



## Effectiveness of dietary vitamin C on the performance of common carp (*Cyprinus carpio*).

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### ABSTRACT

To evaluate the effects of dietary vitamin C on the growth performance of common carp and indices of blood hematology, intestine, and liver histology (*Cyprinus Carpio*), a 60-day study was conducted in well water using 3 experimental treatments (3 replicates per treatment). Three similar diets (isonitrogenous (300 g/kg crude protein) and isoenergetic (13 MJ/kg gross energy)) were formulated as follows: control diet (T<sub>0</sub>) without vitamin added, 100 mg of vitamin C, 200 mg of vitamin C / kg diet were supplemented for T<sub>1</sub> and T<sub>2</sub>, diets, respectively. Each diet was fed to triplicate groups of carp with an initial body weight of 2.03g ± 0.02g in 90-L tanks at a stock king density rate of 30 fish /tank. The results showed that the groups fed with vitamin C-supplemented diets recorded a higher final weight, weight gain (WG), specific growth rate (SGR), and an improving feed conversion ratio (FCR) compared to the control group (T<sub>0</sub>, p<0.05). Regarding hematological parameters indices, white blood cell (WBC), red blood cell (RBC), hematocrit (Ht), haemoglobin (Hb), lymphocyte, platelets count and immunoglobulin (IgM) concentrations were significantly higher in fish-fed T<sub>2</sub> diet than other groups. In terms of the normal cellular structure, intestinal villi heights, goblet cell counts, and infiltrating leukocytes, all treatment groups outperformed the control group in intestinal histology. While liver and intestine tissues were less damaged with the increase of vitamin C concentration in the diet. Our findings demonstrate that common carp diets supplemented with 200 mg of vitamin C per kg of feed help enhance growth performance, improve the structure of the intestinal mucosal epithelium, and have a positive impact on the hematological parameter (*Cyprinus Carpio*).

### INTRODUCTION

An essential aspect of aquaculture is the relationship between fish nutrition and welfare. Mortality rates increase as a result of the insufficient nutritional needs of fish, and this calls for the development of artificial diets that meet the needs of fish under the intensive culture system. (Reddy, 2018). Immunological depression and infectious disease outbreaks have been brought on by the fast proliferation of modern aquaculture as

a result of an increase in the world population (Gobi *et al.*, 2018). Vitamins are predominant requisite nutrients for aquatic animals. Vitamin deficiency causes many morphological and functional abnormalities in different fish species (Liang, *et al.*, 2017). Nutritional adjustments appear to be a potential method for increasing fish's stress tolerance (Hardie, *et al.*, 1990).

Vitamin C, or ascorbic acid, is an essential vitamin that has many uses, the most important of which is that it is needed to enhance the immune response (Hardie *et al.*, 1991). This vitamin helps aquatic creatures' immune systems respond better and reduces the detrimental consequences of stress (Henrique *et al.*, 1998, Petric *et al.*, 2003; Sarma *et al.*, 2009). Additionally, vitamin C is a powerful antioxidant that protects against harmful free radicals created by regular cellular function and other forms of stress in animals (Chew, 1995). The antioxidant properties of certain micronutrients may improve immunity by maintaining the structural and functional integrity of immune cells (Innocent *et al.*, 2011). Additionally, it functions as a co-factor in the hydroxylation of proline residues in collagen and connective tissue in vertebrates (Wang *et al.*, 2003; Zhou *et al.*, 2003). Therefore, dietary vitamin C consumption is necessary for the fish body's proper physiological activities (Zou *et al.*, 2020).

There is no gluconolactone oxidase enzyme in most aquatic species, so they are unable to synthesize ascorbic acid from D-glucose. (El Basuini *et al.* 2021; Fonseca *et al.* 2013). So, many fish's livers and kidneys lack the L-gluconolactone oxidase enzyme necessary for the manufacture of vitamin C, dietary inclusion is necessary to provide the nutrients needed for the developing fish to operate at their best (Wilson, 1973; Dabrowski, 1990; Fracalossi *et al.*, 2001; Dabrowski, 1990 and Ai *et al.*, 2004).

The amount of vitamin C needed for fish depends on their age, size, species, and conditions of upbringing (Chen *et al.*, 2015; Dawood & Koshio, 2016; Lin & Shiau, 2004; Xu *et al.*, 2016; Zhou *et al.*, 2012 and NRC, 2011). Fish needs for vitamin C range between 20 and 50 mg/kg feed, according to research on these vitamin C-supplemented feed requirements in numerous fish species (NRC, 1993). Dietary ascorbic acid (vitamin C) intake guidelines range from 10 to 10,000 mg/kg (Webb and Villamor, 2007). The minimum requirement of dietary vitamin C to support the maximum growth rate (WG) was 53–186 mg/kg in Nile tilapia (*Oreochromis niloticus*), 1200 mg/kg in Pacific bluefin tuna (*Thunnus Orientalis*), and 5000–1000 mg/kg in kuruma shrimp (*Marsupenaeus japonicas*) (Schleicher, *et al.*, 2009). The optimum vitamin C levels have been determined to be 13.6 mg/kg in cobia (*Rachycentron canadum*) (Zhou *et al.*, 2012), 28.2 mg/kg in large yellow croaker (*Pseudosciaena crocea*) (Ai *et al.*, 2006), range from 92.8 to 129.8 mg/kg in grass carp (*Ctenopharyngodon Idella*) (Xu *et al.*, 2016), 142.2 mg/kg in Yellow drum (*Nibea albiflora*) (Wang *et al.*, 2017) and 700 mg/kg in Wuchang bream (*Megalobrama amblycephala Yih*) (Ming *et al.*, 2012). Studies on every fish species are necessary to determine the ideal vitamin C requirements because a previous study

suggested that the GIFT tilapia (*Oreochromis niloticus*) would develop and survive best with 150 mg of vitamin C per kg of diet ( Baroi *et al.* , 2019 and Zou *et al.* , 2020).

The common carp (*Cyprinus carpio*), as a commercial freshwater fish, is the most widely farmed fish in the world (Modanloo *et al.*, 2017). Similar to rainbow trout, common carp is well-liked throughout most of Europe and is one of the freshwater fish with a good nutritional value (KBobukowski *et al.*, 2018 ). The kind of diet has a considerable impact on the flesh yield and quality of common carp muscle tissue, notably the quantity of protein, total fat, and cholesterol (Steffens and Wirth, 2007). Therefore, the goal of the current study was to determine how different vitamin C dosages affected the growth rates and intestinal histology of common carp (*Cyprinus Carpio*).

## MATERIALS AND METHODS

### 1. The experiments location

The experiments were conducted in the fish Nutrition lab., National Institute Oceanographic and fishers (Qanater Khaireya) Egypt.

### 2. Installation and water quality

The investigation was carried out in 100 L plastic water tanks with a well water supply, exchanging water of tanks 3 times weekly. An air pump was used to aerate the tanks to keep the oxygen levels in the testing tanks at the optimum levels. The water's temperature during the experiment varied between 25 and 30 C<sup>0</sup>. At 8:00 and 15:00 h each day, dissolved oxygen and temperature were measured using (Professional Plus, USA). Throughout the experiment, a pH meter was used to test the pH directly in the water column of plastic tanks every week (Orion pH meter, Abilene, TX, USA).

### 3. Experimental fish

Common carp with an initial body weight of 2.02 g were obtained from Kafr El-Sheik governorate, Egypt. Before the start of the experiment, the fish was acclimatized to laboratory conditions for 7 days and fed a basal diet of 30% crude protein (CP). The fingerlings were stocked into 9 plastic tanks (with a water capacity of 90 L each) at a rate of 30 fish/tank. The tanks were supplied with a well water source. Aeration was continuously provided using an air blower. Six days week<sup>-1</sup> fish was fed the experimental diets Three same isonitrogenous (30% CP) and isoenergy 13 MJ/kg. GE) diets were formulated (Table 1). All diets were different in level additives ascorbic acid control without additives, T1 additives 100 mg/kg feed, and T2 additives 200 mg/kg feed. Fish in each replicate aquarium were weighed every week.

