Himalayan lapsi, *Choerospindias axillaris* (Roxb.) enhances concentration of vitamin C in tissues of rohu (*Labeo rohita H*) cultured at Chitwan (Nepal)

**Abstract**

*Labeo rohita* lacks the enzyme for endogenous synthesis of vitamin C and lapsi fruits are rich in vitamin C. A study was conducted to examine the concentration of vitamin C in the blood serum, brain and liver of *L. rohita* through lapsi fruits extract supplemented in the diets. Six groups of *L. rohita* were fed experimental diets containing lapsi fruits extract supplemented at 0 mg kg\(^{-1}\) (D1), 100 mg kg\(^{-1}\) (D2), 200 mg kg\(^{-1}\) (D3), 400 mg kg\(^{-1}\) (D4), 800 mg kg\(^{-1}\) (D5) and 1600 mg kg\(^{-1}\) (D6) for 90 days. Growth parameters (WG, SGR and FCR) and Vitamin C concentration in blood serum, brain and liver were evaluated during the experimental trial. Carps fed with a lapsi fruits extract supplemented diet showed higher specific growth rate (SGR) compared with control diet fed carps. Results from this study help to establish the beneficial effect of vitamin C rich lapsi fruits on growth and imunnunomodulation in rohu. It can be concluded that lapsi fruits extract supplemented diet can be used to improve the immune system of *L. rohita* as indicated by enhancement of vitamin C in the serum, brain and the liver.

**Keywords:** growth, vitamin c, brain, liver, serum, *choerospindias axillaris*, *labeo rohita*

**Introduction**

Himalayan lapsi, *Choerospindias axillaris* (Roxb.) is native to Nepal and is also reported from south-east Asian countries.\(^1\) Its fruits containing vitamin C.\(^2\) Phenol and flavonoid compounds\(^3,4\) are consumed to enhance the immunity\(^5\) and neutralize free radicals formed in the body. Vitamin C is required to form collagen, growth, reproduction, resist diseases and for immunity in many fishes.\(^6\) Oxygen present in air, high temperature, enzymes and multivalent cations destroy it. In the manufacturing process and storage of diet Vitamin C supplemented in it is lost.\(^7\) Many structural and functional abnormalities result in fishes due to insufficient supply of vitamin C.\(^8\) Teleost fishes like rohu lacking GLO enzyme\(^9\) needs supply of vitamin C along with the diet.\(^10\) Many researches on the effect of vitamin C on growth, its concentration in different tissues and stress overcome in fishes are available.\(^11\) But the work on the effect of lapsi extract on growth and its concentration in brain, liver and blood in *L. rohita* is not available.

**Materials and methods**

**Experimental design and set up**

About four hundred farm-raised fingerlings of *Labeo rohita* (3.2 ±0.014 g) were selected from the nursery pond and transferred them to the stocking pond for their proper acclimatization. Altogether six test diets D1 (0), D2 (100), D3 (200), D4 (400), D5 (800) and D6 (1600) were prepared along with other standard ingredients (Table 1). Eighteen rectangular nylon happas (1m×1.5m×1m) were suspended in the experimental pond with ropes and bamboos. Two hundred seventy fingerlings of rohu (2.32±0.017 cm and 3.43±0.113 g) were selected for the experimental pond with ropes and bamboos. Two hundred Seventy Eighteen rectangular nylon happas (1m×1.5m×1m) were suspended in

---

**Keywords:** growth, vitamin c, brain, liver, serum, *choerospindias axillaris*, *labeo rohita*
Himalayan lapsi, *Choerospondias axillaris* (Roxb.) enhances concentration of vitamin C in tissues of rohu (*Labeo rohita H*) cultured at Chitwan (Nepal)

