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Effects of dietary lapsi (*Choerospondias axillaris* Roxb.) on survival, growth and protein profile of common carp (*Cyprinus carpio* L) fingerlings

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Abstract
This study was conducted to evaluate the effects of dietary lapsi *Choerospondias axillaris* (Roxb.) on survival, growth and protein profile of *Cyprinus carpio* (L) fingerlings. Six practical diets were formulated to contain 0.0 (control), 100, 200, 400, 800, and 1600 mg ethanol extract of lapsi fruits equivalent kg ($^{-1}$) diet. Each diet was fed to triplicate groups of fingerlings of *C. carpio* (4.71± 0.012 g) in 100-L glass aquaria as T1, T2, T3, T4, T5 and T6 @ 3% of their body weight twice daily for 70 days. The results showed statistically significant (P<0.05) increased in weight gain, SGR, total protein and globulin which may be considered as a sign of improvement in immune system. It may be due to presence of antioxidant properties (vitamin C) in lapsi fruit extract which act as an antioxidant. Finally, it has been concluded that a minimum amount of 0.4 g lapsi fruit extracts kg^{-1} is sufficient to be added in diet for increment of good growth and serum protein in common carp.

Keywords: Carp, protein, growth, SGR, lapsi, *Choerospondias axillaris*

1. Introduction
Aquaculture is the fastest-growing food-production sector in the world, now providing almost half of the global fish supply. Increases in demand for fish indicate that aquaculture needs to expand, particularly in Asia. To meet this growing demand, World Fish uses its technical and scientific expertise in fisheries and aquaculture to promote evidence-based development solutions and increase aquaculture productivity, while minimizing impacts on the environment by developing technologies, improving resource management, securing access to essential inputs and improving connections to markets. Development of sustainable fish feeds represents a key component of future program. Aquaculture in Nepal is basically small and new that contributes 3% to the agricultural GDP. Rivers are the major source of capture fishery covering 395000 ha. of the surface natural water resources. Around 75000 people are engaged in aquaculture with net fish production of 64,900 Mt. (culture fisheries 43,400 Mt. and capture fisheries 21,500 Mt.) in the year 2014 [1] against 57,500 Mt. in fiscal 2012-13. The present annual fish production in Nepal is 69,500 Mt [2]. The country's fish production has not been able to meet local demand despite a rapid growth in fish farming however; around 80 percent of the domestic requirement of fish is fulfilled by local production while the rest is met by imports. In Nepal, many fishermen, their families and others are engaged in capture fisheries, which represent nearly 0.28% of the total population of Nepal.

Lapsi *Choerospondias axillaris* (Roxb.) [3] 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 [4]. The fruits are rich in vitamin C content [5] and are used as a medicinal plant to enhance the immune system of the body [6]. Phenol and flavonoid compounds [7, 8] present in the fruit of lapsi serve as antioxidants. So there are potential benefits of consuming phenolic rich foods [9]. Thus, keeping these things in mind an experiment was carried out in the wet laboratory of Central Department of Zoology, Tribhuvan University, Kirtipur (Nepal) to study the effect of lapsi extract supplemented diets on survival, growth and protein profile of common carp *Cyprinus carpio* fingerlings.

2. Materials and Methods
2.1 Preparation of lapsi fruits supplemented artificial diets
The crude extract of the pulp of lapsi fruits was prepared by using ethanol (70%) as described by Labh *et al*., [8].10 g of lapsi fruit powder was taken in conical flask and added 500 ml of 70 % ethanol. The flask was sealed by cotton plug and aluminum foil and then kept in orbital shaker for 48 hrs. The mixture was then filtered using Whatman filter paper No.1 and filtrate was centrifuged at 10,000 rpm for 5 minutes to collect the supernatant. The supernatant was concentrated at 70 °C using the water bath. Finally, a greasy substance (crude extract) of the lapsi fruit was obtained which was transferred to screw-cap bottle labeled and stored at 4° C until use. Altogether six treated 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 100, 200, 400, 800 and 1600 mg kg $^{-1}$ lapsi fruit extracts. Other standard ingredients were used during feed preparation (Table1).

