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Effects of dietary lapsi, *Choerospondias axillaris* (Roxburgh, 1832) fruit extract on haematological parameters in *Cyprinus Carpio* (Linnaeus, 1758) fingerlings

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Abstract
An indoor experiment was conducted to study the effects of Lapsi *Choerospondias axillaris* on some haematological parameters of common carp *Cyprinus carpio* fingerlings.
Two hundred seventy fingerlings (average weight 4.71±0.012g) were randomly distributed in six treatment groups in triplicates of control (T1), 0.1 g kg^{-1} (T2), 0.2 g kg^{-1} (T3), 0.4 g kg^{-1} (T4), 0.8 g kg^{-1} (T5) and 1.6 g kg^{-1} (T6) supplemented with ethanol extract of lapsi fruits in the diet containing 40% protein. Fingerlings were fed at 3% of body weight twice daily. Significant differences (P<0.05) were observed in haematological parameters of treated diets fed groups to that of control diet fed group. Haemoglobin (Hb), red blood cells (RBC), packed cell volume (PCV), White blood cells (WBC) and other blood indices were observed to be significantly higher in the treated groups as compared to the control. It was concluded that a minimum amount 0.4% (0.4g kg^{-1}) of lapsi fruit extracts in fish feeds elicited more increase in haematological parameters of common carp. Inclusion of lapsi fruit extract at 0.4% concentration is therefore beneficial for use in aquaculture to enhance immunity in common carp.

Keywords: Aquaculture, haemoglobin, packed cell volume, ethanol extract

1. Introduction
Common carp, *Cyprinus carpio*, is one of the most important fish species in aquaculture [1]. Common carp is an economically significant fish species cultivated mainly in Asia and Europe. Global production of cultivated common carp was about 6.14% of the global aquaculture production [2]. It is a warm water freshwater fish species that is native to Asia. It is cultivated commercially [3] in other parts of the world, including Nepal, because of its fast growth rate, facile cultivation and high feed efficiency ratio [4]. China is by far the widest commercial manufacturer of common carp, which reports nearly 70% of the country's freshwater fish production [5]. In the last two decades the annual production of common carp raised exponentially and obtained more than 3 million tons in 2010 [6]. Currently, it represents 14% of the total world freshwater aquaculture production and is mostly cultivated in Asian countries, especially in China which accounts for 70% of the total global production [6].
Lapsi (*Choerospondias axillaris)* is one of the known medicinal plants rich in vitamin C content [7] and used as a medicinal plant to enhance the immune system of the body [8]. The constituents of lapsi fruits have been investigated chemically and shown to include phenolic compounds and flavonoid content [9]. The ability of phenolic compounds to serve as antioxidants has been recognized, leading to speculation about the potential benefits of ingesting phenolic rich foods [10]. It is assumed that the antioxidant activity of fruits of lapsi *Choerospondias axillaris* (Roxb.) may enhance the blood parameters of carp by improving immunity in the body so that carp can survive in adverse conditions and have capacity to fight against the diseases.
The haematological parameters are used as health indicators in aquatic medicine following different stress conditions [11, 12]. Haematological parameters are therefore ready tools used by fish biologists and researchers in many parts of the world. The knowledge of the haematological characteristics can be used as an effective and sensitive index to monitor physiological and pathological changes in fishes [13]. These parameters are also closely related to the response of the animal to the environment, an indication that the environment where

fish lives could exert some influence on the blood characteristics [14, 15]. Thus, to the best of our knowledge, there have been no studies conducted in *Cyprinus carpio* fingerlings fed with lapsi fruit extract supplemented diet. Hence, the present study was aimed to evaluate the effects of dietary supplementation of lapsi fruit on some blood parameters in *Cyprinus Carpio* fingerlings.

2. Materials and Methods

2.1 Experimental site and experimental fish

The experiment was conducted at wet laboratory of Central Department of Zoology (CDZ), Tribhuvan University, Nepal. Common carp *Cyprinus carpio* (4.71±0.012 g) fingerlings were procured from local hatchery and selected for this experiment.

2.2 Preparation of crude extract of lapsi fruits

Lapsi fruits were taken to laboratory soon after their collection from lapsi garden. The crude extract of the pulp of lapsi fruits was prepared by using ethanol (70%) as described by Labh *et al*. [16]. The known quantity (10 g) of lapsi powder was taken in a conical flask and added 500 ml of 70% ethanol. The flask with its content 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 was obtained which was transferred to screw-cap bottle labeled and stored at 4 °C until use.

