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Effects of lapsi *Choerospondias axillaris* (Roxb.) on survival, growth and hepatic enzyme activities in *Cyprinus carpio* fingerlings

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Abstract
Altogether, two hundred seventy fingerlings of *C. carpio* (4.71±0.012g) were randomly distributed in six treated groups in triplicates form. Carp were fed with basal diet containing 40% protein supplemented with ethanol extract of lapsi fruit at 0, 0.1, 0.2, 0.4, 0.8 and 1.6 g kg -1 @ 3% of their body weight twice daily for 70 days. Cent per cent survival rate were observed in T3 and T4 diet fed group while the survival rate was 91.11% in T1 control diet fed group. For growth profile final weight gain, final length gain, specific growth rate and feed conversion ratio were measured. Similarly, proximate analyses of all the treated diets were assayed with SGOT, SGPT and ALP. There was significant (p<0.05) differences in weight gain, length gain and specific growth rate in treated diet fed group to that of control diet fed group. Carp fed with T4 diet (0.4%) showed higher length gain, weight gain and SGR as compared to others while higher decreasing trend were observed in feed conversion ratio (FCR) of T4 diet. Growth rate was 87.89% higher in T4 diet fed group while it was only 53.68% in control diet fed group. Significant decreasing trend were observed in SGOT, SGPT and ALP in all the treated diet fed group but the lowest decreasing trend were in T4 diet fed group. It can be concluded that a minimum amount of 0.4 g lapsi fruit extracts kg^{-1} is sufficient to be added in diet for good serum enzymes levels and growth performances of common carp.

Keywords: Carp growth, proximate analysis, survival, SGOT, SGPT and ALP

1. Introduction
Aquaculture is probably the fastest growing food-producing sector, now accounts for nearly 50 percent of the world's food fish. Aquaculture in Nepal is basically small but contributes 3% to the agricultural GDP. River is the major source of capture fishery covering 3, 95, 000 ha. of the surface of natural water resources. Around 75,000 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 tonnes 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.) of family Anacardiaceae is a large, dioecious and deciduous fruit tree. The tree is native to Nepal [3] found growing in hills between 850-1900 m above the sea level and has also been reported from various countries like India, China, Thailand, Japan, Vietnam 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]. The lapsi fruits contain phenolic and flavonoid compounds [7, 8]. The secondary products of medicinal plants and many edible plants contain phenolic compounds [9] which serve as antioxidants. So there are potential benefits of consuming phenolic rich foods [10]. Thus, keeping these things in mind an experiment was carried out in the wet laboratory of Central Department of Zoology, Tribhuvan University, Kirtipur to understand the antioxidant activities of lapsi fruits on carp growth.

2. Materials and Methods

2.1 Preparation of Crude Extract of Lapsi Fruits

The crude extract of the pulp of lapsi fruits was prepared by using ethanol (70%) as described by [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.

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

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 0.1, 0.2, 0.4, 0.8 and 1.6% lapsi fruit extracts. Other standard ingredients were used during feed preparation (Table 1).

2.3 Experimental design

A total of two hundred seventy fingerlings of *Cyprinus carpio* with an average weight of 4.71±0.012g and average length 6.78±0.01 cm 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. The uneaten feed and faecal matters were siphoned daily and two third of the aquarium water was replaced at weekly intervals. 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 composition of the experimental diets (Table 2) was analysed following the standard methods of the Association of Official Analytical Chemists (AOAC, 1995). The moisture content was determined by drying at 105 ºC 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).

2.5 Examination Procedures

2.5.1 Growth profiles

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

$$\text{LG}\,(\%) = \text{Final length} - \text{Initial length}\,/\text{Initial length} \times 100$$
$$\text{WG}\,(\%) = \text{Final weight} - \text{Initial weight}\,/\text{Initial weight} \times 100$$
$$\text{SGR} = ((\text{In Wf} - \text{In Wi}))/\text{t} \times 100$$

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.

2.5.2 Blood collection

At the end of the feeding trial, three fish in triplicates from each of the control and experimental groups were anaesthetized with tricaine methane sulfonate (MS-222) (5 mg l^{-1}). Blood were collected from the caudal vein using a syringe with 25 gauge needle. The blood samples were then transferred immediately to eppendort 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. Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT) and alkaline phosphatase (ALP) were determined calorimetrically by using available kits [11].

