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**Mahima Tamta**  
Department of Fisheries  
Resource Management, College  
of Fisheries, G.B.P.U.A. & T.,  
Pantnagar, Uttarakhand, India

**Amita Saxena**  
Department of Fisheries  
Resource Management, College  
of Fisheries, G.B.P.U.A. & T.,  
Pantnagar, Uttarakhand, India

## Effect of amla (*Emblica officinalis*) on the hematology and serum biochemical parameters of grass carp fingerlings in Tarai conditions of Uttarakhand

**Mahima Tamta and Amita Saxena**

### Abstract

*Emblica officinalis*, is also known as amla, has been used in Ayurveda, the ancient system of Indian medicine. Amla has been used for curing many disorders such as common cold, scurvy, cancer and heart diseases in humans. It is supposed that the major factor responsible for all these curing activities is Vitamin C (Ascorbic acid), which is contained in large proportion in the pulp of amla. The present study was conducted to study the effect of different concentrations of amla as a feed additive on the hematology and serum biochemical parameters of Grass Carp fingerlings. Four experimental diets, T0, T1, T2 and T3, were prepared with dried amla powder as a feed additive mixed @ 0% (Control), 1%, 5% and 10% of feed, respectively. The results showed that the haematological and serum biochemical parameters were better in all the fishes fed with T3 (10% dried amla per kg feed).

**Keywords:** Vitamin C, *Emblica officinalis*, carps, hematology, serum biochemical parameters

### Introduction

The goal of the fisheries industry is to optimize the growth and to produce quality fish but the diseases outbreak in fish farming is a major concern, the high vulnerability of fish to stress and the disease have forced fish farmers to shift their focus towards maintaining the fish against infectious disease, so that they can achieve sustainable economic performances. In order to rectify this problem the use of immune-stimulants is an easy tool the use of which can enhance activities in the nonspecific defense mechanism, increase resistance to infectious disease by enhancing inherent humoral and cellular defense mechanisms and ultimately enhance growth and overall improvement in fish. In this respect, vitamins prove to be one of the major immune-stimulants with special importance in modulating the immune system of fish. Vitamins are organic substances that are essential for growth and maintenance of animals, but they are required in small extent. As fish is unable to synthesize vitamins at all or can only synthesize them in inadequate quantities for basic development, growth and maintenance, they must be supplied by means of the diet (Falahatkar *et al.*, 2011) [6].

Antioxidant L-ascorbic acid (vitamin C) is an important vitamin for growth and physiological function in fish. It plays a considerable role in the growth, collagen formation, iron metabolism haematology, reproduction response to stressors, wound healing and immune response. Adequate quantity of ascorbic acid in fish diet, predominantly in early life stages, plays a vital role in disease resistance followed by fish immunity and survival. Majority of the teleosts are incapable to synthesize vitamin C from D-glucose due to the lack of L-gulonolactone oxidase which is responsible for the synthesis of antioxidant vitamin C (*de novo*). Therefore, a supplementary source of antioxidant vitamin C is obligatory in fish diet. However, ascorbic acid need is inconsistent among diverse aquatic species and is dependent on fish size, diet composition, and cultural system (Ai *et al.*, 2004; Garcia *et al.*, 2007) [1, 9].

Blood is a liquid tissue which comprises of plasma, erythrocytes, leukocytes and thrombocytes and is enclosed within the cardiovascular system. It comprises 1.3 to 7% of the total body weight of fishes and is one of the most active constituents that contribute to the metabolic course of actions by ensuring the gas exchange within the body and between the fish and surroundings. Any dysfunction of the blood can have severe impacts on the physiological processes of fish. The most important role of blood in fishes as in other vertebrates also is the transportation of oxygen and nutrition to tissues, maintenance of acid-base balance, and

**Correspondence**  
**Mahima Tamta**  
Department of Fisheries  
Resource Management, College  
of Fisheries, G.B.P.U.A.&T.,  
Pantnagar, Uttarakhand, India

removal of metabolic waste products from tissues etc. Hematological studies had been used as an efficient and sensitive index to monitor physiological and pathological changes in fish.