Table (1): Formulation g/kg and proximate composition (% dry matter basis) of the experimental diets

	control	T1	T2
<b>Soybean meal</b>	250	250	250
<b>Corn gluten meal</b>	200	200	200
<b>Yellow corn</b>	100	<b>100</b>	<b>100</b>
<b>Wheat bran w</b>	140	140	140
<b>Rice polishing</b>	200	200	200
<b>Linseed oil</b>	40	40	40
<b>Starch</b>	50	<b>49.9</b>	<b>49.8</b>
<b>Ascorbic Acid (vitamin C)*</b>	0	<b>0.1</b>	<b>0.2</b>
<b>Mono Calcium Phosphate</b>	1	1	1
<b>Lysine</b>	5	5	5
<b>Methionine</b>	5	5	5
<b>Premix</b>	9	9	9
<b>Proximate composition</b>			
Dry Matter %	90.01	90.01	90.01
Crude protein %	29.74	29.74	29.74
GE MJ/kg**	12.709	12.709	12.709
Crude Fat %	5.17	5.17	5.17
Ash %	5.89	5.89	5.89

T1 additives 100 mg/kg feed, and T2 additives 200 mg/kg feed \*Ascorbic Acid (L(+))tp (vitamin C) C<sub>6</sub>H<sub>8</sub>O<sub>6</sub> ascorbyl-2-polyphosphate (Chem-Lab NV, Belgium- [www.chem-lab.be](http://www.chem-lab.be)) \*\* calculated GE MJ/kg accorded (Brett and Groves 1979).

#### 4. Proximate composition

According to AOAC (2012) guidelines, diets and fish carcass samples were examined for dry matter (DM), ash content, and crude protein (N x 6.25) using a Kjeltex auto-analyzer. According to Bligh and Dyer's (1959) techniques, crude fat was measured. Using conversion factors of 23.7, 39.5, and 17.2 kJ/g for protein, fat, and carbohydrate, respectively to calculate dietary gross energy (GE) According to (Brett and Groves 1979).

#### 5. Blood Collection

By the end of the experiment, 7 fish per treatment had blood samples taken from the caudal peduncle. Serum samples were maintained at -20 °C for further analysis using the technique described in (Abou Shabana *et al.*, 2018)

##### 5.1. Haematological analyses and Immune Parameters

Within 2 hours after the sampling, all hematological values were examined. A Neubauer hemocytometer (Marienfeld Superior, Lauda Königshofen, Germany) was used

to measure hematocrit, total red blood cells (RBC), total white blood cells (WBC), and mean corpuscular volumes (MCV). Microhematocrit capillaries were used to collect heparinized and EDTA-treated blood, which was then centrifuged for five minutes at 12,000 rpm to determine the hematocrit value. Mean corpuscular volume (MCV), one of the blood indicators, was determined (Ahmed, and Maqbool 2014). Total immunoglobulin (IgM) was determined according to Lim *et al.* (2009).

## 6. Histology and morphometric methods

At the end of the feeding period, anterior segments of the intestine and three samples of liver tissue from each group were obtained. Subsequently, the specimens were fixed in 10 % neutral buffered formalin, followed by embedding in paraffin, sectioning at 5  $\mu\text{m}$  thickness using a microtome (Leica®, Wetzlar, Germany), and staining with hematoxylin and eosin (H&E). Slide examinations and photographing, as well as morphometric measurements, were performed under a microscope (Ceti England) equipped with a digital camera (AmScope) (Bancroft and Layton, 2013). Twenty images per fish, covering 10 fields for each slide, were captured at 4x and 40x magnifications for morphometric measurements. using AmScopeToupView software version 3.7 (AmScope, United States).

## 7. Statistical analysis

The results of triplicate experiments are reported as mean values S.D., and any statistical analyses would employ a rejection level of ( $P>0.05$ ). Using statistical software SPSS 18 (SPSS, Chicago, IL, United States), the data were submitted for one-way analysis of variance (ANOVA). Duncan's multiple range tests (Duncan, 1955) were used to detect individual differences between treatment means.

## 8. Equations

Specific growth rate (SGR), feed conversion ratio (FCR) protein efficiency ratio (PER), is calculated as follows:

$$\text{Survival \%} = (\text{Ne} / \text{Ns}) * 100$$

Ne: number of fish at end of the experiment

Ns: number of fish at the start of the experiment

$$\text{SGR (\%/day)} = 100 (\ln W_2 - \ln W_1) / T$$

$W_2$ : is the final weight of fish in g.

$W_1$ : is the initial weight of fish in g. ln: is the natural log.

T: is the time in days

FCR = Feed intake (g)/ Weight gain (g).

PER = Weight gain, g / Protein intake, g.

## RESULTS

### 1. Water quality

Throughout the trial, under natural light (12:12 h light: dark cycle), water quality was in the appropriate range for common carp (*Cyprinus Carpio*), and measures of water temperature between 28-30 c, dissolved oxygen between 4.5-6.5 mg/l, pH between 8.01-8.69, and ammonia between 0.01-0.03 mg/l were in the safe range.

### 2. Growth performance and Feed efficiency:

Table 2 provides a summary of the growth performance indicators and survival (%) data after 60-days of feeding. The initial fish weight (IBW) did not differ significantly ( $P>0.05$ ) between the fish treatments. The weight gain (g/fish) and specific growth rates (%/day) of common carp (*Cyprinus Carpio*) fed at various vitamin C concentrations were significantly higher than the control group. By the conclusion of 60 days with T2 (200 mg vitamin C/kg diet), the weight gain had risen considerably ( $p<0.05$ ) in comparison to the results in the control group.

There was a significant difference between the control and other treatment diets in terms of average food consumption ( $P<0.05$ ) (Table. 2). Fish fed diet without ascorbic acid consumed considerably less feed and had a worse protein efficiency ratio (PER) than fish fed diets supplemented with ascorbic acid ( $P<0.05$ ). The control group (T<sub>0</sub>) had a considerably greater feed conversion ratio (FCR) than the other groups (T<sub>1</sub>, T<sub>2</sub>) ( $P<0.05$ ). While there were no significant differences between the groups T<sub>1</sub> and T<sub>2</sub>. Significant differences ( $P<0.05$ ) were recorded in the survival of *Cyprinus carpio* among experimental diets. Survival increased from 94.44 to 98.88 %, with ascorbic acid levels.

### 3. Body composition:

Carcass protein and ash contents were influenced by dietary treatments (Table 3). Carcass analysis of the experimental fish showed significantly ( $P < 0.05$ ) highest crude protein in fed fish T<sub>2</sub> ( $59.98\pm 0.04$ ) and lowest in control fed fish diet ( $57.49\pm 0.32$ ). The ash content was significantly decreased ( $P < 0.05$ ) with the increased varying dietary level of ascorbic acid. However, lipid percentage in carcass analysis was not influenced by dietary treatments (Table 3).

Table (2): Growth performance, feed efficiency, and survival (%) of common carp *Cyprinus Carpio* reared for 60-days.