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 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 survival were 97.78±2.22, 95.56±2.22, 95.56±2.22 and 91.11±2.22 respectively (Figure 1). In the beginning of the experiment the average initial length was 6.78±0.01 cm in control diet fed group which increased gradually to 7.18±0.01, 7.63±05, 8.13±0.02, 8.84±0.02 and 9.47±0.16 cm on 14th, 28th, 42nd, 56th and 70th day respectively which further increased at the highest level in T4 diet fed carp to 7.52±0.03, 8.33±0.045, 9.23±0.063, 10.24±0.08 and 11.24±0.15 on 14th, 28th, 42nd, 56th and 70th day respectively. The highest 66.08% length increment was recorded in T4 diet fed carp as compared to

control (39.68%) on 70th day of feeding trial (Figure 2). This indicates that treated diet fed group showed better length increment as compared to the control diet fed group. Significantly higher (p<0.05) weight gain was observed in T4 diet fed group (8.84±0.01 g) on 70 days of feeding trial followed by group fed with diet T3 (7.53±0.03g), T5(8.84±0.01g), T6 (7.87±0.01g) and the lower in the group fed with T1 (7.23±0.03 g) (Table 3; Figures 3).

The weight gain (%) increment in T1 diet fed group recorded were 12.89, 24.50, 34.56, 47.38 and 53.68% on 14th, 28th, 42nd, 56th and 70th day of the experiment respectively. In T2 diet fed group the percent weight increment recorded were 15.51, 27.2, 38.6, 53.4 and 60.06%; in T3 19.55, 32.01, 43.63, 59.92 and 76.49%; in T423.87, 38.03, 51.2, 68.2 and 87.89%; in T5 19.52, 30.98, 43.49, 61.17, and 76.17% and in T6 815.56, 27.44, 39.11, 81±0.000), T5 (0.81±0.000), T6 (0.73±0.000), T2 (0.67±0.000) and T1 (0.61±0.000) diet fed group. The highest weight increment percent of 87.89% and lowest of 53.68% in group fed with T4 and T1 diets respectively were recorded on the 70th day of the experiment (Table 3 and Figure 4).

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 and Figure 5).

3.2 Hepatic enzyme activities

Hepatic enzyme activities are the most important analysis for the physiological aspects; hence SGOT, SGPT and ALP were estimated. An inverse relationship was observed between the dose of lapsi fruit extract and serum enzymes such as SGOT, SGPT and ALP. It was found that as the dose of lapsi fruit extract increased in the diets the amount of SGOT and SGPT decreased in blood serum of common carp. A higher SGOT (94.10±3.07) and SGPT (47.76±2.90) level were recorded in blood serum of group fed with T1 diet while the lowest SGOT (31.23±2.78) and SGPT (13.34±0.21) level were recorded in blood serum of group fed with T6 diet. The SGOT level in T5, T4, T3 and T2 were 35.64±1.21, 38.62±0.90, 65.50±2.88 and 80.52±3.16 while SGPT level were 19.96±1.28, 34.27±0.27, 65.50±2.88, 45.99±3.34 respectively. Similar results were obtained in ALP estimated from the blood serum of group fed different doses of lapsi diets. ALP level was found higher (134.46±6.27) in blood serum of group fed with Tl diet while the lowest ALP level (93.89±3.51) in blood serum was recorded in group fed withT4 diet followed by T2 (109.60±1.181), T5 (113.26±0.55),T3 (119.48±2.71) and T6 (123.07±5.42) (Table 4; Figure 6).

Table 1: Composition of experimental diets (%)

Ingredients (g/100g)	Experimental diets (% Inclusion) g/kg					
	C (Control)	T1	T2	T3	T4	T5
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	T5
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)

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

S.N.	Parameters	T1	T2	T3	T4	T5	T6
1	IL(cm)	6.78±0.057	6.79±0.017	6.77±0.013	6.76±0.013	6.77±0.031	6.77±0.061
2	FL(cm)	9.47±0.161	10.45±0.033	10.57±0.058	11.24±0.091	10.66±0.060	10.40±0.024
3	LG (cm)	2.69±0.16	3.66±0.03	3.80±0.24	4.47±0.14	3.89±0.06	3.63±0.03
4	LGP (%)	39.68±2.28	54.28±0.49	56. 18±3.55	66.07±2.07	57.46±0.77	53.59±0.38
5	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
6	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
7	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
8	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
9	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
10	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
11	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.

