International Journal of Chemical Studies

P-ISSN2349–8528
E-ISSN 2321–4902
IJCS 2016; 4(4): 199-205

Received: 27-05-2016
Accepted: 28-06-2016

Shubha Ratna Shakya
Central Department of Zoology, University Campus, Tribhuvan University, Kirtipur, Nepal

Shyam Narayan Labh
Department of Zoology, Amrit Campus, Tribhuvan University, Kathmandu Nepal

Correspondence
Shubha Ratna Shakya
Central Department of Zoology, University Campus, Tribhuvan University, Kirtipur, Nepal

Fruits of lapsi *Choerospondias axillaris* enhances ascorbic acid level in brain and liver of common carp (*Cyprinus carpio* L) during intensive aquaculture

Shubha Ratna Shakya and Shyam Narayan Labh

Abstract
Teleost fish lack the enzyme for endogenous synthesis of ascorbic acid (AA), an essential micronutrient for fish and fruits of lapsi are rich in vitamin C. Thus, the aim of this study was to examine the effect of higher levels of dietary vitamin C on growth and protein levels in the brain and liver of common carp, *Cyprinus carpio* through lapsi fruits supplemented in the diets. Six groups of *C. carpio* were fed with experimental diets containing lapsi fruits supplemented at 0 mg kg^{-1} (T1), 100 mg kg^{-1} (T2), 200 mg kg^{-1} (T3), 400 mg kg^{-1} (T4), 800 mg kg^{-1} (T5) and 1600 mg kg^{-1} (T6) for 70 days. Growth parameters (WG, SGR and FCR) and concentrations of vitamin C in brain and liver were estimated. Fish fed with lapsi fruits supplemented diet showed higher weight gain and specific growth rate (SGR) up to 400 mg kg^{-1} compared with control fish. Concentrations of vitamin C was found higher in liver of T4 diet fed group as compared to brain. In both tissues (brain and liver) the lowest vitamin C concentrations were always in control T4 diet fed carp. Results from this study help to establish the beneficial effect of lapsi fruits on growth and immunmodulation in *C. carpio*.

Keywords: Growth, vitamin C, brain, liver, lapsi, *Choerospondias axillaris*, carp

1. Introduction
Vitamins are the important essential nutrients for most animal species. Vitamin deficiencies in fish under aquaculture are known to produce biochemical dysfunction, leading to tissue and cellular level clinical manifestations. Several morphological and functional abnormalities have been reported in various fish species deprived of vitamins. Vitamin C is synthesized in animals from either D-glucose or D-galactose as part of the glucuronic acid pathway [1]. Branching from L-gulonic acid, the biosynthetic pathway of vitamin C comprises three consecutive steps: first, the enzymatic lactonization of L-gulonic acid catalyzed by L-gulonolactone hydrolase [2]; second, the oxidation of L-gulonolactone catalyzed by L-gulonolactone oxidase (GLO); and third, the spontaneous isomerization of 2- keto-L-gulonolactone leading to vitamin C [3]. The general view is that the animals lacking GLO are not able to synthesize vitamin C and thus depend upon a dietary source of the vitamin [4]. Among the fishes analyzed to date, only those retaining numerous ancestral characters, such as lamprey, shark, ray, lungfish and sturgeon [5-10] have been shown to have GLO in the kidney, whereas teleost fish lack GLO activity [11].
Lapsi *Choerospondias axillaris* (Roxb.) [12] of family Anacardiaceae is a large, dioecious and deciduous fruit tree found growing in hills between 850-1900 m above the sea level in Nepal and has also been reported from various countries like India, China, Hong Kong, Thailand, Japan, Vietnam, Thailand, and Mongolia [13]. The fruits are rich in vitamin C content [14] and are used as a medicinal plant to enhance the immune system of the body [15]. Phenol and flavonoid compounds [16, 17] present in the fruit of lapsi serve as antioxidants. So there are potential benefits of consuming phenolic rich foods [18]. Thus, keeping these things in mind an experiment was conducted to understand the effects of lapsi fruits supplemented diets on carp growth and the concentrations of vitamin C (ascorbic acid) in some tissues (brain and liver) of common carp *C. carpio* fingerlings.

2. Materials and Methods
2.1 Preparation of ethanol extract of lapsi fruits
The ethanol extract of lapsi fruits was prepared as described by Labh *et al*., [17] with slight modifications.

2.2 Feed formulation and preparation of lapsi fruit extract supplemented artificial diets

Altogether six experimental diets T1, T2, T3, T4, T5 and T6 were prepared in which T1 was treated as control while rest of the diets were supplemented with 0.1, 0.2, 0.4, 0.8 and 1.6% lapsi fruit extracts. Other ingredients used during feed preparation s were as per the standard methods (Table 1).