Vitamin C estimation in blood serum, brain and liver

Vitamin C in the blood serum, tissues of brain and liver were estimated according to the method described by. Preweighed brain and liver tissues were homogenized in ice-cold 250 mM HClO₄ containing 5% trichloro acetic acid (TCA) and 0.08% EDTA. The homogenates were centrifuged at 27000 g for 30 min at 4°C. 25 µl of 0.2% dichloro phenolindophenol (DCIP) were added to the 250 µl of deproteinsed samples. The same amount was added to a blank and then the mixtures were incubated at 37°C for 1 hour. After that 25 µl of 1% KBrO₃ were added and mixtures were incubated at 37°C for further 1 hour. Then 250 µl of 2% thiourea in 5% meta-phosphoric acid was added followed by an equal volume of 2% of 2,4-dinitro phenyl hydrazine (DNPH) in 12 M H₂SO₄. All samples were incubated for 3 hour at 60°C after which 0.5 ml of ice-cold 18 M H₂SO₄ were added. The samples were transferred into Eppendorf tubes and centrifuged at 11300 g 3 minutes. The absorbance was recorded at 524 nm with a spectrophotometer. Standard (20-200 µg/ml) were prepared with vitamin C (l-ascorbic acid, HiMedia).

Statistical analysis

Value for each parameter measured has been expressed as mean ± standard error of mean. One-way Analysis of Variance (ANOVA) was used to analyze the data followed by Duncan’s Multiple Range Test to find the difference at 5% (P<0.05) level.

---

Citation: Shakya SR, Labh SN. Himalayan lapsi, *Choerospondias axillaris* (Roxb.) enhances concentration of vitamin C in tissues of rohu (*Labeo rohita H*) cultured at Chitwan (Nepal). Int J Biosen Bioelectron. 2018;4(3):152–156. DOI: 10.15406/ijbsbe.2018.04.00116
Results

Growth measurements and Survival

After the completion of the experiment cent per cent survival rate was observed in D4 and D5 fed groups followed by 97.78±6.65 % in D2, D3 and D6 and 93.33±11.54 % in D1. Significant (P < 0.05) differences were observed in the treated groups (D2, D3, D4, D5 & D6) of rohu fed lapsi after 90 days of feeding trials as compared to control (D1) diet fed group. Average initial length of rohu was 2.32±0.017 cm in the beginning of the experiment while, highest 15.49±0.199 cm length was recorded in D4 diet fed group. In other treated groups the average final length recorded were 13.57±0.254 cm (D3), 13.13±0.412 cm (D5), 12.02±0.96 cm (D6) and 11.42±0.165 cm (D2) in control diet fed group the final length recorded was 10.68±0.375 cm (D1). The length gain in all the treated and control groups were 8.36±0.373 cm (D1), 9.1±0.176 cm (D2), 11.25±0.25 cm (D3), 13.17±0.19 cm (D4), 10.81±0.41 cm (D5) and 9.71±0.96 cm (D6). The length gain (%) was found high in D4 (567.5±0.462) diet fed group followed by D3 (484.8±0.977), D5 (465.75±0.794), D6 (418.01±0.903), D2 (392.32±0.952) and D1 (360.47±0.902). Similarly, a higher 57.43 % increase in length was observed in D4 diet fed group as compared to control D1 diet fed group and the length gain increment in other treated groups were 34.5 (D3), 29.21 (D5), 15.96 (D6), and 8.85 (D2) respectively. Similar results were found in the average final weight and weight gain (% of different diets fed groups. The average initial weight was 3.43 ± 0.11 g in the beginning of experiment which after 90 days of feeding trials, highest average final weight and average weight gain were recorded in D4 (22.42±0.23 g, 19.45±0.54 g) diet fed group. The average weight gain % was found high in D4 (567.5±0.88) diet fed group followed by D3 (484.82±0.01), D5 (465.75±0.77), D6 (418.13±0.71), D2 (392.38±0.31) and D1 (360.47±0.04). Similarly, a higher 67.5 % increase in weight was observed in D4 diet fed group as compared to control D1 diet fed group and the weight gain increment in other treated groups were 40.2 (D5), 35.64 (D3), 26.66 (D6), and 12.64 (D2) respectively. Significant results were observed in SGR among all the treated and control diet fed groups after 90 days of feeding trial. SGR level was found high in D4 (2.17±0.019) followed by D5 (2.0±0.03), D3 (1.97±0.05), D6 (1.91±0.15), D2 (1.80±0.021) and D1 (1.73±0.06) diet fed groups. FCR level was found decreased up to D4 diet fed group but in D5 and D6 it showed a bit increasing trend. The Highest FCR was recorded in control D1 (2.18±0.03) diet fed group.