Table 4: SGOT, SGPT and ALP in the serum of *Cyprinus carpio* fed with control and experimental diets for 70 days

Parameters of Blood	Treatments					
	T1	T2	T3	T4	T5	T6
SGOT	94.10±3.07	80.52±3.16	65.50±2.88	38.62±0.90	35.64±1.21	31.23±2.78
SGPT	47.76±2.90	45.99±3.34	31.17±0.88	34.27±0.27	19.96±1.28	13.34±0.21
ALP	134.46±6.27	109.60±1.181	119.48±2.71	93.89±3.51	113.26±0.55	123.07±5.42

SGOT= Serum glutamate oxaloacetate transaminase; SGPT-Serum=Serum glutamate pyruvate transaminase; ALP= Alkaline phosphatase; Values are provided as mean ± SE.

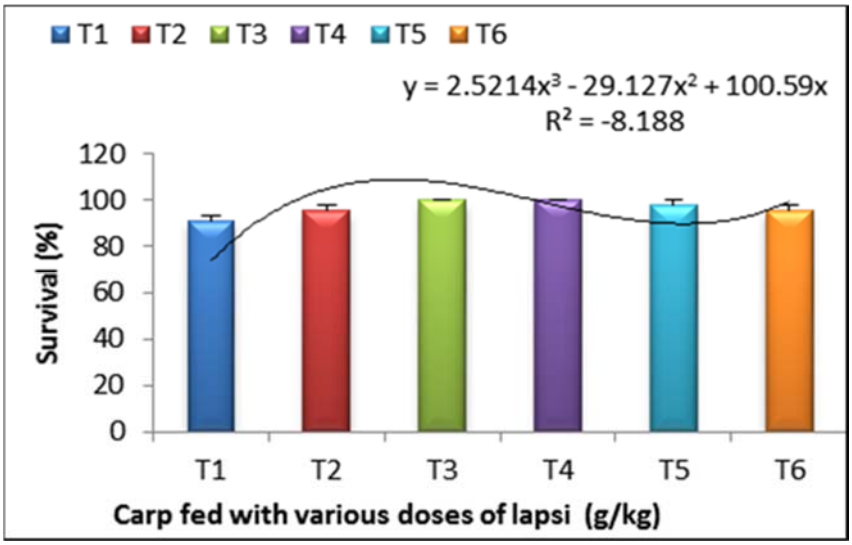

Fig 1: Carp fed with lapsi supplemented diets and survival % during the experiment.

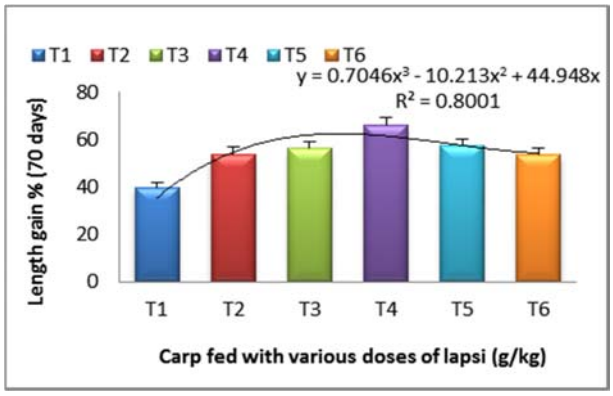

Fig 2: Carps fed with lapsi supplemented diets and length gain % in 70th day of culture.