*Emblica officinalis*, is also known as *Phyllanthus emblica* or amla, has been used in Ayurveda, the ancient system of Indian medicine. Amla has been used for curing many disorders such as common cold, scurvy, cancer and heart diseases. It is supposed that the major factor responsible for all these curing activities is Vitamin C (Ascorbic acid), which is contained in large proportion in the pulp of amla. Amla has various properties viz. antioxidant, anti-inflammatory, anti-mutagenic, anti-fungal and antimicrobial activity (Ihantola-Vormisto *et al.*, 1997; Bhattacharya and Ghosal 2000; Khopde *et al.* 2001) [10, 4, 11].

Based on these facts and studies, the present study was conducted to study the effect of different concentrations of amla as a feed additive on the hematology and serum biochemical parameters of fingerlings of Grass carp. Seeing the importance and all the qualities of amla, fruit pulp was incorporated in the diet of fish fingerlings.

### Materials and Methods

The present research work was conducted to estimate the effect of amla as a feed additive used in different concentrations on the haematology and serum biochemical parameters of the experimental fish fingerlings.

### Experimental Site

The experiment was conducted at College of Fisheries, Pantnagar, geographically located at 29° N latitude, 79.3 degree longitude and an altitude of 243.3 m above mean sea level (MSL), in Tarai belt of the Shivalik range of Himalaya. The site had humid sub-tropical climate characterized by very hot and dry summer and very cold winter. The study was carried out for 3 months from March 2017 to June 2017.

### Experimental Fish and Setup

The experimental fish fingerlings were collected during the month of March 2017 from fish seed hatchery of College of Fisheries and kept in F.R.P. tanks in wet laboratory of the college. The carp species selected for the experiment had the same length and weight. The experiment was setup in triplicates with four feeding groups.

### Feed Formulation and Feeding Schedule

The feed for fish fingerlings was prepared with three basal ingredients i.e. rice bran, mustard oil cake, and soyabean oil cake, in the ratio of 1:1:1 along with little amount of wheat flour as binder, 1% Agrimin Forte (vitamin-mineral mixture) and vitamin E powder was also mixed in the feed that was put constant (5 gm per kg feed) in all the experimental feeds. Four experimental diets, T0, T1, T2 and T3, were prepared with dried amla powder as a feed additive mixed @ 0% (Control), 1%, 5% and 10% of feed, respectively. The proximate composition of basal feed i.e., moisture content, crude protein level, crude fat level and ash, was also calculated prior the starting of experiment. Feeding was done @ 6% of average body weight of fingerlings twice a day.

### Feed Ingredient Analysis and Feed Formulation

The basal feed ingredients for the feed formulation were collected from the feed mill of College of Fisheries Govind Ballabh Pant University of Agriculture and Technology,

Pantnagar, Udham Singh Nagar, Uttarakhand India. The feed for fish fingerlings was prepared with three basal ingredients i.e. rice bran, mustard oil cake, and soyabean oil cake, in the ratio of 1:1:1.

**Collection of Amla** – The amla fruit was purchased from local markets (Haat) of pantnagar. The fruit was divided into small pieces and then shade dried. After drying the fruit was grinded using ball mixture. Amla powder was then stored in sealed plastic boxes at room temperature and was kept away from the sun light and heat.

### Results and Discussion

#### Proximate composition of feed

Proximate composition of feed was calculated and values were found to be as given in Table 1.

**Table 1:** Proximate Composition of Feed

S. No.	Contents	Percentage%
1	Moisture	10.09
2	Ash	7.0
3	Crude protein	28.7
4	Crude fat	7.56
5	carbohydrate	46.65

**Hematological parameters-** The value of haemoglobin for grass carp in the group T0 i.e. the control group was  $\mu = 9.01 (\pm 0.86)$  mg%, while in treatment T3 the mean value of haemoglobin was  $9.27 (\pm 1.11)$  mg%. As the amount of amla powder (vitamin C) was increased the mean Hb content was also increased. The mean value of the total leucocytes count was  $8.07 (\pm 0.47) \times 10^3/\text{mm}$  in the control group, and the highest value was obtained in treatment T1 i.e.  $8.03 (\pm 0.44) \times 10^3/\text{mm}$ . The mean value of Total erythrocyte count was lowest  $1.98 (\pm 0.26) \times 10^6/\text{mm}$  in fingerlings fed with the control diet, and highest value of TEC was recorded in treatment T3 i.e.  $2.11 (\pm 0.34) \times 10^6/\text{mm}$ , which clearly indicated that with the elevated amount of vitamin C in the diet of fingerlings the erythrocyte count was also increased.