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.
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2.2 Collection of fish, experimental design and feeding trial

Fingerlings of common carp *Cyprinus carpio* were procured from local hatchery and after acclimatization in laboratory conditions, a total of two hundred seventy fingerlings (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 80 l throughout the experimental period. Fingerlings were fed twice daily at 3% of the body weight for 70 days. The uneaten feed and faecal matters were siphoned daily and two third of the aquarium water was replaced at weekly intervals. Temperatures were ranged from 25^0 C to 29^0 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. A randomly 5 fingerlings were weighed randomly from each aquarium on every 14 days interval to adjust the feeding status of carp.

2.3 Proximate analysis of feed

The proximate composition of the experimental diets (Table 2) was analyzed following the standard methods of the Association of Official Analytical Chemists [10]. The moisture content was determined by drying at 105 ^{0}C to a constant weight. Nitrogen content was estimated by automated Kjeldahl apparatus (2200 Kjeltec Auto distillation, Foss Tecator, Sweden) and crude protein was estimated by multiplying nitrogen percentage by 6.25. Ether extract (EE) was measured using a Soxtec system (1045 Soxtec extraction unit, Tecator, Sweden) using diethyl ether (boiling point, 40-60 ºC) as a solvent and ash content was determined by incinerating the samples in a muffle furnace at 600 ºC for 6 hours. Nitrogen free extract (NFE) was calculated by difference i.e., NFE = 100 − (CP + EE+ CF+ Ash).

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)

2.4 Examination Procedures

2.4.1 Growth and survival profiles

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

$$\text{WG (\%)} = \text{Final weight} - \text{Initial weight} / \text{Initial weight} \times 100$$

$$\text{SGR} = ((\text{In Wf} - \text{In Wi}))/\text{t} \times 100$$

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

$$\text{FCR} = \text{F}/(\text{Wf} - \text{Wo})$$

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.

$$\text{Survival (\%)} = Nf/Ni \times 100$$

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

2.4.2 Blood collection protein estimation

At the end of the feeding trial, three fish from each of the control and experimental groups were anaesthetized with tricaine methane sulfonate (MS-222) (5 mg l^{-1}) for 2-3 minutes. Blood were collected from the caudal vein using a syringe with 25 gauge needle. The blood samples were then transferred immediately to eppendorf tubes and allowed to clot for a while then centrifuged for 5 min at 3000×g and thus collected serum was stored at -20 °C for further analysis. Total serum protein content was determined by biuret method developed by Doumas (1975) [11] using a kit whereas albumin was determined by BCG (Bromo Cresol Green) method developed by Doumas [12]. Globulin was calculated by the deduction of albumin from total protein while albumin - globulin ratio was calculated by dividing albumin values by globulin values.

2.5 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

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. 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).

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 Protein profiles

A direct relation was observed between the doses of lapsi fruit extract in the diet of common carp and protein concentration in serum. The concentration of total protein was found significantly (P<0.05) high in the serum of carp fed with diet T4 (32.38±0.495 µg/dl) followed by diet T3 (28.42±0.4085 µg/dl), diet T2 (18.93±0.6609 µg/dl), T5 (17.87±0.2303 µg/dl), T6 (15.57±0.4017 µg/dl) and control diet T1 (7.01±0.4270 µg/dl). The concentration of total protein was five folds higher in the carp fed with diet T4 as compared to the carp fed with control diet T1. Similar trend of average albumin concentration was recorded from common carp fingerlings after the feeding trials of 70 days with different concentration of lapsi extract. The quantitative estimation of albumin in the serum of carp showed that the concentration of albumin in the serum increased as the dose of lapsi extract in the diet increased. The concentration of albumin level was significantly (P<0.05) high in the fish fed with diet T4 (12.51±0.4706 µg/dl) followed by diet T3 (11.04±0.6982 µg/dl), diet T2 (7.72±0. µg/dl), T5 (6.37±0.2809 µg/dl), T6 (4.88±0.5578 µg/dl) and control diet T1 (2.55±0.1755 µg/dl). Albumin level was 20.38.47% higher in the carp fed with diet T4 compared to control diet T1. Similar trend of average albumin concentration was recorded from common carp fingerlings after the feeding trials of 70 days with different concentration of lapsi extract. The quantitative estimation of albumin in the serum of carp showed that the concentration of