Parameters	Treatments		
	(control)	T <sub>1</sub>	T <sub>2</sub>
<b>Initial body weight (g)</b>	2.02 ± 0.01 <sup>a</sup>	2.04 ± 0.02 <sup>a</sup>	2.03 ± 0.01 <sup>a</sup>
<b>Final body weight (g)</b>	17.61 ± 0.65 <sup>c</sup>	20.76 ± 0.35 <sup>b</sup>	22.09 ± 0.46 <sup>a</sup>
<b>Gain (g)</b>	15.58 ± 0.66 <sup>c</sup>	18.72 ± 0.33 <sup>b</sup>	20.07 ± 0.46 <sup>a</sup>
<b>SGR (%/day)</b>	3.61 ± 0.07 <sup>c</sup>	3.86 ± 0.01 <sup>b</sup>	3.98 ± 0.04 <sup>a</sup>
<b>Feed intake (g)</b>	27.58 ± 1.32 <sup>c</sup>	32.25 ± 0.47 <sup>b</sup>	34.71 ± 0.64 <sup>a</sup>
<b>FCR</b>	1.77 ± 0.01 <sup>a</sup>	1.72 ± 0.00 <sup>b</sup>	1.73 ± 0.01 <sup>b</sup>
<b>PER</b>	2.26 ± 0.01 <sup>b</sup>	2.32 ± 0.00 <sup>a</sup>	2.31 ± 0.01 <sup>a</sup>
<b>Survival (%)</b>	94.44 ± 1.92 <sup>a</sup>	97.77 ± 3.84 <sup>a</sup>	98.88 ± 1.92 <sup>a</sup>

Data are mean±SD. Numbers with different superscripted letters within the same row mean a significant difference (P<0.05). T1 additives 100 mg/kg feed, and T2 additives 200 mg/kg feed

Table (3): Whole body composition (% on dry matter basis, DM) of common carp reared for 60-days

	Control (T <sub>0</sub> )	T <sub>1</sub>	T <sub>2</sub>
<b>Moisture (%)</b>	78.09±0.23 <sup>a</sup>	78.26±0.31 <sup>a</sup>	77.79±0.39 <sup>a</sup>
<b>Protein (CP, %)</b>	57.49±0.32 <sup>b</sup>	57.80±0.16 <sup>b</sup>	59.98±0.04 <sup>a</sup>
<b>Ether extract (EE, %)</b>	21.89±0.45 <sup>a</sup>	21.81±0.89 <sup>a</sup>	21.43±0.42 <sup>a</sup>
<b>Ash (%)</b>	17.31±0.30 <sup>a</sup>	15.69±0.27 <sup>b</sup>	15.46±0.48 <sup>b</sup>

Data are mean±SD. Numbers with different superscripted letters within the same row mean a significant difference (p<0.05). T1 additives 100 mg/kg feed, and T2 additives 200 mg/kg feed

#### 4. Effect of vitamin C on hematological

The hematological characteristics are presented in Table 4. Hematological values increased significantly (P<0.05) with dietary ascorbic acid levels. A significant reduction in the total number of RBCs in the control treatment whereas increase in T<sub>2</sub> compared to T<sub>0</sub> at the end of the experimental period. WBCs count increased significantly in the T<sub>1</sub> and T<sub>2</sub> groups than in the control. The hemoglobin (Hb) level was decreased in T<sub>0</sub> and an increment was observed in both the T<sub>2</sub> group compared to the control (T<sub>0</sub>). The

hematocrit (Hct) is significantly influenced in both groups (T<sub>0</sub>, T<sub>1</sub>, and T<sub>2</sub>) at the end of the experimental period. The Immunoglobulin M (IgM), Lymphocytes, and Platelets counts were significantly in the T<sub>1</sub> group and elevated in the T<sub>2</sub> group as compared to the control (T<sub>0</sub>). No significant effect of vitamin C supplementation was observed on the blood levels of RBCs, WBCs, and Hb (Table 4) at the end of the experiment.

Table (4): Haematological parameters and immunological parameters for common carp after rearing 60 days.

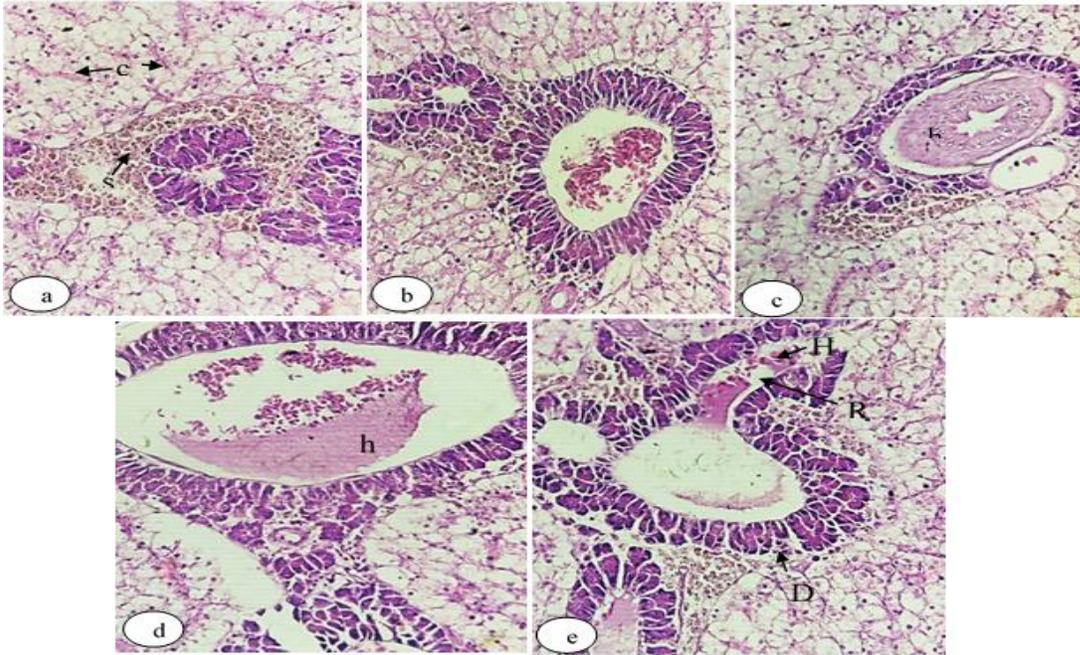
treatments	Control (T <sub>0</sub> )	T <sub>1</sub>	T <sub>2</sub>
<b>Red Cell</b> 10 <sup>6</sup> /ul	1.17±0.06 <sup>b</sup>	1.48±0.05 <sup>a</sup>	1.71±0.05 <sup>a</sup>
<b>Hemoglobin</b> g/dl	9.22±0.00 <sup>b</sup>	10.86±0.21 <sup>a</sup>	11.10±0.02 <sup>a</sup>
<b>Hematocrit</b> %	13.79±0.08 <sup>c</sup>	21.63±0.40 <sup>b</sup>	23.51±0.13 <sup>a</sup>
<b>Lymphocytes</b> 10 <sup>3</sup> cells/μL	62.64±1.23 <sup>b</sup>	74.24±0.58 <sup>ab</sup>	82.65±0.87 <sup>a</sup>
<b>Platelets Count</b> (10 <sup>3</sup> /uL)	52.20±0.92 <sup>b</sup>	232.58±6.89 <sup>a</sup>	298.41±3.79 <sup>a</sup>
<b>White Cell</b> (10 <sup>3</sup> /mm)	37.32±0.61 <sup>b</sup>	60.32±4.11 <sup>a</sup>	64.38±1.74 <sup>a</sup>
<b>IgM</b>	2.92±0.13 <sup>b</sup>	3.13±0.08 <sup>b</sup>	3.69±0.06 <sup>a</sup>

Data are mean±SD. Numbers with different superscripted letters within the same row mean a significant difference (p<0.05). T1 additives 100 mg/kg feed, and T2 additives 200 mg/kg feed

### 5. Liver and Intestine Histology:

Intestine histology of common carp fingerlings fed vitamin C enriched diets shown in Fig. (1) shows a pathological picture of liver tissue; congested blood vessels (c) and blood sinusoids, hemorrhage (H), Hemolysis of blood (h), presence of hemosiderin pigment(s), anastomosis and degeneration of pancreatic structure surround blood vessel (D), rupture of the blood vessel wall (R) in treatment control.