**Serum biochemical parameters** -When serum biochemical parameters were estimated it was found that the lowest values of total protein, albumin and globulin recorded in the fingerlings that were fed with the control diet. The mean value of total protein in control group was  $2.41 (\pm 0.09)$  g/dl and the highest value was obtained in the T3 group i.e.  $2.69 (\pm 0.22)$  g/dl. Albumin was lowest in the control group i.e.  $1.36 (\pm 0.08)$  g/dl, and the highest value of albumin was recorded in T3 group, and it was  $1.63 (\pm 0.23)$  g/dl. The amount of globulin followed the same trend; it was equal in control diet, i.e.  $1.05 (\pm 0.01)$  g/dl, and T3 group, i.e.  $1.05 \pm 0.06$  g/dl. When the amount of serum creatinine was estimated it was found that the lowest mean value of serum creatinine was obtained in group T3 i.e.  $0.46 (\pm 0.03)$  g/dl and the highest value of serum creatinine was recorded in the group of fingerlings that were fed with control diet T0 i.e.  $0.49 (\pm 0.05)$  g/dl. The average value of blood calcium was lowest in control group i.e.  $9.37 (\pm 0.13)$  g/dl and average highest value was recorded in treatment T3 group i.e.  $9.38 (\pm 0.08)$  g/dl. When the alkaline phosphate was estimated the lowest value of alkaline phosphate was obtained in the control group i.e.  $8.31 (\pm 0.15)$  IU/dl and the highest value was recorded in the fingerlings of T3 group i.e.  $8.61 (\pm 0.12)$  IU/dl.