2.3 Experimental design

After acclimatization, a total of two hundred seventy fingerlings of *Cyprinus carpio* with an average weight of 4.71±0.012g were distributed in six treatment groups in triplicates following a completely randomized design. The experimental rearing system consisted of 18 uniform size rectangular glass aquaria (100 L capacity) containing 15 fish per aquarium (12 inch x 24 inch x 18 inches). The total volume of the water in each tank was maintained at 80L throughout the experimental period. Fingerlings were fed twice daily at 3% of the body weight for 70 days. Temperature ranged from 250 C to 290 C and PH ranged from 7.53 to 7.92 throughout the study. DO was maintained above 5 gm/L with the help of aerators. The uneaten feed and faecal matters were siphoned daily and two third of the water was replaced at weekly intervals from each aquarium. A randomly 5 fingerlings were weighed randomly from each aquarium on every 14 days interval to adjust the feeding status of carp.

2.4 Proximate analysis of feed

The proximate compositions of the experimental diets (Table 2) were analyzed following the standard methods of the Association of Official Analytical Chemists [19].

Table 1: Composition of experimental diets (%)

Ingredients (g/100g)	Experimental diets (% Inclusion) g/kg					
	T1	T2	T3	T4	T5	T6
Fish Meal†	29.31	29.31	29.31	29.31	29.31	29.31
Soya meal‡	14.52	14.52	14.52	14.52	14.52	14.52
Groundnut oil cake†	9.17	9.17	9.17	9.17	9.17	9.17
Rice Powder†	14.16	14.16	14.16	14.16	14.16	14.16
Wheat Flour†	14.43	14.43	14.43	14.43	14.43	14.43
Corn flour†	11.37	11.37	11.37	11.37	11.37	11.37
Sunflower oil†	3	3	3	3	3	3
Cod liver oil†	2	2	2	2	2	2
Vitamin & Mineral Premix§	1	1	1	1	1	1
C. axillaris extract†	0	0.01	0.02	0.04	0.08	0.16
Betain Hydrochloride††	0.02	0.02	0.02	0.02	0.02	0.02
BHT (Butylated hydroxytoluene)††	0.02	0.02	0.02	0.02	0.02	0.02
CMC (Carboxymethyl cellulose) ††	1	0.99	0.98	0.96	0.92	0.84
Total	100	100	100	100	100	100

†Ingredients like fish meal, soya meal, groundnut oil cake, rice powder, wheat flour, corn flour, sunflower oil and Cod Liver Oil were procured from local market of Kathmandu Valley.

‡Ruchi Soya Industries, Raigad, India.

§Composition of vitamin mineral mix (EMIX PLUS) (quantity 2.5kg $^{-1}$)

Vitamin A 55,00,000 IU; Vitamin D_3 11,00,000 IU; Vitamin B_2 2,000 mg; Vitamin E 750 mg; Vitamin K 1,000 mg; Vitamin B_6 1,000 mg; Vitamin B_{12} 6 µg; 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 mg; Ca 500 g; P 300g; L- lysine 10 g; DL-Methionine 10 g; Selenium 50 mgl^{-1}; Selenium 50 mgl^{-1}; Satwari 250 mgl^{-1}; (Lactobacillus 120 million units and Yeast Culture 3000 crore units).

†Fruits of C. Axillaris were obtained locally and then extracts were prepared from the pulp of lapsi fruits.

††Himedia Laboratories, Mumbai, India.

Table 2: Proximate composition (%DM) of experimental diets (%)

Ingredients	Experimental diets (% Inclusion)					
	T1 (Control)	T2	T3	T4	T5	T6
Dry Matter (DM)	97.15	97.43	97.59	97.71	96.93	97.014
Moisture	2.85	2.57	2.41	2.29	3.07	2.986
Crude Protein (CP)	31.16	31.07	31.32	31.14	31.22	31.239
Ether Extract (EE)	6.56	6.37	6.11	6.98	6.755	6.855
Crude Fiber	8.32	8.32	8.43	8.79	8.845	8.997
Ash	9.23	8.73	9.53	7.69	7.84	7.458
NFE#	44.73	45.51	44.61	45.4	45.34	45.451

#Nitrogen Free Extract (NFE) = 100-(CP+EE+CF+Ash)

2.5 Examination Procedures

2.5.1 Growth and survival profiles

Before harvesting, fingerlings were fasted for 24 hours and then final weight of individual carp was measured for growth profiles. Weight gains (%), specific growth rate (SGR) and feed conversion ratio (FCR) were calculated survival percentages using the following equations:

WG (%) = Final weight - Initial weight /Initial weight ×100

SGR= ((In Wf –In Wi))/t×100

Where, Wi and Wf are the initial and final body weights and t the total duration of the experiment in days.

FCR=F/(Wf –Wo)

Where F is the weight of food supplied to fish during the experimental period; W_o is the live weight of fish at the beginning of the experimental period; W_f is the live weight of fish at the end of the experimental period.

Survival (%)=Nf/Ni×100

Where Nf is the number of fish harvested and Ni the initial number of fish.

2.5.2 Collection of tissues

At the end of the experiment 3 fingerlings from each treatment were collected and anesthetized with (5 mg/l) tricaine methane sulfonate (MS-222) for 2-3 minutes. Fingerlings were dissected properly to obtain the required tissues. Brain and liver were collected through dissection and stored at -4 ºC using buffer for further analysis.