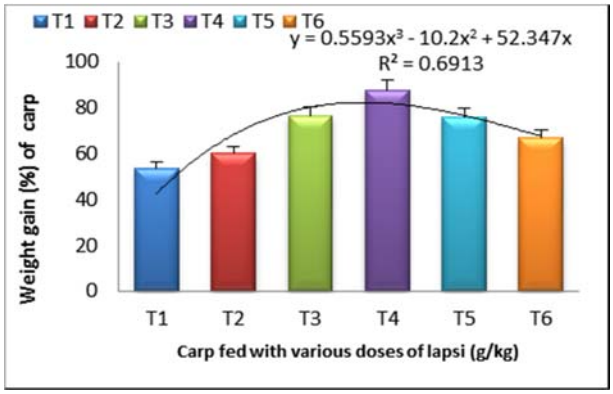

Fig 3: Carps fed with lapsi supplemented diets and weight gain % in 70th day of culture

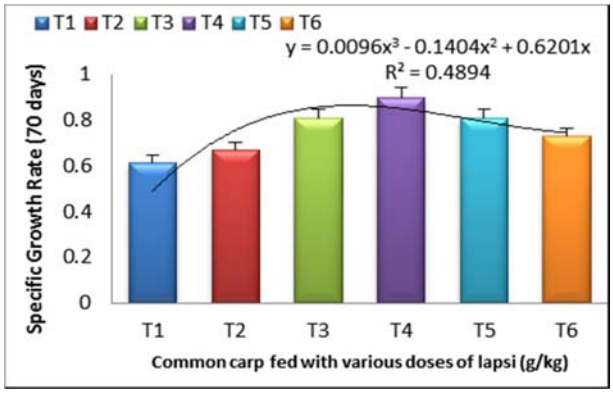

Fig 4: Carps fed with lapsi supplemented diets and SGR in 70th day of culture.

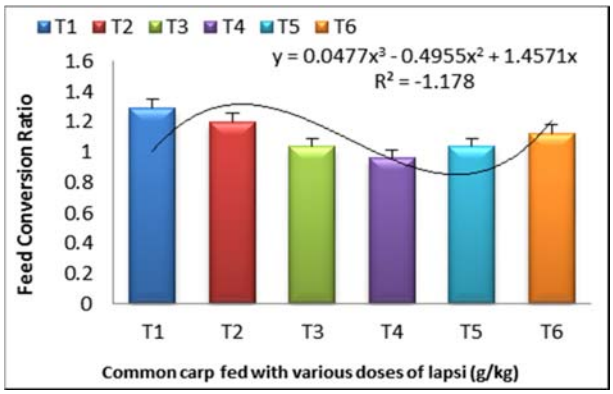

Fig 5: Carps fed with lapsi supplemented diets and FCR in 70th day of culture.

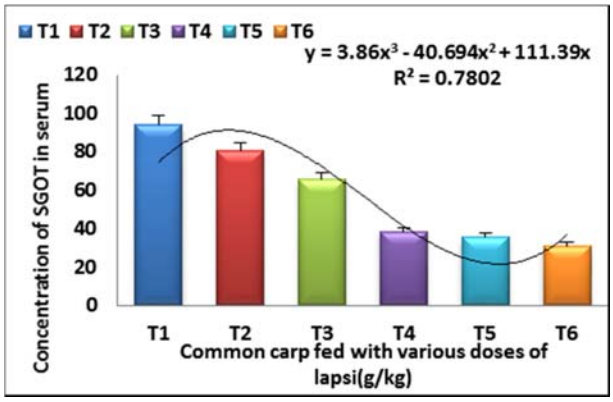

Fig 6: Carps fed with lapsi supplemented diets and SGOT in 70th day of culture.

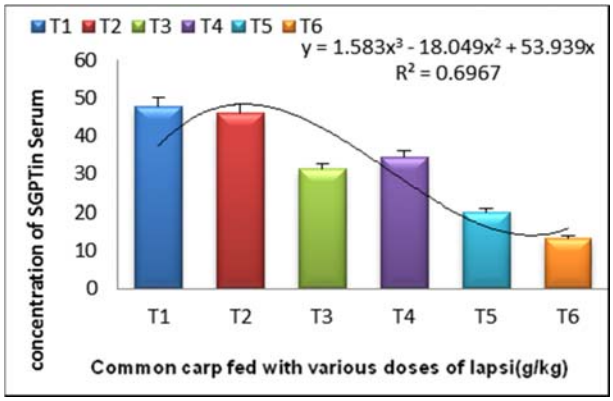

Fig 7: Carps fed with lapsi supplemented diets and SGPT in 70th day of culture.