Fig. (2) shows inflammation of intestinal tissue; hyperplasia of mucosal layer leading to fusion of intestinal villi (H), hyperplasia of goblet cells (g), and degeneration of submucosa (D) in control. Liver histological (Fig. 3) shows severe congestion of hepatic vessels and blood sinusoids(c), degeneration of hepatic cells (D), rupture of blood vessels and hemorrhage (H), and anastomosis of blood vessels in T<sub>1</sub>. The intestinal histological section (Fig. 4) revealed severe degeneration and necrosis of intestinal tissue (D), hyperplasia of the d mucosal layer and submucosa (H), edema, and separation in the muscular layer (E) at the common Carp in T<sub>1</sub>. Liver cross-section (Fig. 5a, 4X) showed healthy liver tissue with some anastomosis of hepatic blood vessels and blood hemolysis ; (Fig. 5b, 40X) revealed some congestion of blood sinusoids. (Fig. 5c,40X) revealed degeneration of pancreatic structure and congestion of blood sinusoids to some extent. (Fig. 5d, 40X) shows little rupture of the hepatic vessel and hemorrhage at the common Carp in T<sub>2</sub>. Histological (Fig. 6) of intestinal tissue revealed healthy intestinal tissue with slight degeneration and edema of the muscular layer and submucosa at the common Carp in T<sub>2</sub>.



Fig(1): showing a pathological picture of liver tissue; congested blood vessels (c) and blood sinusoids, hemorrhage (H), Hemolysis of blood (h), presence of hemosiderin pigment(s), anastomosis and degeneration of pancreatic structure surround blood vessel (D), rupture of the blood vessel wall (R) at common Carp in treatment control.

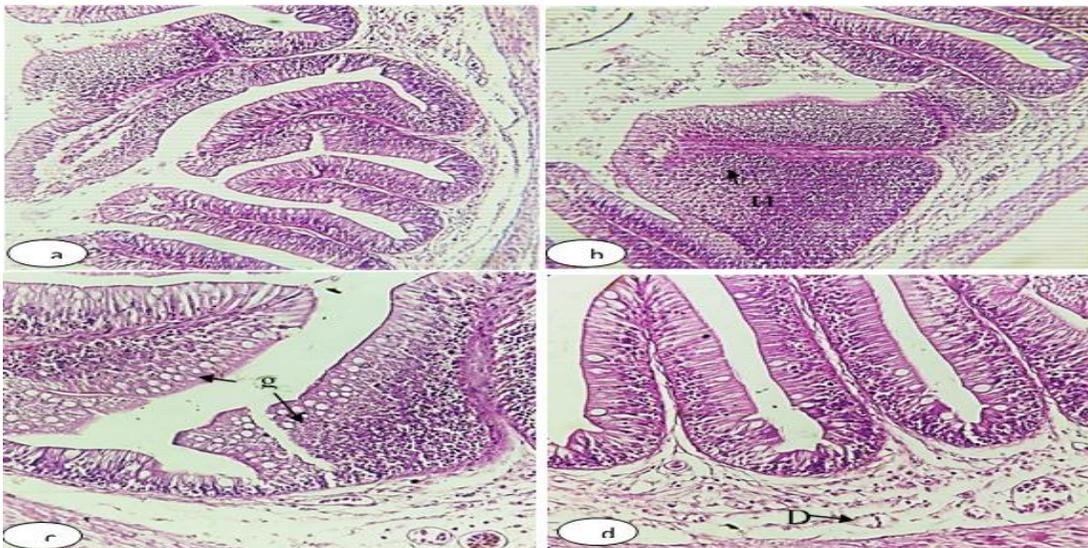


Fig. (2): showing inflammation of intestinal tissue; hyperplasia of mucosal layer leading to fusion of intestinal villi (H), hyperplasia of goblet cells (g), degeneration of submucosa (D) at common carp in control.

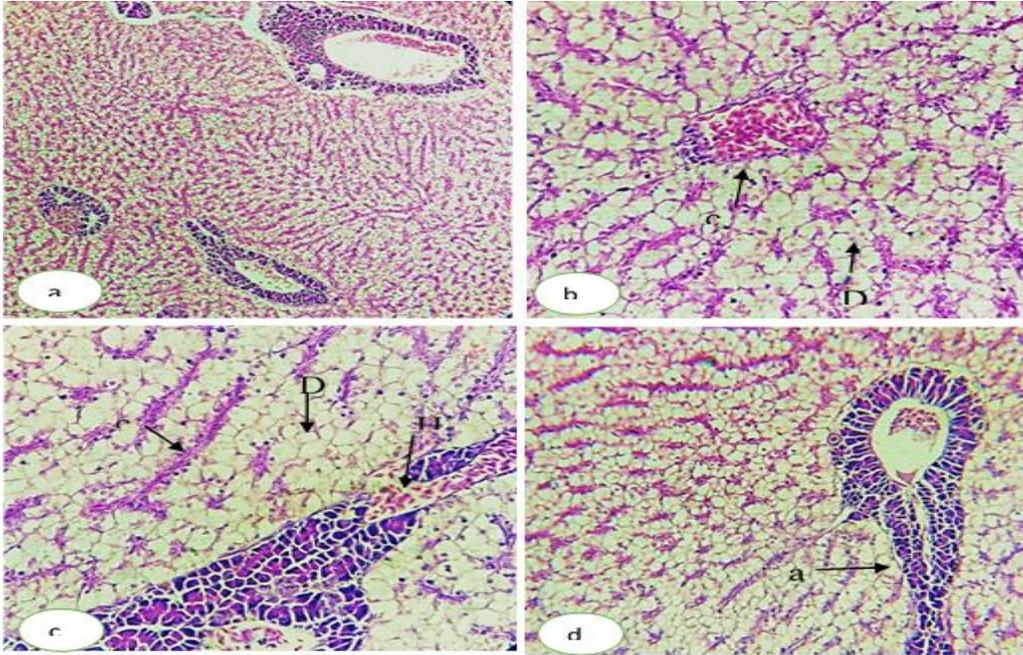


Fig. (3): liver histological picture showing severe congestion of hepatic vessels and blood sinusoids(c), degeneration of hepatic cells (D), rupture of blood vessels and hemorrhage (H), and anastomosis of blood vessels at common Carp in T<sub>1</sub>.

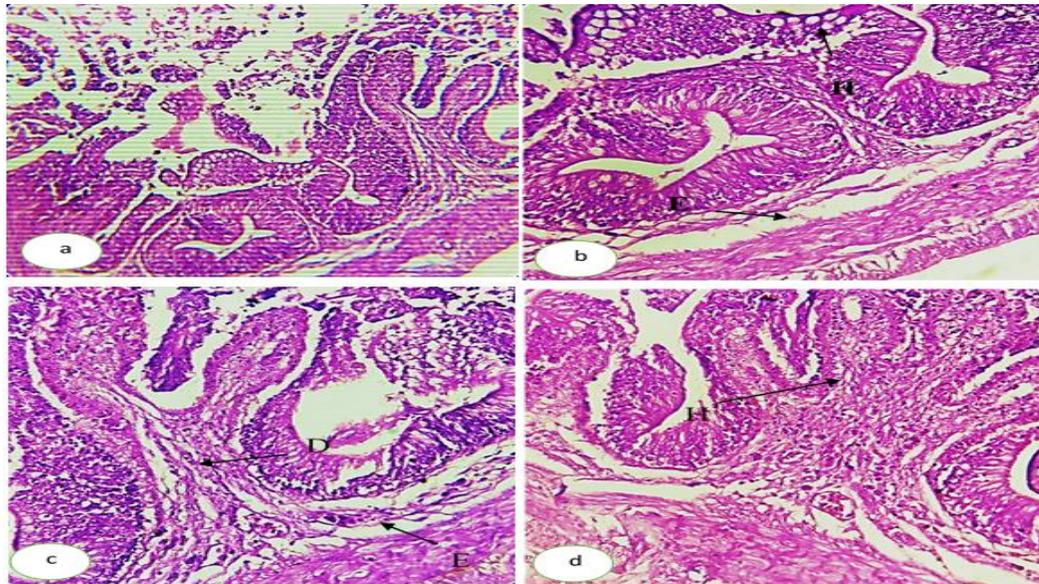


Fig. (4): intestinal histological section revealed severe degeneration and necrosis of intestinal tissue (D), hyperplasia of the mucosal layer and submucosa (H), edema, and separation in muscular layer (E) at common carp in T<sub>1</sub>.