2.5.3 Estimation of Vitamin C from the tissues (brain and liver)

The assay of vitamin C from the tissues of brain and liver were followed by the method described by Dabrowski and Hinterleitner [20]. Pre-weighed tissues of brain/liver were homogenized in ice-cold 250 mM $HClO_4$ containing 5% trichloro acetic acid (TCA) and 0.08% ethylene diamine tetraacetic acid (EDTA). The homogenates were centrifuged at 27000 g for 30 min at 4 ºC. 25 µl of 0.2% dichlorophenolindophenol (DCIP) were added to the 250 µl of deproteinized sample and to a blank and then the mixture were incubated at 37 °C for 1 hour. Then 25 µl of 1% $KBrO_3$ were added and mixtures were incubated at 37 °C for a further 1 hour. The blank was prepared in exactly the same manner as described above except that deproteinizing buffer was used instead of the sample extract. After the incubation of 1 hour at room temperature, 250 µl of 2% thiourea in 5% meta-phosphoric acid was added followed by an equal volume of 2% of 2, 4-dinitrophenylhydrazine (DNPH) in 12 M H_2SO_4. All samples were incubated for 3 hour at 60 °C after which 0.5 ml of ice-cold 18M H_2SO_4 were added. The samples were transferred into eppendorf tubes and centrifuged at 11300 g for 3 minutes. After that an absorbance was recorded at 524 nm with a spectrophotometer. Standard (20-200 µg/ml) were prepared with vitamin C (l-ascorbic acid, HiMedia).

2.6 Statistical Analysis

Value for each parameter measured has been expressed as mean ± standard error of mean. The results were analyzed by one-way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test. Significance was tested at $P<0.05$ level.

3. Results

3.1 Survival and growth performances

In the beginning of the experiment the initial weight of fish was 4.71±0.014 g and after 10 weeks of feeding trials, significantly higher ($p<0.05$) weight gain was observed in T4 diet fed group (4.14±0.006 g) on 70 days of feeding trial followed by group fed with diet T3 (3.60±0.003 g), T5(3.59±0.012 g), T6 (3.15±0.003 g), T2 (32.83±0.037 g) and the lower in the group fed with T1 (2.53±0.032 g). The highest weight increment percent was in T4 (87.96±0.59) and the lowest was in T1 (53.68±0.603) group fed carp while the weight gain (%) increments in T2, T3, T5 and T6 were 60.057±0.844, 76.57±0.043, 76.19±0.239 and 66.90±0.073% respectively. The SGR level was significantly higher ($p<0.05$) in T4 diet (0.90±0.000) followed by T3 (0.81±0.00), T5 ((0.81±0.00), T6 (0.73±0.00), T2 (0.67±0.00) and T1 (0.61±0.00) diet fed group. A higher 47.54% increment of SGR was found in T4 diet fed group as compared to control group at the end of the experiment. The FCR level found lower in T4 (0.96±0.000), followed by T3 (1.04±0.000), T5 (1.04±0.000), T6 (1.12±0.000) and finally in T2 (1.2±0.010) diet fed group. FCR level was 1.29±0.010 in control T1 diet fed group (Table 3). At the end of 70 days of feeding trials, cent per cent survival rate was observed in T3 and T4 diet fed group while in T5, T6, T2 and T1 the percent of survival were 97.78±2.22, 95.56±2.22, 95.56±2.22 and 91.11±2.22 respectively.

Table 3: Growth profiles of *C. carpio* fingerlings fed various doses of lapsi supplemented diets on 70th day of sampling

S. No.	Parameters	T1	T2	T3	T4	T5	T6
1	IW (g)	4.71±0.007	4.71±0.014	4.70±0.013	4.70±0.014	4.71±0.007	4.71±0.014
2	FW (g)	7.23±0.033	7.53±0.033	8.31±0.058	8.84±0.091	8.30±0.060	7.87±0.024
3	WG (g)	2.53±0.032	2.83±0.037	3.60±0.003	4.14±0.006	3.59±0.012	3.15±0.003
4	WGP (%)	53.68±0.603	60.057±0.844	76.57±0.043	87.96±0.59	76.19±0.239	66.90±0.073
5	SGR(%/day)	0.61±0.000	0.67±0.000	0.81±0.000	0.90±0.000	0.81±0.000	0.734±0.000
6	FCR	1.29±0.010	1.2±0.010	1.04±0.000	0.96±0.000	1.04±0.000	1.12±0.000
7	S (%)	91.11±2.22	95.56±2.22	100.00±0.00	100.00±0.00	97.78±2.22	95.56±2.22

IL= Initial length; FL=Final length; LG= Length gain; LGP= Length gain in percentage; IW= Initial weight; FW= Final weight; WG= Weight gain; WGP= Weight gain in percentage; SGR=Specific growth rate; FCR= Feed conversion ratio; S=Survival rate

Values are provided as mean ± SE.