Vitamin C concentration in blood serum, brain and liver

Vitamin C (L-ascorbic acid) levels of blood serum, brain and liver were also estimated. Significant differences (P<0.05) were found in the vitamin C concentration in blood serum and tissues of brain and liver of all the treated diet fed groups in comparison to control diet fed group. The highest vitamin C concentration in blood serum was observed in D4 diet fed group (15.38 ± 0.329µg/mg). In liver also similar trend was recorded. The vitamin C concentration in D4 diet fed group was 191.83 ± 3.29 µg/mg followed by groups fed with diet D3 (148.51 ± 9.07 µg/mg), D6 (142.63 ± 23.08 µg/mg), D2 (136.72 ± 11.11 µg/mg), D5 (135.49 ± 14.87 µg/mg) and the minimum was in D1 (127.52 ± 5.80 µg/mg) diet fed group. In the brain highest vitamin C concentration was 91.197 ± 3.59 µg/mg in D4 diet fed group followed by 81.86 ± 0.02 (D3), 78.93 ± 0.97 (D2), 65.86 ± 0.01 (D5), 59.02±06 (D6) and 57.373 ± 3.318 (D1) (Table 2).

Discussion

Many herbs are used in aquatic animals including fish to promote growth.14,15 All the rohu fed with diets supplemented with ethanol extract of lapsi fruit showed better growth than control group. The maximum growth was observed in group fed with D4 diet. These results indicates that rohu needs vitamin C supplemented diet for better growth which agreed well with the works of Goullous-Coustans et al.,26 Shiu & Hus27 and Wang et al.28 Several species of fish including rainbow trout and Korean rockfish fed with diet containing sufficient vitamin C showed better growth.19,19 The recommended ascorbic acid need for optimum growth of channel catfish is 10 to 25 mg per kg of diet.20 Cyprinus carpio and for newly hatched Cirrhinus mirgalla21 is 650 to 700 mg per kg diet. Growth rates in fishes depend upon the amount of Vitamin C present in the diet. The fish fed with diet containing more vitamin C grew more and the fish fed with diet containing fewer vitamins C grew less. The fish feed with diet without vitamin C showed less growth.21 The herbal drugs promote growth, boost stress resistance boosters and prevent infections. Most fishes, including rohu, cannot synthesis vitamin C21 due to lack of L-gulonolactone oxidase.22 Stickney et al.,23 reported supplementation of 50 mg of ascorbic acid in one kilogram diet resulted in maximum weight gain without any deficiency signs in blue tilapia (Oreochromis aureus). Similarly 79 mg ascorbic acid in one kilogram diet was the required level for maximum weight gain of hybrid tilapia (Oreochromis niloticus x Oreochromis aureus).24 Many studies have shown that fish with high concentration of vitamin C in tissues can tolerate ambient pollution and are better resistant to

Table 2 Concentrations of vitamin C in blood serum, liver and brain of rohu fed varied doses of lapsi up to 90 days of trial

| S.N. | Parameters | D1 | D2 | D3 | D4 | D5 | D6 |
|------|------------|----|----|----|----|----|----|
| 1    | Vit-C S    | 7.54±0.661 | 12.16±1.169 | 12.52±0.617 | 15.38±0.329 | 14.46±0.320 | 13.34±0.320 |
| 2    | Vit-C L    | 110.52±5.80 | 136.72±1.11 | 148.51±9.07 | 191.83±3.29 | 135.49±14.87 | 142.63±23.08 |
| 3    | Vit-C B    | 57.373±3.318 | 78.930±1.977 | 81.867±3.241 | 91.197±3.598 | 65.867±9.111 | 59.020±4.064 |