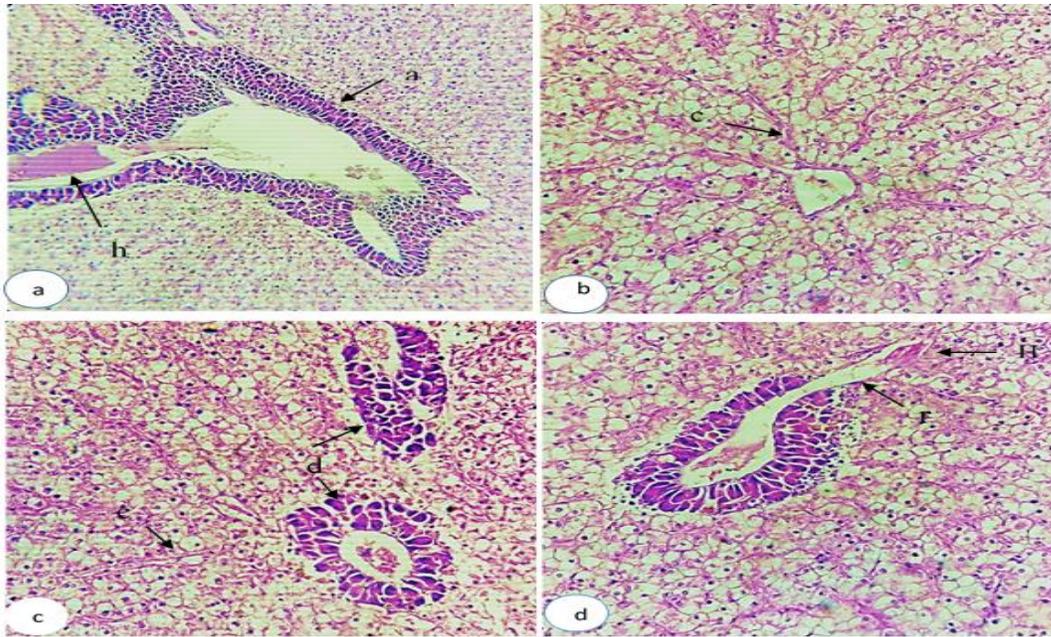


Fig. (5): (a) liver cross-section 4X showing healthy liver tissue with some anastomosis of hepatic blood vessels and blood hemolysis ; (b) 40X revealed some congestion of blood sinusoids. (c) 40X revealed degeneration of pancreatic structure and congestion of blood sinusoids to some extent. (d) 40X showing little rupture of hepatic vessel and hemorrhage at common carp in T<sub>2</sub>.

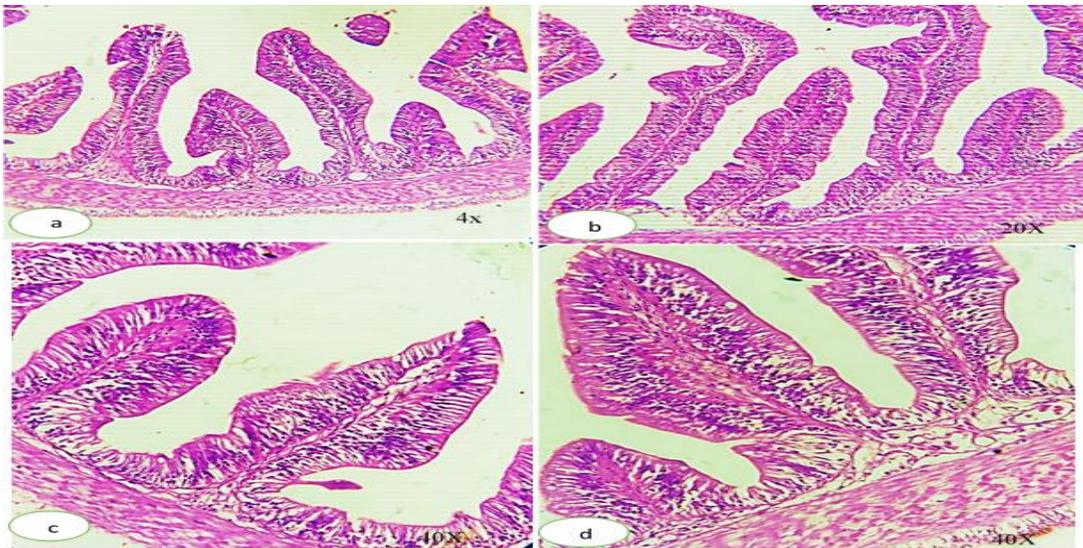


Fig. (6): histological picture of intestinal tissue revealed healthy intestinal tissue with slight degeneration and edema of the muscular layer and submucosa at common Carp in T<sub>2</sub>.

## DISCUSSION

Since most fish species can't convert L-gluconolactone into 2-keto-L-gluconolactone, they need dietary vitamin C to sustain normal development (NRC, 1993). For better survival and growth of economically significant species such as tilapia (Al-Amoudi *et al.*, 1992), yellow croaker (Ai *et al.*, 2006), parrot fish (Wang *et al.*, 2003), soft-shelled turtles (Zhou *et al.*, 2003), and parrot fish, sufficient vitamin C supplementation in fish feeds is required (Lin & Shiau, 2005).

In the current study, food supplementation with various vitamin C concentrations considerably enhanced common carp (*Cyprinus Carpio*) growth performance ( $T_1$ ,  $T_2$ ) as compared to zero-level vitamin C supplementation ( $T_0$ ). Given the decrease in growth performance of fish fed the control diet in the current study, it appears that vitamin C, as initially proposed by Ram (1966). This may be explained by vitamin C's involvement in elevating growth hormone levels in the blood, altering the intestinal shape, and strengthening the absorptive surface of the gut in fish (Abdel Rahman *et al.*, 2018). Vitamin C's ability to stimulate protein synthesis may explain why common carp grow faster and gain weight after receiving vitamin C supplements (Chagas and Val, 2003; Faramarzi, 2012).

The results of the present study are also in line with previous results in fish when fed a vitamin C diet. Ibrahim *et al.* (2020) in Nile tilapia, *Oreochromis niloticus*, Nsonga *et al.* (2009) in juvenile Tilapia, *Oreochromis karongae*, and Fracalossi *et al.* (1998) observed a similar trend in juvenile Oscars (*Astronotus ocellatus*) cichlids. Also, these findings are in by results presented Vitamin C on Akhoundian (2017) on barbel sturgeon (*Acipenser nudiventris*), on Nile tilapia (*Oreochromis niloticus*) (El Basuini *et al.*, 2021), and large yellow croaker (*Pseudosciaena crocea*) (Ai *et al.*, 2006). Similarly, studies with common carp (Sobhana, *et al.* ,2002), hybrid tilapia, *Oreochromis niloticus* (Stickney,2000), Japanese seabass, *Lateolabrax japonicas* (Gouillou-Coustans *et al.* 1998), juvenile grouper, *Epinephelus malabaricus* (Shiau, SY. and Hsu 2002 ) and juvenile cobia, *Rachycentron canadum* indicate a positive effect of vitamin C on the growth.