2.3 Preparation of lapsi fruits supplemented artificial diets

Altogether six practical diets (40% protein) were prepared for this experiment. Diet one was control diet (0.0 g kg-1) without the extract of lapsi fruits where as in the remaining five diets (0.1, 0.2, 0.4, 0.8 and 1.6 g kg^{-1}) extract of lapsi fruits were supplemented along with other important ingredients (Table 1) and thus all six treated diets were called as T1, T2, T3, T4, T5 and T6 respectively. Dry fish were procured from the market and grounded in a grinder and then sieved (mesh size: 500µ). Thus obtained fish powder was dried in sun and stored. Ingredients were procured from the local market and grounded in a grinder and then sieved (mesh size: 500µ). The fishmeal powder was mixed thoroughly with wheat flour, other ingredients and lukewarm water was added in required amount and mixed well to make dough. The well prepared dough was steamed for 30 minutes to make gelatinous. It was cooled and then Cod liver oil, sunflower oil, premix of vitamins and minerals, Betaine Hydrochloride, BHT and CMC were added, mixed well to spread all the ingredients homogeneously. For control diet no lapsi extract was added in them but for treatment diets, extract of lapsi fruits were added (0.1, 0.2, 0.4, 0.8 and 1.6 g kg^{-1}) in the final dough separately. Thus, final gelatinous dough was again mixed well and was passed through a feed maker using 1 mm die; the threads obtained were spread on the newspaper and kept in the oven @ 30 °C for properly dried. Finally, the dried threads were further chopped into small pieces of required sizes of pellets manually and then passed through a sieve to obtain equal sized particles. All six types of test diets (T1, T2, T3, T4, T5 and T6) were stored at normal temperature until used.

Table 1: Ingredients and proximate composition of experimental diets (%).

Ingredients	Experimental diets (% inclusion)					
	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
Betaine 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
Proximate composition						
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 (CF)	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

†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.

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

2.4 Proximate analysis of feed

The experimental diets were analyzed for proximate composition following AOAC (1995) standard methods (Table 1). Moisture content was determined by conventional method using oven at 105 ºC to a constant weight. Nitrogen content was estimated by automated Kjeldahl apparatus (2200 Kjeltec Auto distillation, Foss Tecator, Sweden) and CP 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 h. Nitrogen free extract (NFE) was calculated by difference i.e., NFE = 100 − (CP + EE+ CF+ Ash).

2.5 Experimental design and feeding

A total of two hundred fifty two fingerlings of *C. carpio* with an average weight of 4.71±0.012g were distributed in six treatment groups in triplicates of TI, T2, T3, T4, T5 and T6 (Table 1). Fingerlings were fed for 70 days twice daily at 3% of the body wt and adjusted on every 15 days accordingly based on the biomass gain during the trial. Fish were fed equal portions twice daily (11:00 h and 17:00 h). The experimental rearing system consisted of 18 uniform size rectangular glass aquaria (100 l capacity) containing 15 fish per aquarium. The total volume of the water in each tank was equally maintained throughout the experimental period. The uneaten feed and faecal matters were removed by siphoning and two third of the aquarium water was replenished on alternate days and continuous aeration was provided to all the tanks.

2.6 Sampling, blood collection and analysis

At the end of the feeding trial, five fish in triplicates from each of the control and experimental groups were anaesthetized with tricaine methane sulfonate (MS-222) (5 mg l^{-1}). Blood samples were obtained from the caudal vein using a syringe and immediately transferred to eppendorf tubes containing EDTA powder and shaken gently to prevent haemolysis of blood. The bloods were diluted with appropriate diluting fluids for RBC and WBC counts and were determined using improved Neubauer haemocytometer and calculated [17]. Replicated counts were made for each blood samples to minimize the error. The hemoglobin was determined by cyanmethemoglobin method [18]. Haematocrit (PCV) was determined by the microhematocrit method [19]. Mean Corpuscular Volume (MCV) was calculated according to [20]. Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin concentration (MCHC) were calculated [21].

2.7 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 (DMRT). Significance was tested at $P<0.05$ level. The software SPSS (version 20) was used to compare the target parameters.