3.2 Concentrations of vitamin C in tissues

A direct relationship was found between the dose of lapsi fruits in the diet of carp and the concentration of vitamin C in the brain of fish (Fig.1 Table 4). Vitamin C level was significantly ($P<0.05$) higher in the carp fed with diet T4 (74.50±2.33 µg/mg) followed by fish fed with diet T6 (64.62±1.69 µg/mg), T5 (64.10±1.68 µg/mg), T3 (53.06±1.65 µg/mg), T2 (25.76±1.45µg/mg) and the minimum was in fish fed with diet T1 (17.68±1.73 µg/mg). A 45.7 to 321.38% higher vitamin C level was recorded in the fish fed with 800 mg vitamin C incorporated diet compared to others (Fig.1 Table 4). Similar trend for vitamin C was also recorded in liver (Fig.2 Table 4). The concentration of vitamin C was significantly ($P<0.05$) higher in the carp fed with diet T4 (82.77±1.28 µg/mg) followed by fish fed with diet T5 (76.11±1.28 µg/mg), T6 (69.46±1.28 µg/mg), T3 (61.36±4.63 µg/mg), T2 (59.04±3.64 µg/mg) and the minimum was in T1 (48.03±2.29 µg/mg) diet fed group. The vitamin C level was 73.2% higher in T4 diet fed fish compared to the fish fed with diet T1; whereas it was 22.9% higher in T2 compared to the fish fed with diet T1(Fig.2 Table 4).

Table 4: Concentrations of vitamin C in carp fed varied doses of lapsi up to 10 weeks of trials.

S. No.	Parameters	T1	T2	T3	T4	T5	T6
1	Vit-C B	17.68±1.73	25.76±1.45	53.06±1.65	74.50±2.33	64.10±1.68	64.62±1.69
2	Vit-C L	48.03±2.29	59.04±3.64	61.36±4.63	82.77±1.28	76.11±1.28	69.46±1.28

Vit-C B = Vitamin C in Brain; Vit-C L = Vitamin C in Liver

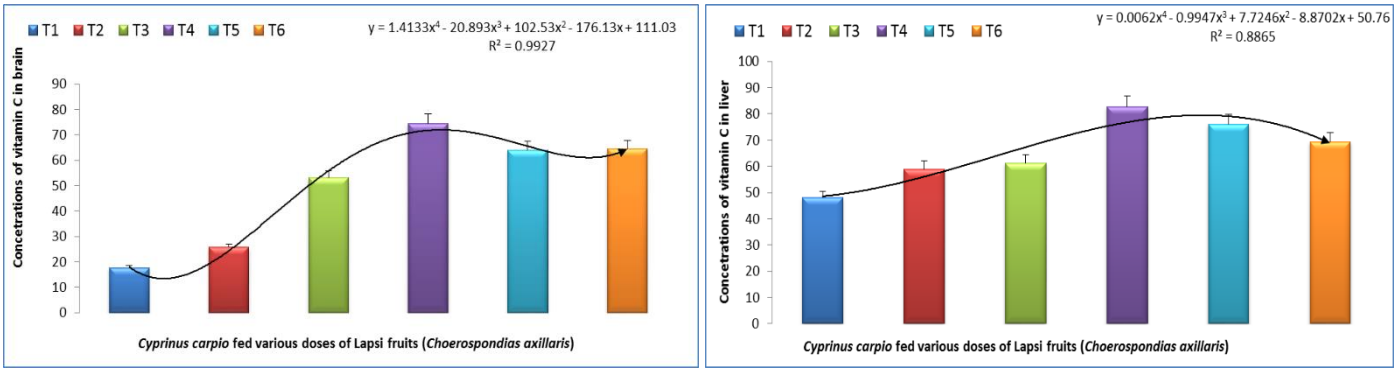

Fig 2: Concentrations of vitamin C in liver after *Cyprinus carpio* fed with diets containing six different doses of lapsi fruits.

4. Discussion

4.1 Effects of lapsi fruits on growth of carp

To develop alternative practice for growth promotion and disease management in aquaculture, attention has also been focused in identifying novel drugs, especially from plant sources. These drugs may be delivered to the cultivable organisms either through feed supplementation or oral delivery through predator larvae or any other micro particulate diets mode. Several herbs have been tested for their growth promoting activity in aquatic animals [21-23]. The present study demonstrated the diets supplemented with ethanol extracts from medicinal plant fruits of lapsi enhanced growth and immunostimulant in common carp fed with 70 days. The growth of the carp has been increased in all experimental groups when compared to control except and among the tested diets T4 showed highest rate of growth and the enhanced growth rate could be due to the growth promoting effect of lapsi extract. The increased growth rates and feed efficiency in several fish species fed diets sufficient in vitamin C are well documented elsewhere [24-26]. Navarre and Halver [27] reported that a higher weight gain was observed in rainbow trout fed high dietary AA (500 to 2000 mg/Kg diet). The minimum requirement to support optimal growth was estimated to be between 10 and 25 mg ascorbic acid equivalent/Kg diet for channel catfish [28], rainbow trout [29], hybrid striped bass [30] and hybrid tilapia [31]. Lee and Bai [32] reported that Korean rockfish, *Sebastes schlegelii* (Hilgendorf), fed 1500 mg/Kg diet showed the highest weight gain compared to fish fed 25 to 150 mg vitamin C/Kg diet. Gouillou-Coustans *et al.* [33] reported that the growth rate of *Cyprinus carpio* larvae reached a maximum at a vitamin C level of 90 mg/Kg dry diet, while body vitamin C concentrations reached plateau values at or above 270 mg/Kg dry diet. The vitamin C for optimum growth of *Cyprinus carpio* [33] and newly hatched *Cirrhinus mrigala* [34] was 650 to 700 mg/Kg. Dabrowski *et al.* [35] reported that the growth rates were significantly affected by dietary ascorbic acids levels and growth of fish fed the high-ascorbate diet was greater than in low or required-ascorbate level diets. Lee and Dabrowski [36] showed a typical growth trend in which growth rate is higher in the fish fed vitamin C supplemented diets than in fish fed a diet devoid of vitamin C. Tewary and Patra [37] reported that in *Labeo rohita* maximum growth (50.88±0.18) was observed in fish fed with 1000 mg AA/Kg diet, while the lowest growth (30.83±0.12) was observed in control diet fed fish.