The findings of the current inquiry, however, go against some earlier research on several fish species. According to Ai *et al.* (2006), the development characteristics for juvenile large yellow croaker were unaffected by dietary vitamin C levels. Individual differences in size, stage of development, the environment of cultivation, variation in experimental conditions, such as levels of nutrient interaction in treatment diets, other feed components like other vitamins, like vitamin E, and the response variable used in the analysis, could all be contributing factors (Lovell, 1989 and NRC, 2011). However, due to differences in their sizes and metabolic activity, various fish species may have varying needs (Dabrowski, 1991 Chen *et al.*, 2015). Additionally, the observed discrepancies may potentially be explained by the usage of different forms of vitamin C, which have

different bioavailability (Dabrowski, 1994) and efficacy (Lin & Shiau, 2005). Additionally, the nutritional composition of the prepared feed used may vary depending on the type of fish (Chen *et al.*, 2015), which may also affect the amount of vitamin C needed. However, it is probable that carnivorous species, like trout and pintado, are more vulnerable to this vitamin shortage and thus require these vitamins at a higher level than omnivore species (Abimorad and Carneiro, 2007; Fujimoto and Carneiro 2001; Mitoma and Smith, 1960).

Fish-fed diets without ascorbic acid had significantly lower ( $P<0.05$ ) feed intake and protein efficiency ratio (PER) than fish fed with ascorbic acid-supplemented diets. While, the feed conversion ratio (FCR) in groups ( $T_1$ ,  $T_2$ ) was improvement significantly ( $P<0.05$ ) than in the control group ( $T_0$ ). According to a study by Baroi *et al.* (2019), GIFT Tilapia fed a non-supplemented vitamin C diet had low feed conversion ratios whereas fish fed a supplemented diet had the best feed conversion ratios. This study also indicated that the dietary amount of vitamin C had an impact on feed consumption. For fish (*Heterobranchus logfiles*) fingerlings fed diets with graded levels of vitamin C, Ibiyo *et al.* (2007) found significant ( $P<0.05$ ) progressive improvement in feed conversion ratio (FCR) and protein efficiency ratio and the same authors reported that the best achieved with 100 mg vitamin C/kg diet supplementation. A similar pattern was seen in young Oscars (*Astronotus ocellatus*) cichlids, according to Fracalossi *et al.* (2001).

According to Zou, *et al.*, (2019), the nutrients in the feeds supplied were used effectively, which resulted in greater protein and fat content in the fish-fed diets enriched with vitamin C. *Catla Catla* without ascorbic acid supplementation had considerably lower specific growth rates, feed conversion ratios, protein conversion ratios, and protein efficiency ratios ( $P<0.05$ ) than fish fed ascorbic acid-supplemented diets, according to Reddy (2018).

A few research showed that dietary vitamin C decreased feed intake and resulted in fish growing slowly because dietary lipid oxidation altered the diet's flavor and smell (Simic and Karel, 1980; Gao *et al.*, 2011). However, according to Lewis- McCrea, and Lall (2007), dietary vitamin C did not influence Atlantic halibut's feed consumption. In our study, increasing the vitamin C concentration from 100 mg/kg to 200 mg/kg significantly increased the BWG, SGR, and FCR of common carp (*Cyprinus Carpio*). Numerous studies have shown that vitamin C supplementation improves growth performance and prevents indications of vitamin C deficiency such as hemorrhage, scoliosis, and dysplasia. According to Zhou *et al.* (2012), juvenile cobia *Rachycentron canadum* needed just 13.6 mg/kg of vitamin C for normal development performance, however signs of deficiency such as spinal deformity slowed growth, and caudal fin erosion showed up in diets with low vitamin C. According to Xiao *et al.* (2010), vitamin C dosages of 44.7, 53.9, and 104 mg/kg resulted in the best growth performance in cobia, *R. canadum*, while a vitamin C-free diet resulted in slow development and high mortality rates. Additionally, it stated that increasing vitamin C supplementation improved the

growth and survival of Japanese seabass, *Lateolabrax japonicus* and that the minimum amount of vitamin C needed for optimal growth was 53.5 mg/kg. Deficiency symptoms like scoliosis, lordosis, and caudal fin erosion were also noted in vitamin C-free diets (Ai *et al.*, 2004). Kumari and Sahoo, (2005) found that Asian catfish, *Clarias batrachus*, fed vitamin C 1000 mg/kg revealed a considerable rise in SGR for 2 weeks and that the least amount of vitamin C 500 mg/kg was needed to maintain fish health status. Vitamin C 200 mg/kg was suggested for optimal development performance in Indian big carp *Labeo rohita*, which was fed vitamin C at a rate of 500 mg/kg. This fish demonstrated a greater SGR (Misra *et al.*, 2007).

Our findings showed that given diets containing vitamin C significant ( $P < 0.05$ ) effect on body composition (crude protein and ash). According to Liu *et al.* (2011), vitamin C-fed *C. carp* had considerably higher body protein and fat levels. Furthermore, Awad *et al.* (2013) showed that vitamin C is crucial for the metabolism of both proteins and lipids. The findings of Zou, *et al.*, (2019) showed that increasing dietary vitamin C levels from 85.01 to 715.46 mg/kg increased the crude protein contents of the total body and muscle, demonstrating that vitamin C acted as a cofactor to boost protein synthesis in juvenile Chu's croaker (Biswas *et al.*, 2013; Chagas & Val, 2003; Chen *et al.*, 2015).

Tewary and Patra (2008) reported that increasing vitamin C supplementation up to a particular level (1000 mg) improved the quality of the carcass composition, possibly as a result of the antioxidant's capabilities and its capacity to stop the peroxidation of unsaturated fatty acids. According to Chatterjee 1967; Shiao and Jans 1992), vitamin C is a crucial chemical for animal health in general and has a significant function in several areas of protein metabolism. The considerable increase in the fish's whole-body crude protein composition, according to Ibiyo *et al.* (2007), is proof of the significance of vitamin C for body protein metabolism. A similar improvement was seen in tilapia (Soliman *et al.*, 1994). Vitamin C is an essential coenzyme in certain oxidative processes, including the oxidation of tyrosine and phenylalanine (Brander and Pugh, 1977). This likely explains the discrepancies between the vitamin C-free and enriched groups' weight growth and levels of total body crude protein.

The highest levels of dietary vitamin C in groups T<sub>1</sub> and T<sub>2</sub> had slightly lower carcass lipid concentrations than the control group (Table 3), which may allow the animals to store more energy for faster metabolism and better growth performance (Xu *et al.* 2019) as well as prevent fatty liver disease (Pes *et al.* 2016). Additionally, compared to fish-fed vitamin C diets, fish fed the baseline diet had decreased crude lipid levels in their whole bodies and muscles, which is in line with findings for largemouth bass (Chen *et al.*, 2015) and Chinese sucker (Huang *et al.*, 2015). According to the same authors, increased protein and lipid levels in the body as a whole and muscle were employed to, respectively, construct muscle and supply energy, which led to higher growth performance. Additionally, in this trend, VC increases the number of intestinal villi and goblet cells as well as the intestinal microbiota population (Abdel *et al.* 2018). These

adjustments regulate the host's digestive physiology, promoting feed digestion and supplement absorption (Li *et al.* 2009; Dong *et al.* 2018). However, Rahimnejad, *et al.* (2021) discovered that dietary treatments had no effect on the muscle protein, lipid, or ash contents and that Rainbow Trout at 1,000 mg /kg vitamin C and 800 mg /kg vitamin E had decreased muscle moisture content (*Oncorhynchus mykiss*). Similar results were obtained by Gao *et al.* (2013) when they examined the muscle vitamin C and vitamin E contents of red sea bream-fed meals with various levels of oxidation and vitamin C. These disparities may result from varying rates of lipid oxidation product absorption in various species, experimental settings, varying levels of oil oxidation, and supplementary dosages of vitamin C and vitamin E in the baseline diet (Fatima *et al.*, 2019).