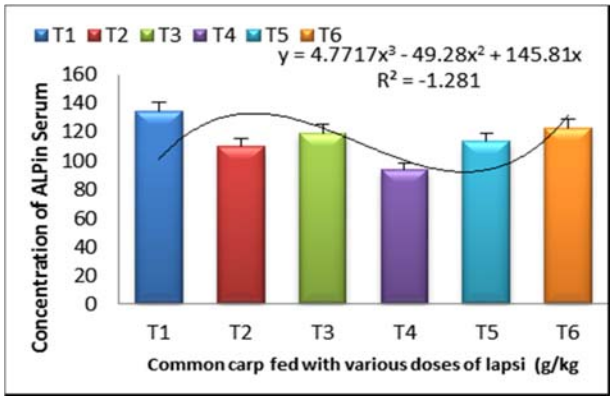

Fig 8: Carps fed with lapsi supplemented diets and ALP in 70th day of culture.

4. Discussion

Several herbs such as garlic, onion have been tested and evaluated for promoting growth [12], feed conversion ratio [13], enhancement of protein digestion [14] in fishes and other aquatic organisms. Significant increase in weight gain (WG) and specific growth rate (SGR) of Nile tilapia fed diet containing 3% garlic powder (GP) was reported by [15]. Similarly, [16] mentioned feeding *O. niloticus* with diet containing 2.5% garlic resulted in the highest growth performance. In the same species, [17] found positive improvement in biomass and SGR with garlic supplementation [18]. Al so mentioned that the best performance was obtained in Nile tilapia fed with diet containing 3.2% GP. Similarly a significant increase in

growth, feed conversion and protein efficiency was shown in rainbow trout when fed diet with 1.0% garlic [19]. In our study better weight gain and SGR were observed which is similar to the result obtained by [20] 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 [21]. 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 [22-24] but were lower than the FCR of 2.6 to 3.0 in tilapia fed on on-farm formulated diets in fertilized ponds [25]. It is evident that lapsi supplemented diet promotes growth in common carp. Higher growth in the common carp was supported by the increment of SGR and body weight gain %. This improvement in carp may be due to the effect of the chemical constituents such as antioxidant, antibacterial and antifungal activities on performance, stimulation and metabolism in lapsi. In addition, these effective components have a strong stimulating action on bile secretion as well as antispasmodic and anti-inflammatory effects [16]. Similar results have been reported for medicinal plants in diets for *O. niloticus* fingerlings by [26]. Finally, on the basis of above results, it could be recommended that extract of lapsi fruits may be used as a growth promoter and alternative to antibiotic for the treatment or prevention of diseases and for enhancing carp tolerance to environmental stress [27]; therefore extract of lapsi fruits should be added to the diets of common carp during aquaculture for better production.

Alteration in the serum biochemical profile specifically reflects the condition of the animal which occurs under influence of certain internal and external factors [18]. In this experiment serum enzymes such as SGOT, SGPT and ALP were estimated. The quantitative estimation of blood serum showed that the concentration of SGOT and SGPT level decreased as the dose of lapsi fruit extract increased in the diets. Serum glutamic oxaloacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), and alkaline phosphatase (ALP) were significantly (P<0.05) higher in rohu fed the control diet than in rohu fed the supplemented diets while myeloperoxidase was lower in the former [28]. Similar results have been reported for medicinal plants in diets for Nile tilapia (*O. niloticus*) fingerlings by [29-32, 13]. A large number of plants have been used in traditional medicine for the treatment and control of several diseases. Three of such plants are mistletoe (*Viscum album*), nettle (*Urtica dioica*), and ginger (*Zingiber officinale*) [33]. Some of the medicinal plants have been used as the phytogenic basis immunostimulatory preparations. Such preparations have been used, as such as adjuvant therapy, in cancer and AIDS treatment [34-36]. In Mongolian medicine lapsi is used in the treatment of myocardial ischemia, calming nerves, ameliorating blood circulation and improving microcirculation [37, 10].

5. Conclusion

In many countries such as Mongolia, China, Vietnam and India the lapsi is used to cure different diseases but in Nepal it is used as immunositimulant and antioxidant for human health as well as for animal growth. We have tested its properties on fish growth and found excellent result in 70 days of feeding trial. Thus, it can be concluded that antioxidant properties in lapsi fruits enhances the fish growth and improves immune system in the body.

6. Acknowledgements

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