In recent times, a large portion of the world population, especially in developing countries depends on the traditional system of medicine for a variety of diseases. Therefore, the herbal drugs (medicinal plants) are used not only against diseases but also as growth promoters, stress resistance boosters and preventatives of infections. The phytochemicals, such as tannins, alkaloids and flavonoids present in lapsi (*C. axillaris*) may have antimicrobial activity. Herbal drugs are used not only against diseases but also as growth promoters, stress resistance boosters and preventatives of infections. The herbs can also act as immunostimulants, conferring the non-specific defense mechanisms of fish and elevating the specific immune response. Studies have proved that herbal additives enhance the growth of fishes and protect them from diseases. Inclusion of herbal additives in diets often provides cooperative action to various physiological functions. Beneficial role of vitamins C have been reported in fish nutrition, reproduction, growth and related indices. In addition, vitamins C and E are credited with modulating the stress response in fish. The biological role played by vitamins C is very vital for the sustained growth and health of many living organisms as well as fish. Dietary vitamins have antibody enhancement effects in fish. The herbal drugs, viz., ginger, nettle and mistletoe have been used as an adjuvant therapy in rainbow trout fish through feed. The disease resistant of catla fish has been produced through the immersion herbal treatment (neem, garlic and turmeric) of spawn. In the present article, therefore, the beneficial effects of certain herbal supplements on the health and disease resistance of fish have been elucidated.

The common carp *Cyprinus carpio* Linnaeus (1758) has been one of the oldest domesticated species of fish for food in Nepal. Common carp have been introduced in Nepal since 1950 [38]. The current report is based on the graded level of ethanolic extract of lapsi fruits supplementation in the food of carp for better production. Vitamin C (ascorbic acid) is an essential nutrient in aqua-feeds. It is an indispensable nutrient required to maintain physiological processes such as normal growth, immunity and reproduction of different animals including fishes [39]. Ascorbic acid is water soluble and is essential for several metabolic functions including the antioxidant system. Most fishes, including common carp, are not capable of vitamin C biosynthesis [40] due to the absence

of the enzyme L-gulonolactone oxidase, which is responsible for synthesis of ascorbic acid [41]. Recent studies indicate that ascorbic acid derivatives that include sulfate and phosphates are more resistant to oxidation and retain ascorbic acid activity in fish [42]. Ascorbic acid requirements of some fish species have been investigated. Stickney *et al.*, [43] reported the fortification of 50 mg of ascorbic acid equivalent kg-1 diet as the level that allows for maximum weight gain and absence of deficiency signs in blue tilapia *(Oreochromis aureus).* A diet of 79 mg ascorbic acid kg^{-1} diet was found to be the requirement level for maximum weight gain of hybrid tilapia *(Oreochromis niloticus x Oreochromis aureus)* [44]. Studies have shown that high ascorbic acid concentration in tissue determines higher tolerance to ambient pollution and better resistance to bacterial infection (Li & Lovell, 1985) [45]. Abdel- Tawwab *et al.*, [46] found that a high level of ascorbic acid enhanced the weight gain, specific growth rate and survival rate of tilapia exposed to sub lethal dose of mercury. There is a relationship between tissue ascorbate and the fish health [47].

After 10 weeks of feeding trials in the present experiment a direct relationship was found between the doses of lapsi fruit extracts in the diet of common carp and the concentration of vitamin C in the brain and liver of fish. Vitamin C level in brain and liver were significantly (P<0.05) higher in the carp fed with diet T4 diet followed by carp fed with T6, T5, T3 and T2 diets and minimum in group fed with diet T1 diet. This is due to the presence of vitamin C in lapsi fruit [14]. The diet without ascorbic acid supplementation decreased the specific growth rate (0.32% day^{-1}) of juvenile *O. karongae* and this is in accordance with studies conducted by Ai *et al.* [48] who also observed declining specific growth rate with ascorbic acid deficient diet for seabass *(Scophthalmus maximus).* The concentration of vitamin C in various tissues is related to the dietary intake in diets. Some tissues such as brain and liver accumulate high concentrations of vitamin C [49].