Studies of hematological factors have gained relevance as an auxiliary tool for the identification of metabolic abnormalities, reproductive dysfunctions, and disease (Fazio, 2019) and the diagnosis (Tavares-Dias and Moraes, 2004) of fish health status (Fazio *et al.*, 2012). Although the proteins and hormones that make up fish blood change in response to their nutritional and physiological condition, the study of hematological parameters is widely used to evaluate the fish's physiological status and ascertain their nutrient status (Satheeshkumar *et al.*, 2012). Therefore, hematological measures can be used to evaluate the physiological consequences of an ascorbic acid (AA) diet. According to research by Ortun'o *et al.* (1999), vitamin C has been shown to temporarily boost phagocytic activity, natural complement, and respiratory burst activity in gilthead sea bream *Sparus aurata*. Additionally, they proposed that vitamin C is very interactive and that maintaining appropriate vitamin C levels may directly strengthen antioxidant defense and improve immunological response. Additionally, it has potent anti-stress properties and can boost the nonspecific immune response in Atlantic salmon subjected to 2 hours of confinement stress (Thompson *et al.*, 1993).

The control diet (T<sub>0</sub>) in the current investigation had the lowest significant results, whereas the diets containing 100 mg and 200 mg of ascorbic acid per kilogram per day had the greatest levels of hematological activity. The decrease in RBC and Hb in blood fish control (T<sub>0</sub>) may be caused by erythropoiesis being inhibited, chemosynthesis being impaired, osmoregulatory malfunction, or an increase in the rate of erythrocyte apoptosis in the hematopoietic organ (Jenkins *et al.*, 2003). Our data indicate that 200 mg of vitamin C can help lessen the negative effects on RBC, Hb, WBC, and Hct ( Narra *et al.*, 2015 ). The same authors demonstrated that WBCs are important in the regulation of immunological activities, and changes in count following exposure to various toxins may signify a decline in the organism's nonspecific immunity.

Although, in the current experiment, the decline in RBC counts in infected fishes fed a control diet may be attributed to the destruction of mature RBCs and inhibition of erythrocyte production as a result of the fungal infection's reduction of hemoglobin synthesis or it may be due to the removal of RBCs from circulation as a result of the fungal infection's induced extravasation of the blood (Innocent, *et al.*, 2011). According

to Fabiana *et al.* (2007), vitamin C and vitamin e are necessary for the preservation of erythrocytes. Similar findings were obtained by Shiau & Jan (1992) in hybrid tilapia without dietary ascorbic acid, which had a lower hematocrit than fish receiving ascorbic acid supplementation. According to the same authors, anemia is a common symptom in animals with ascorbic acid deficiency because there is a decrease in iron absorption and redistribution, which leads to a decrease in the production of hemoglobin. Due to a lack of ascorbic acid supplementation, Fracalossi *et al.* (1998) also reported scurvy symptoms in Oscars (*Astronotus ocellatus*), including stunted development, decreased collagen synthesis, and lordosis. Since Vitamin C, an antioxidant prevents the oxidation of red blood cell membranes, RBC, Ht, and Hb values can be kept at optimal levels even during oxidative stress, which is a current trend (Narra, 2017). Additionally, vitamin C not only increases iron intake by converting ferric ions to ferrous ions but also facilitates the release of iron from transferrin, which enhances the activities of hemoglobin (Zafar and Khan, 2020). These vitamin C responses may enhance the delivery of oxygen to tissue and shield fish from anemia (Affonso *et al.*, 2007). RBC, Ht, and Hb levels significantly increased in the matrix, *Brycon amazonicus*, pirarucu, *Arapaima gigas* (de Menezes *et al.*, 2006), and *Cirrhinus mrigala* (Affonso *et al.*, 2007). (Zehra and Khan, 2012). The vitamin C diet had no discernible effect on the juvenile yellow catfish, *Pelteobagrus fulvidraco's* RBC or Ht levels, but it did dramatically boost Hb levels (Liang *et al.*, 2017). According to Yu and Kang (2020), fish-fed diets containing L-ascorbyl-2-monophosphate (AMP) 800 mg/kg at 2 weeks and above AMP 200 mg/kg at 4 weeks had significantly greater total RBC counts than fish fed other diets.

A powerful immunostimulant that improves lymphocyte activity is vitamin C (Head 1998 and Innocent 2011). Fish fed a control diet showed a little drop in lymphocytes, but fish fed fish supplemented with 100% vitamin C showed an increase in lymphocytes. Innocent, *et al.* (2011) noted that all the infected fishes had a drop in lymphocytes along the same trend, although the decline in fishes receiving an Immunostimulant diet was low. When *Piaractus mesopotamicus* was fed diets fortified with vitamins C and E and challenged with *Aeromonas hydrophila*, it was noted that the numbers of neutrophils and monocytes rose while the total leukocyte counts, lymphocytes, and eosinophils dropped (Fabiana *et al.*, 2007).

In a similar vein, vitamin C enhances lysozyme activity and phagocytosis in leukocytes (WBCs), including neutrophils and phagocytes (Affonso *et al.*, 2007; Overland 2010). The phagocytes of rainbow trout and gilthead seabream, *Sparus aurata*, also accumulated vitamin C. The neutrophils accumulate vitamin C levels ranging from 2mM to 10mM and use vitamin C as a reductant to produce hydrogen peroxide for destroying pathogens and cell debris (Johnston *et al.*, 2007; Mulero *et al.*, 1998). This buildup of AA can affect fish lysozyme activity and phagocytic activity, two non-specific immune responses. Numerous publications claimed that AA supplementation improved non-specific immunological indicators like lysozyme and phagocytosis. The vitamin C

supplementation increased lysozyme activity in Japanese eel, *A. japonica* (Shahkar et al., 2015), Indian major carp, *L. rohita* (Misra et al., 2007), Nile tilapia (Ibrahim et al., 2020), Japanese seabass, *L. japonicus* (Ai et al., 2004), tiger puffer, *Takifugu rubripes* (Eo and Lee, 2008) and large yellow croaker, *P. crocea* (Ai et al., 2006). In addition, vitamin C administration enhanced the phagocytosis in Indian major carp, *L. rohita* (Tewary and Patra, 2008), large yellow croaker, *P. crocea* (Ai et al., 2006), gilthead seabream, *S. aurata* (Ortuno et al., 2001) and grouper, *Epinephelus malabaricus* (Lin and Shiau, 2005). Similar to earlier research, our findings showed that lysozyme activity and phagocytosis improved dramatically with increasing AA concentration. This finding shows that the AA diet benefits starry flounder immunity. However, as compared to the control (T0), the Immunoglobulin M (IgM), Lymphocytes, and Platelets counts were considerably higher in the T1 group and lower in the T2 group. Leukocytes and thrombocytes are both thought to play a role in fish defense mechanisms and have hemostatic activities, according to Bozzo et al. (2007) and Tavares-Dias et al. (2007). Even though Williams and Barclay (1988) and Uribe et al. (2011) shown that immunoglobulins or antibodies, which are heterodimeric glycoproteins that are members of the large Ig superfamily and play a crucial role in adaptive immune responses (IgSF).