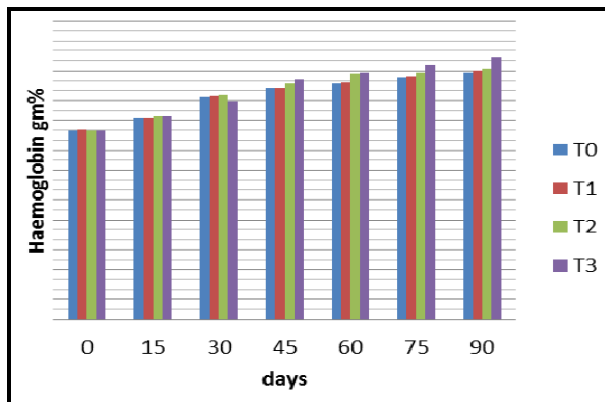


Fig 1: Haemoglobin content

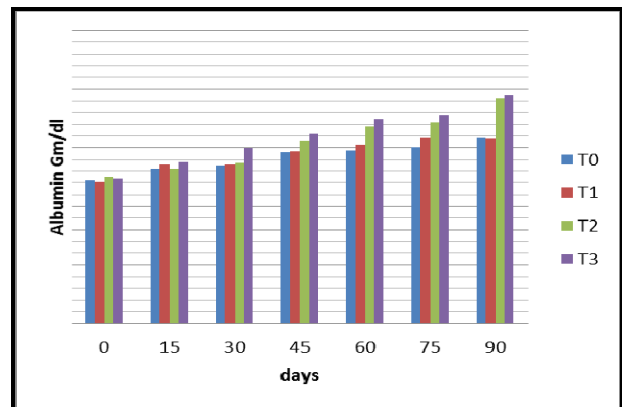


Fig 5: Total albumin content

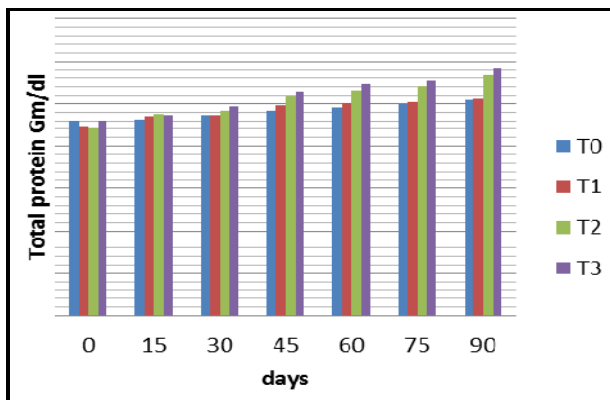


Fig 2: Total protein content

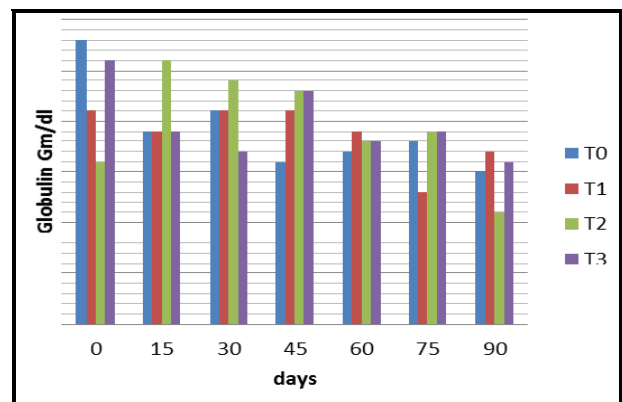


Fig 6: Total globulin content

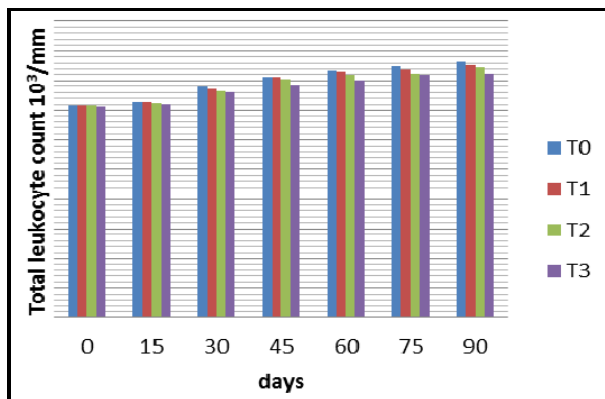


Fig 3: Total leukocyte count

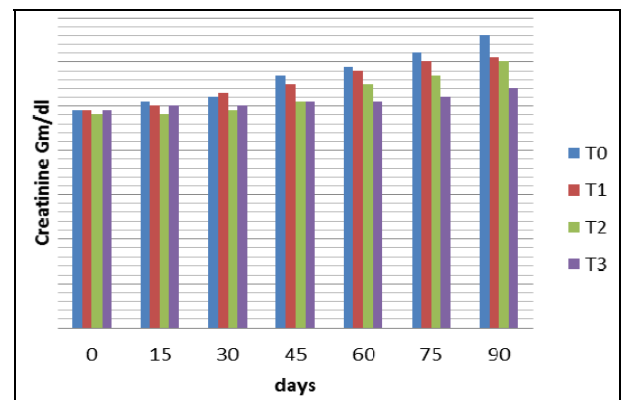


Fig 7: Creatinine content

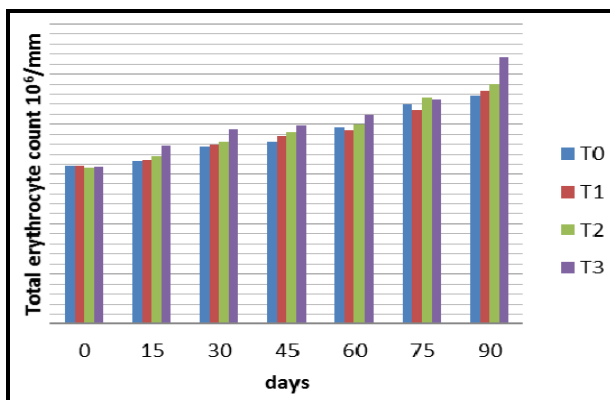


Fig 4: Total erythrocyte count

### Discussion

The proximate composition of formulated feed was analysed and the amount of moisture found was 10.09%, protein content 28.70%, fat content 7.56%, carbohydrate 46.65%, and ash content was 7.00%. The haematological parameters such as haemoglobin percent, total leukocyte count, total erythrocyte count, were best obtained by the fingerlings those were fed with the highest amount of vitamin C. For Grass carp the haemoglobin was lowest in control group i.e. 9.01 ( $\pm 0.86$ ) mg%, in treatment T3 the mean value of haemoglobin was 9.27 ( $\pm 1.11$ ) mg%. The value of the total leukocytes count was 8.07 ( $\pm 0.47$ )  $\times 10^3/\text{mm}$  in the control group, and the highest value was obtained in treatment T1 i.e. 8.03 ( $\pm 0.44$ )  $\times 10^3/\text{mm}$ . Total erythrocyte count was lowest 1.98 ( $\pm 0.26$ )  $\times 10^6/\text{mm}$  in fingerlings fed with the control diet, and highest

value of TEC was recorded in treatment T3 i.e. 2.11 ( $\pm 0.34$ )  $\times 10^6/\text{mm}$ , total protein in the control group was 2.41 ( $\pm 0.09$ ) g/dl and the highest value was recorded in the T3 group i.e. 2.69 ( $\pm 0.22$ ) g/dl. Albumin was lowest in the control group i.e. 1.36 ( $\pm 0.08$ ) g/dl, and the highest was recorded in T3 group, i.e. 1.63 ( $\pm 0.23$ ) g/dl. The amount of globulin followed was equal in the control diet, i.e. 1.05 ( $\pm 0.01$ ) g/dl, and T3 group, i.e. 1.05  $\pm 0.06$  g/dl. Lowest value of serum creatinine was recorded in group T3 i.e. 0.46 ( $\pm 0.03$ ) g/dl and the highest value of serum creatinine was recorded in the control diet, T0 i.e. 0.49 ( $\pm 0.05$ ) g/dl. So from these findings it was clear that the antioxidant vitamin C had a significant role in elevating the amount of haemoglobin, total serum protein, albumin and globulin, and