In the present study, the concentration of vitamin C in brain and liver of common carp fed with various doses of lapsi were evaluated. Vitamin C concentration in brain was found higher in the carp fed with T4 diet compared to the carp fed with other groups. Lim and Lovell [50] and Murai *et al.* [51] found a good correlation between the dietary and liver AA concentrations in channel catfish. Liver ascorbic acid content is usually considered as an indicator of the vitamin C status [52-54]. In red drum, liver storage of ascorbic acid in response to graded levels of dietary vitamin C was not as readily influenced as seen in other species such as channel catfish [55]. However, levels of total ascorbate in liver of red drum diets with supplemental vitamin C were generally similar to those reported for some other fish species [56-58]. Hilton *et al.*, [59], found the rainbow trout maintain relatively constant liver AA levels when fed diets supplemented with 80 to 320 mg AA/Kg, but when fed a diet supplemented with 1280 mg AA/Kg (12 times the requirement for normal growth), liver AA increased to a level more than double that in the fish supplemented with 320 mg AA/Kg. The AA concentrations of five tissues (brain, heart, liver, head kidney and muscles) in fish fed the diet supplemented with 800 mg AA/Kg (10 times the optimum dietary level for maximum growth) were more than 2 to 3 times those of fish fed the diets supplemented with 25 to 150 mg AA/Kg [60]. The liver AA content of fish fed diet containing 762 mg AA/Kg was significantly *(P*<0.05) higher than fish fed diets containing 32 mg AA/Kg [61]. Ibiyo *et al.* [62] found that in the vitamin C concentration in the liver, kidney, gills and muscles of *Heterobranchus longifilis* (2.3±0.3 g) fed graded levels of ascorbic acid were positively correlated with dietary level of the vitamin.

5. Conclusion

In conclusion, we found that dietary supplementation with *Choerospondias axillaris* fruits enhanced the growth of common carp *Cyprinus carpio*, provided protection against oxidative stress, and prevented tissue damage and immunity in the body. The *C. axillaris* fruits improved the overall health status of the carp. Incorporation of *Choerospondias axillaris* fruits at 0.80% proved effective for this early developmental stage of carp. *Choerospondias axillaris* fruits have immense potential for supporting the production of healthy fish during aquaculture.

6. References

1. Hassan M, Lehninger AL. Enzymatic formation of ascorbic acid in rat liver extracts. J Biol. Chem. 1956; 223:123-138.
2. Stubbs DW, Haufrect DB. Effects of actinomycin D and puromycin on induction of gulonolactone hydrolase by somatotrophic hormone. Arch. Biochem. Biophys. 1968; 124:365-371.
3. Chatterjee IB, Chatterjee GC, Ghosh NC, Ghosh JJ, Guha BC. Biological synthesis of L-ascorbic acid tissues: conversion of L-gulonolactone into L-ascorbic acid. Biochem. J. 1960; 74:193-203.
4. 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.
5. Dykhuizen DE, Harrison KM, Richardson BJ. Evolutionary implications of ascorbic acid production in the Australian lungfish. Experientia. 1980; 36:945-946.
6. Dabrowski K. Primitive Actinopterigian fishes can synthesize ascorbic acid. Experientia 1994; 50:745-748.
7. Touhata K, Toyohara H, Mitani T, Kinoshita M, Satou M, Sakaguchi M. Distribution of L-gulono-1,4-lactone oxidase among fishes. Fish. Sci. 1995; 61:729-730.
8. Moreau R, Kaushik SJ, Dabrowski K. Ascorbic acid status as affected by dietary treatment in the Siberian sturgeon_*Acipenser baeri* Brandt. Tissue concentration, mobilisation and L-gulonolactone oxidase activity. Fish Physiol. Biochem. 1996; 15:431-438.
9. Moreau R, Dabrowski K. Body pool and synthesis of ascorbic acid in adult sea lamprey _ *Petromyzon marinus*. An agnathan fish with gulonolactone oxidase activity. Proc. Natl. Acad. Sci. USA. 1998; 95:10279-10282.
10. Mæland A, Waagbø R. Examination of the qualitative ability of some cold water marine teleosts tosynthesise ascorbic acid. Comp. Biochem. Physiol. 1998; 121A:249-255.
11. 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.
12. Roxburgh W. Didynamia Angiospermia. Flora Indica. W. Thacker & Co.: Serampore Calcutta, Edn 2. 1832; 3:1-116.
13. Paudel KC, Pieber K, Klumpp R, Laimer M. Evaluation of lapsi tree *Choerospondias axillaris* (Roxb.) for fruit production in Nepal, Bodenkultur-Wien and Munchen. 2003; 54(1):3-10.