Figures (1- 6) depict the histological appearance of hepatic and intestinal samples, respectively. According to logic, there are two explanations for the observed results: (a) the dietary vitamin C absorbed in the intestine was first transported to the liver to fulfill the needs of physiological functions, and the excess was then transported from the liver to muscle; and (b) the liver is a metabolically active organ, so it has lower vitamin C requirements than muscle (Xiao et al., 2010). Understanding pathological alterations linked to fish nutrition sources requires knowledge about histological change (Shi et al., 2017). Nile tilapia, (*Oreochromis niloticus*) with almost normal intestinal mucosal and sub-mucosal coatings, enlarged lacteals, and moderate submucosal inflammatory lymphocytes were investigated in sections by Ibrahim et al. (2020) in this trend. The fish intestinal sections from the vitamin C treated group showed remarkably tall and dense villi with localized inflammatory lymphocyte infiltrations. Few residual villi that were tall, thick, and short were seen in the 300 mg /kg vitamin C group, whereas healthy-looking villi with prominent broad cup-shaped ends were seen in the 400 mg /kg vitamin C group. The same scientists also looked at fish liver samples from the group that had been fed a diet containing 0 mg /kg of vitamin C, and they found that the hepatic architectures were virtually normal, with just a little localized edema and a few inflammatory cells. The liver sections of the group supplied with 200 mg /kg vitamin C showed distinct normal healthy hepatic histo-structures with congested sinusoids, whereas the group treated with 300 mg /kg vitamin C showed normal hepatic tissues with a few Kupfer cell hyperplasia. The 400 mg /kg vitamin C-supplemented group had hepatic tissues that seemed normal and had a few fatty vacuoles (Ibrahim et al., 2020).

In a recent study, Abdel Rahman *et al.* (2018) found that Nile tilapia fed a vitamin C-supplemented diet for 28 days had better intestinal anatomy. The liver sections from the fish supplemented with 300 mg/kg of vitamin C showed normal hepatic tissues with only a few Kupfer cell hyperplasia, while the liver sections from the fish fed with a diet containing 0 mg /kg of vitamin C showed nearly normal hepatic architectures, with mild focal edema and a few inflammatory cells. Kupfer cells are essential for removing from circulation macromolecules, immune complexes, poisons, and degenerated cells, all of which are thought to be hepatoprotective under steady-state settings and at low concentrations (Deshane *et al.*, 2005; Vickers, 2017). Additionally, fish treated with 400 mg /kg of vitamin C showed normal liver architecture and a few lipid vacuoles, which was likely due to the antioxidant activity of vitamin C's hepatoprotective effects (Ozmen *et al.*, 2004). Intestinal tissues of fish fed with the control diet displayed abnormalities including severe necrosis in the intestinal villi, intact mucosa with moderate mucinous degeneration, swelling of goblet cells, reduced villi height, increase in the number of goblet cells, less crypt depth, and increased goblet cell number, according to Rathore *et al.*, (2019) research on mono-sex Nile tilapia (*Oreochromis niloticus*). According to research by Pirarat *et al.* (2011), the height of the intestinal villi and the number of goblet cells in fish intestinal histology are major indicators of the gut's capacity for absorption and digestion. These results show that the intestine's absorptive capacity has increased by providing more surface area for nutrient absorption and by enhancing the immune system's defenses against pathogens by using viscous mucin layers that spread out the infectious agent receptors present on the intestinal mucosa (Ringo *et al.*, 2003). In contrast, Rahimnejad, *et al.*, (2021) demonstrated that no histopathological changes were seen in the intestines of rainbow trout (*Oncorhynchus mykiss*) fed oxidized fish oil in their study of the effects of vitamin C and E supplementation on growth, fatty acid composition, innate immunity, and antioxidant capacity. Even though they discovered the mucosal epithelium to be a single, fully developed layer, they also noticed that the epithelial cells were uniform in size and arrangement, showing no signs of hypo/hyperplasia or hypo/hypertrophy, atrophic or necrotic alterations of the epithelial cells, or signs of inflammation from the infiltration of leukocytes or lymphocytes. The same scientists discovered that fish hepatocytes had varied numbers of vacuoles and mild to moderate granulation in the cytoplasm, but no statistically significant changes were discovered between the treatment groups. In this investigation, vitamin C and vitamin E supplementation also partially decreased hepatocyte vacuolation, showing their protective benefits (Chen *et al.*, 2012; Kjaer *et al.*, 2014).

## CONCLUSION

According to the present results, vitamin C in the diet has a beneficial impact on growth performance, immunological indices, oxidative capacity, and the histomorphology of liver and gut tissue. The ideal dietary amount of vitamin C supplementation for common carp was found to be 200 mg kg<sup>-1</sup>.

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## الملخص العربي

فعالية فيتامين ج الغذائي في أداء أسماك المبروك (*Cyprinus carpio*)

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تم إجراء بحث عن تغذية أسماك المبروك لمدة ٦٠ يومًا في ٩ خزانات تجريبية في نظام مياه جوفية لتقييم آثار فيتامين ج الغذائي على أداء نمو المبروك ، ومؤشرات الدم ، وأنسجة الأمعاء والكبد. تم تكوين ثلاثة علائق متساوية في محتواها من البروتين الخام (٣٠٠ جم / كجم) ومتساوية الطاقة (١٣ ميغا جول / كجم من الطاقة الإجمالية) على النحو التالي: عليقة مقارنة (T<sub>0</sub>) ، و ١٠٠ مجم / كجم علف من فيتامين ج ، و ٢٠٠ مجم من فيتامين ج / كجم علف (T<sub>1</sub> و T<sub>2</sub>) ، على التوالي. (تم تغذية كل حمية على ثلاث مجموعات بوزن جسم أولي يبلغ ٢.٠٣ جم ± ٠.٠٢ جم في خزانات سعة ٩٠ لترًا بمعدل ٣٠ سمكه / حوض. وفقًا للنتائج ، أظهرت المجموعات التي تم تغذيتها بالوجبات الغذائية الكاملة بفيتامين ج وزنًا نهائيًا أعلى ، وزيادة في الوزن (WG) ، ومعدل نمو محدد (SGR) ، وتحسن معدل تحويل العلف (FCR) مقارنة بمجموعة التحكم. (p < 0.05) فيما يتعلق بمؤشرات المعلمات الدموية ، كانت خلايا الدم البيضاء (WBC) ، وخلايا الدم الحمراء (RBC) ، والهيماتوكريت (Ht) ، والهيموغلوبين (Hb) ، والخلايا الليمفاوية ، وعدد الصفائح الدموية ، وتركيزات الغلوبولين المناعي (IgM) أعلى بكثير في الأسماك التي تتغذى T2 من المجموعات الأخرى . من حيث التركيب الخلوي الطبيعي ، ارتفاعات الزغابات المعوية ، تعداد خلايا الكأس ، وتسلسل الكريات البيض ، تفوقت جميع المجموعات العلاجية على المجموعة الضابطة في الأنسجة المعوية. بينما كانت أنسجة الكبد والأمعاء أقل تضررًا مع زيادة تركيز فيتامين سي في النظام الغذائي. توضح النتائج التي توصلنا إليها أن إطعام المبروك الشائع بالعلف التكميلي الذي يحتوي على ٢٠٠ مجم من فيتامين ج / كجم من العلف هو إضافة غذائية مفيدة لتحسين أداء النمو. أدت هذه الإضافة الغذائية أيضًا إلى تحسين بنية الظهارة المخاطية المعوية وكان لها تأثير إيجابي على معامل الدم لأسماك المبروك.