albumin in the serum increased as the dose of lapsi extract in the diet increased. The concentration of globulin level was significantly (P<0.05) high in the fish fed with diet T4 (19.87±0.15901 µg/dl) followed by diet T3 (17.39±0.7279 µg/dl), diet T2 (7.72±0.4217 µg/dl), T5 (11.50±0.1561 µg/dl), T6 (10.68±0.1561 µg/dl) and control diet T1 (4.46±0.5773 µg/dl). Globulin level was 22.44 % higher in the carp fed with diet T4 compared to control diet T1. The ratio of albumin and globulin in serum of common carp fingerlings fed with different treatments was found to highest in fish fed with diet T2 (0.71±1.1174) followed by diet T3 (0.64±0.0.617), diet T4 (0.63±0.0242), T1 (0.60±0.1120) and T6 (0.46±0.0590) (Table.4).

Table 4: Protein profile of C. carpio fingerlings fed various doses of lapsi supplemented diets for 70 days

S. N.	Treatments	T1	T2	T3	T4	T5	T6
1	Serum- Protein	7.01±1.279	18.93±1.980	28.42±1.224	32.38±1.483	17.87±0.690	15.57±1.204
2	Serum- Albumin	2.55±0.526	7.72±1.264	11.04±2.092	12.51±1.410	6.37±0.842	4.88±1.671
3	Serum- Globulin	4.46±1.730	11.21±3.240	17.39±2.181	19.87±0.476	11.50±0.468	10.68±0.468

Values are provided as mean ± SE.

4. Discussion

Fish is a highly nutritive and rich source of animal proteins. For the improvement of fisheries and to achieve maximum yields from resources of fresh water, it is necessary to provide an artificial feed, by which fish grow rapidly and attain maximum weight in shortest possible time [13]. One approach is to include new substances into fish diets to improve feed conversion efficiency or elevate general conditions for fish growth and maintenance [14]. Plants are natural source of safer and cheaper chemicals. Beneficial effects of bioactive plant substances in animal nutrition may include the stimulation of appetite and feed intake, the improvement of endogenous digestive enzyme secretion, activation of immune responses and antibacterial, antiviral and antioxidant actions [15].

There is no standard practical grow-out diet for carp in Nepal and only few diets have been used by fish farmers but these diets are still under research and development. Also, most fish farmers in Nepal use diets commercially available, particularly those for tilapia and trout fish. Various extracts from herbs and spices are reported to improve animal performance by stimulating action on gut secretions or by having a direct bactericidal effect on gut microflora and furthermore the herbals active principles in the diets induce the secretion of the digestive enzyme and the growth promoter in herbs induced high protein synthesis [15]. Lapsi is an important medicinal herb extensively cultivated in many countries and has played an important dietary function as well as medicinal role for centuries. In our study better weight gain and SGR were observed which is similar to the result obtained by Abdel-Hakim *et al*., [16] who reported that incorporation of garlic in diet of Nile tilapia (diet with fresh garlic 3 g per kg) resulted in significant improvement in weight gain, feed conversion and protein efficiency. Feed conversion ratio (FCR) is an important indicator of the quality of fish feed, a lower FCR indicate better utilization of the fish feed [17]. In this experiment as the dose of lapsi fruit extracts increased in the diets FCR level decreased and the better lowest FCR was recorded in T4 diet fed carp. The current FCR values coincided with ranges reported for *O. niloticus* ranging from 1.43 to 2.30 [18, 19, 20] but were lower than the FCR of 2.6 to 3.0 in tilapia fed on on-farm formulated diets in fertilized ponds [21]. It is evident that lapsi supplemented diet promotes growth in common carp. Several herbs such as garlic, onion, marjoram, caraway, basil, anise, fennel, licorice, black seed and fenugreek have been tested for growth promoting activities [22-24], feed conversion [25-28] and improvement of protein digestibility and energy retention [26, 27] in aquatic animals.