simultaneously lowered the amount of serum creatinine which in excess amount is not safe for proper renal functioning. Application of hematological techniques is a valuable means for fishery biologists in evaluating the physical condition of fish and monitoring stress. Blood parameters were considered patho-physiological indicators of the body and therefore, were essential in diagnosing the structural and functional status of the fish (Adhikari and Sarkar, 2004) <sup>[1]</sup>. The result of hematology of fish fingerlings was supported by Chen *et al.* (2004). They reported in juvenile golden shiner (*Notemigonus crysoleucas*) that fish fed diets with 98 or 222 mg AA  $\text{kg}^{-1}$  had a highest percentage of lymphocytes. The same result was observed by Nsonga *et al.* (2009) <sup>[12]</sup> they studied in tilapia that the fish fed AA supplemented diets showed a significant increase in hematological indices with increasing dietary vitamin C level

### Conclusion

Antioxidant vitamin C and E helped in elevation of haemoglobin and other haematological parameters, in maintaining fingerlings showed healthiness in all experimental fish fingerlings. The amount of total protein, albumin, globulin, was also increased with increasing amount of vitamin C, but there was a declining trend in the values of serum creatinine. The amount of serum creatinine was greater in the control group, and it was gradually decreased with increasing level of vitamin C. Because the amount of serum creatinine reflects the renal health, low amount of serum creatinine is good for proper functioning of kidney, so it was revealed from the above study that antioxidant vitamin C and E exerted a positive effect on renal functioning.

Thus, it is concluded that the 100 gm amla powder with antioxidant Vitamin C (1000 mg), and E at 5 gm per kg feed were added in the feed gave to fish fingerlings in order to obtain high growth performance and a good health condition of fish. Generally, the diet supplemented with approximately 1000 mg vitamin C (100 gm amla powder) gave the best growth performance compared with the other diets. These results further confirmed that carp fingerlings needed satisfactory exogenous source of vitamin C to maintain normal growth and physiological functions.

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