14. 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.
15. Chunmei Li, Jie He, Yonglin, Gao, Yanli, Xing *et al*. Preventive Effect of Total Flavones of *Choerospondias axillaris* on chemia/Reperfusion-Induced Myocardial Infarction-Related MAPK Signaling Pathway; Cardiovasc Toxicology. 2014; 14:145-152.
16. Zhou J, Huang J, Song XL. Applications of immunostimulants in aquaculture. Marine Fish Research. 2003; 24:70-79.
17. Labh SN, Shakya SR, Kayasta BL. Extract of Medicinal lapsi *Choerospondias axillaris* (Roxb.) exhibit antioxidant activities during in vitro studies. Journal of Pharmacognosy and Phytochemistry. 2015; 4(3):194-197.
18. Shi S, Li ZX, Tian FJ, Bai YF, Tian L, Yang YM. Effect of flavanoid from *Choerospondias axillaris* fruit on left ventricle function and hemodynamics of anaesthesia dog. Inner Mongolia Pharmaceutical Journal. 1985; 2:14-15.
19. AOAC. Official methods of analysis. 16th ed. Association of Official Analytical Chemists; Arlington, Virginia, USA, 1995.
20. 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.
21. Citarasu T, Sekar RR, Babu MM, Marian MP. Developing Artemia enriched herbal diet for producing quality larva in *Peneaus monodon*. Asian Fish Science. 2002; 15:21-32.
22. Sivaram V, Babu MM, Immanuel G, Murugadass S, Citarasu T, Marian MP. Growth and immune response of juvenile greasy groupers (*Epinephelus tauvina*) fed with herbal antibacterial active principle supplemented diets against Vibrio harveyi infections. Aquaculture. 2004; 237:9-20.
23. Immanuel G, Citarasu T, Sivaram V, Michael BM, Palavesam A. Delivery of HUFA, probionts 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.
24. Dabrowski KN, El-Fiky G, Kock Frigg M, Wieser W. Requirement and utilization of ascorbic acid and ascorbic sulfate in juvenile rainbow trout. Aquaculture. 1990; 91:317-337.
25. Dabrowski K, Matusiewicz K, Matusiewicz M, Hoppe PP, Ebeling J. Bioavailability of vitamin C from two ascorbyl monophosphate esters in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquaculture Nutrition. 1996; 2:3-10.
26. Lee SH, Oe T, Blair IA. Vitamin C induced decomposition of lipid hydroperoxides to endogenous genotoxins. Science. 2001; 292:2083-2086.
27. Navarre O, Halver JE. Disease resistance and humoral antibody production in rainbow trout fed high levels of vitamin C. Aquaculture. 1989; 79:207-221.
28. Mustin WG, Lovell RT. Na-L-ascorbyl-2-monophosphate as a source of vitamin C for channel catfish. Aquaculture 1992; 105:95-100. National Research Council Nutritional requirements of fish. National Academy Press, Washington, DC, 1993.
29. Cho CY, Cowey CW. Utilization of monophosphate esters of ascorbicacid by rainbow trout (*Oncorhynchus mykiss*). Pages in S.J. Kanshik and P. Luquet, eds. Fish Nutrition in Practice. Institut National de la Recherch Agronomique, Paris, France, 1993, 149-156.
30. Sealey WM, Gatlin DM. III. Dietary vitamin C requirement of hybrid striped bass (*Morone chrysops* × *M. saxatilis*). Journal of the World Aquaculture Society. 1999.
31. Shiau SY, Hsu TS. Quantification of vitamin C requirement for juvenile hybrid tilapia, *Oreochromis niloticus*, with L-ascorbyl-2-monophasphate Na and L-ascorbyl-2-monophasphate Mg. Aquaculture. 1999; 175:317-326.
32. Lee K-J, Bai SC. Different dietary levels of L-ascorbic acid affect growth and vitamin C status of juvenile Korean rockfish (*Sebastes schlegelii*). Aquaculture. 1998; 161:475-477.
33. Gouillou-Coustans MF, Bergot P, Kaushik SJ. Dietary ascorbic acid needs for common carp (*Cyprinus carpio*) larvae. Aquaculture, 1998, 453-461.
34. Mahajan CL, Agrawal NK. Comparative tissue ascorbic acid studies in fishes. J Fish Biol. 1980; 17:135-141
35. Dabrowski K, Lee KJ, Guz L, Verlhac V, Gabaudan J. Effects of dietary ascorbic acid on oxygen stress, growth and tissue vitamin concentration in juvenile rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 233 2004; 1-4:383-392.
36. 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.
37. Tewary A, Patra BC. Use of vitamin C as an immunostimulant. Effect on growth, nutritional quality, and immune response of *Labeo rohita* (H.) Fish Physiology and Biochemistry. 2008; 34:251-259.
38. Shrestha J. Biodiversity: Fish, In: Biological diversity and conservation (eds.) P.K. Jha, F.P. Neupane, M.L. Shrerstha and I.P. Khanal. Nepal Academy of Science and Technology, Khumaltar, Lalitpur. Nepalpedia series 2. 2013, 69-81.
39. Tolbert BM. Ascorbic acid metabolism and physiological function. International Journal of Veterinary, Nutrition and Research. 1979; 19:127-142.
40. Chatterjee IB. Evolution and the biosynthesis of ascorbic acid. Science. 1973; 182:1271-1272.
41. Wilson RP. Absence of ascorbic acid synthesis in channel catfish, *Ictalurus punctatus* and blue catfish, Ictalurus frucatus. Comp. Biochem. Physiol. 1973; 46B:635-638.
42. Abdelghany A. Growth response of Nile tilapia *Oreochromis niloticus* to dietary L-ascorbic acid, L-ascorbyl-2-sulfate, and L-ascorbyl-2-polyphosphate. Journal of the World Aquaculture Society. 1996; 27:449-455
43. Stickney RR, McGeachin RB, Lewis DH, Marks J, Riggs A, Sis RF *et al*. Response of *Tilapia aurea* to dietary vitamin C. Journal of the World Mariculture Society. 1984; 15:179-185.
44. Shiau SY, Jan FL. Dietary Ascorbic acid requirement of Juvenile tilapia *Oreochromis niloticus* × *O. aurea*. Bulletin of the Japanese Society of Scientific Fisheries 1992; 58:671-675.