3. Results

The results of the haematological parameters of *C. carpio* fingerlings with different feeding regimes are presented in Table 2. Hematological parameters of common carp fingerlings with different feed clearly showed significant enhancement with treated diet when compared with control. Carp fingerlings showed haematological parameters increased maximum in RBC (3.27±0.15), WBC (128400±7718.376), Hb (18.68±0.69) while in PCV (29.27±1.10), MCV (149.33±4.67), MCH (49.35±1.659), and MCHC (36.30±0.631) were observed in T4 diet treated group compared to other and control. The control showed that RBC (1.80±0.12), WBC (97000±6807.596), Hb (3.38±0.83), PCV (21.43±2.24), MCV (112.00±0.58), MCH (41.25±0.682) and MCHC (32.77±0.225) were observed in 70 days (Table 2).

Table 2: Complete blood profile of C. carpio fed with various doses of *C. axillaries* for 70 days

	Haematological Parameters						
	Hb	**Total RBC**	**PCV**	**MCV**	**MCH**	**MCHC**	**Total WBC**
Treatments	**(gm/dl)**	**mill/cu.mm**	**(%)**	**(fL)**	**(pg)**	**(%)**	**No/cu mm**
T1	3.38±0.83	1.80±0.12	21.43±2.24	112.00±0.58	41.25±0.682	32.77±0.225	97000±6807.596
T2	5.19±0.60	2.92±0.01	23.27±1.10	118.67±3.28	47.34±1.651	34.47±0.393	123400±7718.376
T3	7.43±0.87	1.74±0.25	18.60±1.73	135.33±2.33	39.90±1.391	35.33±0.348	85689±6740.291
T4	18.68±0.69*	3.27±0.15*	29.27±1.10*	149.33±4.67*	49.35±1.659*	36.30±0.631*	128400±7718.376*
T5	15.21±0.69	1.80±0.12	21.43±2.24	114.67±1.20	41.31±0.681	34.77±0.237	97000±6807.594
T6	11.55±0.78	1.74±0.25	18.60±1.73	116.00±4.51	39.90±1.394	34.46±0.395	85689.33±6740.602

*Significantly different from control $p<0.05$; Values were expressed as Mean ± standard deviation (n=3)

4. Discussion

There is growing interest in the study of haematological parameters of fish blood cells regarded as important for aquaculture purposes. Blood parameters have been used as indices of fish health status in a number of fish species to detect physiological changes as a result of stress condition such as transportation, handling, hypoxia and acclimation [22, 23]. Haematological studies help in understanding the relationship of blood characteristics to the habitat and adaptability of the species to the environment. Haematological parameters are closely related to the response of the animal to the environment, an indication that the environment where fishes live could exert some influence on the haematological characteristics [24]. These indices have been employed in effectively monitoring the responses of fishes to supplementation of diet and thus their health status under such adverse conditions.

The blood parameters in fishes are influenced by many factors [25]. Quality of water, temperature, food availability and physiological status of fish either directly or indirectly influence on blood constituents of fish [26]. Accordingly the sex, size, season and age of fishes are directly reflected on blood parameters. The values of haematological parameters depend on season and slow or active movement of fishes [27]. Harikrishnana *et al*. [28] reported that haematological parameters are influenced by microbial infection of fish and toxicants. Hematological parameters act as physiological indicators to changing external environment [29] as a result of their relationship with energetics (metabolic levels), respiration (hemoglobin levels) and defense mechanisms

(leucocyte levels), as these parameters provide an integrated measure of the health status of an organism which overtime manifest in changes in weight (growth) [30]. The haematological values such as RBC and WBC count and haemoglobin obtained in the present study almost agree with earlier workers [31]. According to the results, lapsi extract supplemented diets could increase hemoglobin content, WBC and RBC levels in experimental groups compared to control group. In agreement with the present findings, Sahu *et al.,* [32] reported that WBC and RBC counts were higher in *Labeo rohita* fingerlings fed *with Mangifera indica* kernel when compared to control. Gopalakannan and Arul [33] also reported that there was an increase in the WBC count after feeding the common carp with immunostimulants like chitin. The haemoglobin (Hb) levels increased with increasing amount of lapsi in carps may be due to the increase in antioxidant properties as indicated by Labh *et al.* [16].

5. Conclusion

The results of our research provide a contribution to the knowledge of the haematological parameters of the *Cyprinus carpio*, under the supplementation conditions with medicinal lapsi fruits employed in this study. This investigation may be helpful as a tool to monitor the health status of fish. In conclusion, the incorporation of ethanol extract of lapsi fruits in common carp diets improves haematological parameters. Among the various diet supplementations, the 0.4% lapsi fruit supplemented diet was most effective to improve the haematological parameters in fish as compared with other diets. This might be due to rich antioxidant properties present in lapsi fruits.

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