more significant production of lactate, the preferred source for gluconeogenesis in fish during anaerobic metabolism (Moon & Foster, 1995). LDH activity increases in stress condition viz. temperature stress (Grigo, 1975), starvation stress (Vijayaraghavan & Rao, 1986) and confinement stress (Chatterjee et al., 2006). Significantly, lower activity in the treatment groups suggested the mitigating stress effect of dietary plant extracts in *L. rohita* fingerlings. The decreasing trend of LDH activity in *L. rohita* fingerling with supplementation of herbal plant extracts are also reported by Chanu et al. (2013) and Kumar et al. (2014).

Malate dehydrogenase (MDH) is an enzyme of the TCA cycle catalyses the conversion of malate into oxaloacetate and vice versa. The MDH activity is also expected to be higher during glycogenesis from amino acids (Hemre, Mommsen, & Krogdahl, 2002). The current study showed significantly lower MDH activity in the treatment group as compared to the control group. Lower activity of MDH indicates reduced activity of TCA cycle due to decreased energy demands signifying the protective and stress mitigating role of *H. cordata* leaves. This result is in the corroboration with Chanu et al. (2013), who reported that supplementation of *Eclipta alba* extract has reduced the MDH activity in *Labeo calbasu* fingerlings. Gbadamosi, Fasakin, and Adebayo (2016) had also reported that dietary *Moringa oleifera* extract in Nile tilapia decreased the MDH activity in treatment groups in compared to the control.

In fish, a central role in the regulation of a multitude of physiological functions, including growth, differentiation, metabolism, reproduction, osmoregulation and immune responses played by growth hormone (GH), insulin-like growth factor-I (IGF-I), thyroid hormone and corticosteroids (Filby & Tyler, 2007). The influence of growth hormone (GH) secretion from the pituitary is to stimulate the liver to synthesize and release IGF-I and IGF-II (Carnevali et al., 2005; Duguay, Lai-Zhang, Steiner, Funkenstein, & Chan, 1996; Shamblott, Cheng, Bolt, & Chen, 1995). In mammals, it is reported that the dietary supplementation of red clover extract increases the plasma concentrations of GH and IGF-I and also altered physiological mechanisms to enhance the growth rates of animals (Moorby, Fraser, Theobald, Wood, & Haresign, 2004).

Insulin-like growth factor-I has a direct effect on somatic tissues that results in growth. The expression of IGF-I was found to be higher in LM-1.0 group followed by LE-1.0 group and shadowed a similar trend in growth performances in the current study. These data are in agreement with the findings of Duan (1998) who proposed that in fish as in mammals, IGF-I plays a central role in the hormonal control of growth. However, *H. cordata* has been shown to possess anti-hyperglycaemic activity and promote the insulin secretion in rodents (Kumar et al., 2014). Kumar, Prasad, and Hemalatha (2016) reported that *H. cordata* plant consists of flavonoid compounds mainly quercetin that has potent ability to induce the secretion of insulin and finally that up-regulate the IGF-I expression.

This study revealed that inclusion of either leaf meal or leaf extract of *H. cordata* at 10 g/kg level in the diet improved growth

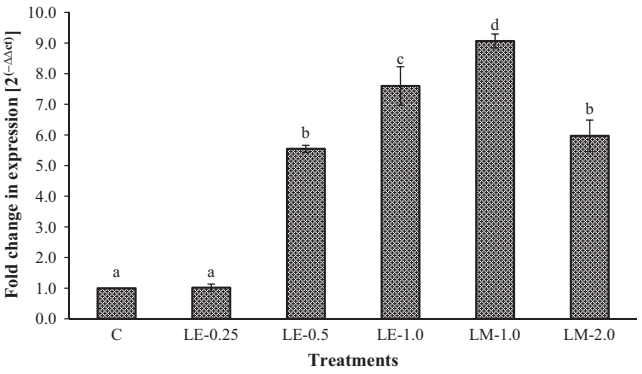


FIGURE 2 IGF-1 expression in the liver of *Labeo rohita* fingerlings fed with different diets. Bars with different superscript differ significantly ($p < 0.05$). Values are expressed as mean ± *SE*, *n* = 3

performances of *L. rohita* fingerlings. The highest expression of growth gene IGF-I was found at 10 g/kg inclusion of leaf meal. It is suggested that dietary inclusion of leaf meal of *H. cordata* at 10 g/kg level is better than leaf extract of the plant as extraction process at high cost during feed preparation is not involved. This information may be useful for further research on the inclusion of leaf meal in practical carp diet for commercial application.

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