45. Li Y, Lovell RT. Elevated levels of dietary ascorbic acid increases immune response in channel catfish. Journal of Nutrition. 1985; 115:123-131.
46. Abdel-Tawwab M, Shalaby AME, Ahmed MH, Khattab YA. Effect of supplement dietary L- ascorbic acid (vitamin C) on mercury intoxication and growth performance of Nile tilapia (*Orechromis niloticus* L.). Annals of Agric. Sci. Moshtoher. 2001; 39(2):961-973.
47. Halver JE. Recent advances in vitamin nutrition and metabolism. In: Cowey, C.B., Mackie, A.M. and Bell, J.G. (Eds.), Nutrition and Feeding in Fish. Academic Press, New York, 1985, 415-429.
48. Ai QH, Mai KS, Li HT, Zhang CX, Zhang L, Duan QY *et al*. Effects of dietary protein to energy ratios on growth and body composition of juvenile Japanese sea bass, *Lateolabrax japonicus*. Aquaculture. 2004; 230:509-516.
49. Gabaudan J, Verlhac V. Biological efficacy of Rovimix Stay-C as a source of vitamin C for Salmonids. Proceeding of the International Symposium on Cultivation of Atlantic Salmon, Bergen, 1992, 16-20.
50. Lim C, Lovell RT. Pathology of the vitamin C deficiency syndrome in channel catfish, *Ictalurus punctatus*. Journal of Nutrition. 1978; 108:1137-1146.
51. Murai T, Andrews JW, Bauerfeind JC. Use of L-ascorbic acid, ethocel coated ascorbic acid and ascorbate-2-sulfate in diets for channel catfish *Ictalurus punctatus*. Journal of Nutrition. 1978; 108:1761-1766.
52. Gabaudan J, Verlhac V, Schuep W. Calcium L-ascorbate-2- monophosphate salt as a source of vitamin C for rainbow trout (*Oncorhynchus mykiss*). In: Wenk, C., Fenster, R. and Volker, L. (eds.), Ascorbic Acid in Domestic Animals. Proceedings of the Second Symposium, Zurich, Switzerland, 1992, 395-401.
53. White A, Fletcher TC, Secombes CJ, Houlihan DF. The effect of different dietary levels of vitmin aC and E and their tissue levels in Atlantic salmon, *Salmo salar* L. Kaushik, S. J. and Luquet, P. (eds.), Fish Nutrition in Practice, Les Colloques Paris, 1991, 203-207.
54. Fournier V, Gouillou-Coustans MF, Kaushik SJ. Hepatic ascorbic acid saturation is the most stringent response criterion for determining the vitamin C requirement of juvenile European sea bass (*Dicentrarchus labrax*). Journal of Nutrition. 2000; 130:617-620.
55. El Naggar GO, Lovell RT. L-Ascorbyl-2-monophosphate has equal antiscorbutic activity as L-ascorbic acid but L-ascorbyl-2-sulfate is inferior to L-ascorbic acid for channel catfish. J. Nutr. 1991; 121:1622-1626.
56. Bai SC, Gatlin DM. III. Dietary rutin has limited synergistic effects on vitamin C nutrition of fingerling channel catfish (*Ictalurus punctatus*). Fish Physiology Biochemistry 1992; 10:183-188.
57. Halver JE, Hardy RW. L-ascorbyl-2-sulfate alleviates Atlantic salmon scurvy. Proceedings of the Society of Experimental Biology and Medicine 1994; 206:421-424.
58. Matusiewicz M, Dabrowski K. Utilization of the bonelliver alkaline phosphatase activity ratio as an indicator of ascorbate deficiency in salmonid fish. Proceedings of the Society of Experimental Biology and Medicine 1996; 212:44-51.
59. Hilton JW, Cho CY, Slinger SJ. Effect of graded levels of supplemental ascorbic acid in practical diets fed to rainbow trout (*Salmo gairdneri*). Journal of Fisheries Research Board of Canada 1978; 35:431-436.
60. Misra CK, Das BK, Mukherjee SC, Pradhan J. Effects of dietary vitamin C on immunity, growth and survival of Indian major carp *Labeo rohita*, fingerlings. Aquaculture Nutrition 2007; 13:35-44.
61. Ren T, Koshio S, Teshima S, Ishikawa M, Saichiro Y, Micheal FR *et al*. Influence of dietary vitamin C and bovine lactoferrin on blood chemistry and non-specific immune response of Japanese eel, *Anguilla japonica*. Aquaculture 2007; 267(1):31-37 ·
62. Ibiyo LMO, Atteh JO, Omotosho JS, Madu CT. Vitamin C (ascorbic acid) requirements of *Heterobranchus longifilis* fingerlings. African Journal of Biotechnology. 2007; 6:1559-1567.