Vit-C S; Vitamin C in Blood serum; Vit-C L; Vitamin C in Liver

Values are provided as mean ± SE, n=3

Citation: Shakya SR, Labh SN. Himalayan lapsi, Choerospondias axillaris (Roxb.) enhances concentration of vitamin C in tissues of rohu (Labeo rohita H) cultured at Chitwan (Nepal). Int J Biosen Bioelectron. 2018;4(3):152–156. DOI: 10.15406/ijbsbe.2018.04.00116
heterotrophic infections. Tilapia exposed to sub lethal dose of mercury showed weight gain, increase specific growth rate and survival rate when fed with diet containing high level of ascorbic acid. Increase in the amount of lapi extract in the diets directly relates with the concentration of vitamin C in the blood serum, brain and liver of rohu. Fish with more vitamin C in tissues are healthier than with less vitamin C. Vitamin C concentration in blood serum, brain and liver were significantly (P<0.05) higher in the rohu fed with D4 diet and other treated groups and minimum in control diet fed group. The diet without vitamin C supplementation decreased the specific growth rate (0.32 % day\(^{-1}\)) in juvenile O. karongae and this is in accordance with studies conducted by Ai et al., who also observed decreasing specific growth rate in sea bass (Scophthalmus maximus) fed with vitamin C deficient diet. The concentration of vitamin C in various tissues is related to the vitamin C taken along with diet. Vitamin C concentration is brain and liver is high concentrations of vitamin C. 

**Conclusion**

*Labeo rohita*, an indigenous major carp, has high market demand in Nepal. Lapi is an indigenous Himalayan medicinal herb and pulp of its fruits having rich in antioxidant properties has high medicinal values. *L. rohita* cannot make vitamin C. The finding of this study along with other findings from different researchers generally recognized that the lapi fruit extract in feed of rohu increase survival rate, power to resist stressful environmental situations and accelerate growth. For successful culture of *L. rohita* in ponds 400 mg lapi extract in one kg diet is recommended.

**Acknowledgements**

None.

**Conflict of interest**

Author declares that there is no conflict of interest.