The results of this study showed that feeding *C. carpio* with supplemented diets containing lapsi fruit extract enhanced total plasma protein, albumin and globulin values in treatment groups. Similar to present observations were obtained by Rao *et al.,* [29] after feeding the rohu fingerlings (*Labeo rohita*) with *Achyranthes aspera* seed. Similar observations were also obtained by Sahu *et al*., [30] who reported that serum protein, albumin and globulin levels in *L. rohita* fingerlings fed with *Magnifera indica* kernel were higher than control. Since serum proteins include various humoral elements of the non-specific immune system, high concentrations of total serum protein, albumin and globulin might be due to the enhancement of non-specific immune response of fishes.

Higher concentration in protein profile of serum in carp fed with lapsi fruit extracts treated diets in the present study may be due to antioxidant property of lapsi fruit extract. In agreement with present findings [31] found that the use of Garlic (*Allium sativum*) and Ginger (*Zingiber officinale*) or mixture increased total plasma protein, albumin and globulin concentration significantly (*P*<0.05) and concluded that they protected the liver against deleterious agents and free radical- mediated toxic damages to the liver cells. The same result was also supported by Metwally [32]. By adding extract of ginger to fish diet increased the total protein level, the highest level of plasma protein was observed in those fishes fed with 1% ginger extract [33]. Sivaram *et al*., [24] used methanolic extracts of *O. sanctum*, *W. somnifera* and *Myristica fragrans* herbs and found significantly improved in albumin–globulin (A/G) ratio along with other immune parameters against *Vibrio harveyi* in juvenile greasy grouper, *Epinephelus tauvinae.*

Plant products have been reported to promote various activities like anti-stress, growth promotion, appetite stimulation and immuno stimulation in aquaculture practices [34, 23, 24]. Garlic (*Allium sativum*) is a perennial bulb-forming plant that belongs to the genus *Allium* in the family *Liliaceae*. Garlic has been a subject of considerable interest for centuries as a flavouring agent, traditional medicine, and a functional food to enhance physical and mental health. Garlic was studied in different forms of extracts: aqueous, ethanol and dried powder [35]. It contains a variety of organosulfur compounds such as allicin, ajoene, S-allylcysteine, diallyl disulfide, S-methylcysteine sulfoxide and S-allylcysteine [36]. A wide array of beneficial effects of garlic such as antihypertensive, antihyperlipidemic, antimicrobial, hypoglycaemic, anticancer, antidote (for heavy metal poisoning), anticarcinogenic, hepatoprotective and immunomodulation have been reported by several researchers

[37, 38, 39, 40, 41, 42]. Studies on garlic as an alternative growth promoter in livestock production were conducted and its beneficial effects on growth, digestibility and carcass traits have been reported [43, 44]. Dietary garlic as a growth promoter in Nile tilapia (*Oreochromis niloticus*) improved body weight gain (WG), feed intake and feed efficiency (FE) [45, 46].

5. Conclusion

After the 70 days of feeding trials with lapsi fruits supplemented diets, we can reach on the conclusion that fruit extracts enhances survival rates and growth capacities of carps. The increase in the concentration of protein, albumin and globulin and A-G ratio in blood serum indicates the enhancement of immunity in the body. Hence, we can consider that better growth and increase in protein profiles are due to presence of antioxidant properties in lapsi fruits.

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