**References**

1. Paudel KC, Pieker K, Klump R. Evaluation of lapi tree *Choerospondias axillaris* (Roxb.) for fruit production in Nepal, Bodenkultur-Wien and München. 2003;54(1):3–10.
2. Shah DJ. Ascorbic acid (vitamin C) content of Lapsi- pulp and peel at different stage of maturation, Res Bull, (2035 BS, Food Research Section, HMGN, Department of Food and Agriculture Marketing Services, Kathmandu). 1978.
3. Zhou J, Huang J, Song XL. Applications of immunostimulants in aquaculture. *Marine Fish Research*. 2003;24:79–79.
4. Labh SN, Shakya SR, Kayasta BL. Extract of Medicinal lapi *Choerospondias axillaris* (Roxb.) exhibit antioxidant activities during *in vitro* studies. *Journal of Pharmacognosy and Phytochemistry*. 2015;4(3):194–197.
5. Chumnei Li, Jie He, Yonglin Gao, et al. Preventive Effect of Total Flavonoids of *Choerospondias axillaris* on chemios/Reperfusion-Induced Myocardial Infarction-Related MAPK Signaling Pathway. *Cardiovasc Toxicology*. 2014;14:145–152.
6. Lim C, Klessis PH, Li MH, et al. Interaction between dietary levels of iron and vitamin C on growth, hematology, immune response and resistance of channel catfish (*Ictalurus punctatus*) to Edwardsiella ictaluri challenge. *Aquaculture*. 2000;185:313–327.
7. Anderson JS, Sunderland R. Effect of extruder moisture and dryer processing temperature on vitamin C and E and astaxanthin stability. *Aquaculture*. 2002;207:137–149.
8. Gouillou-Coustans, Bergot MFP, Kaushik SJ. Dietary ascorbic acid needs for common carp (*Cyprinus carpio*) larvae. *Aquaculture*. 1998;453–461.
9. Dabrowski K. Absorption of ascorbic acid and ascorbic sulfate and ascorbate metabolism in stomachless fish, common carp. *Journal of Comparative Physiology B*. 1990;160:549–561.
10. Sato P, Nishikimi M, Udenfriend S. Is L-gulonolactone-oxidase the only enzyme missing in animals subject to scurvy? *Biochem Biophys Res Commun*. 1976;71:293–299.
11. Dabrowski K. *Ascorbic acid in aquatic organisms*. CRC press; 2001 288p.
12. Dabrowski K. Hinterleitner, S. Applications of a simultaneous assay of ascorbic acid, dehydroascorbic acid and ascorbic sulphate in biological materials. * Analyst*. 1989;114:83–87.
13. Duncan DB. Multiple range and multiple ‘F’ tests. *Biometrics*. 1955:11:1–42.
14. Citrasus T, Sekar RR, Babu MM, et al. Developing Artemia enriched herbal diet for producing quality larva in *Penaeus monodon*. *Asian Fish Science*. 2002;15:21–32.
15. Emanuel G, Citrasus T, Sivaram V. Delivery of HUFA, probiotics and biomedicine through biocapsulated Artemia as a means to enhance the growth and survival and reduce the pathogenicity in shrimp *Penaeus monodon* post larvae. *Aquacult Internet*. 2007;15:137–152.
16. Shiau SY, Hsu TS. Quantification of vitamin C requirement for juvenile hybrid tilapia, *Oreochromis niloticus*, with L-ascorbyl-2-monophosphate Na and L-ascorbyl-2-monophosphate Mg. *Aquaculture*. 1999;175:317–326.
17. Wang XJ, Kim KW, Bai SC, et al. Effects of the different levels of dietary vitamin C on growth and tissue ascorbic acid changes in parrot fish (*Opogonius fasciatus*). *Aquaculture*. 2003;215:21–36.
18. Lee KJ, Bai SC. Different dietary levels of L-ascorbic acid affect growth and vitamin C status of juvenile *Oreochromis niloticus*, with L-ascorbyl-2-monophosphate Na and L-ascorbyl-2-monophosphate Mg. *Aquaculture*. 1998;161:475–477.
19. Lee SH, Oe T, Blair IA. Vitamin C induced decomposition of lipid hydroperoxides to endogenous genotoxins. *Science*. 2001;292:2083–2086.
20. Mustin WG, Lovell RT. Na-L-ascorbyl-2-monophosphate as a source of vitamin C for channel catfish. *Aquaculture*. 1992;105:95–100.
21. Mahajan CL, Agrawal NK. Comparative tissue ascorbic acid studies in fishes. *J Fish Biol*. 1980;17:135–141.
22. Lee KJ, Dabrowski, K. Interaction between vitamins C and E affects their tissue concentrations, growth, lipid oxidation and deficiency symptoms in yellow perch (*Perca flavescens*). *British Journal of Nutrition*. 2003;89:589–596.
23. Chatterjee IB. Evolution and the biosynthesis of ascorbic acid. *Science*. 1973;182:1271–1272.
24. Wilson, RP. Absence of ascorbic acid synthesis in channel catfish, *Ictalurus punctatus* and blue catfish, *Ictalurus furcatus*. *Comp Biochem Physiol B*. 1973;46(3):635-638.
25. Stickney RR, Mc Geachin RB, Lewis DH, et al. Response of *Tileapia aurea* to dietary vitamin C. *Journal of the World Mariculture Society*. 1984;15:179–185.
Himalayan lapsi, Choerospondias axillaris (Roxb.) enhances concentration of vitamin C in tissues of rohu (Labeo rohita H) cultured at Chitwan (Nepal)

Shakya SR, Labh SN. Himalayan lapsi, Choerospondias axillaris (Roxb.) enhances concentration of vitamin C in tissues of rohu (Labeo rohita H) cultured at Chitwan (Nepal). Int J Biosen Bioelectron. 2018;4(3):152–156. DOI: 10.15406/ijbsbe.2018.04.00116