

**RESEARCH ARTICLE**

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## **GROWTH PERFORMANCE, GILL, LIVER AND KIDNEY HISTOMORPHOLOGY OF COMMON CARP (*CYPRINUS CARPIO*) FINGERLINGS FED HUMIC ACID SUPPLEMENTED DIETS**

**ABSTRACT:**

A total of 72 *Cyprinus carpio* fingerlings were used as three dietary groups; one control and two experimental groups, in a feeding trial lasted 10 weeks. Two doses of humic acid were tested; 180 and 360 mg /kg feed in diets 1 and 2 versus the control diet (with no additives). Results indicated that the fish groups fed diets 1 and 2 had significantly ( $P < 0.05$ ) higher but slight weight gain (WG), daily weight gain (DWG) and specific growth rate (SGR) than the control group. The best ( $P < 0.05$ ) feed conversion ratio (FCR) among all dietary groups were obtained for fish fed diet 2 then diet 1. There were no significant differences ( $P > 0.05$ ) between fish groups in condition factor at the end of the feeding trail. The histomorphological effects of using humic acid on gill, liver and kidney tissues of *Cyprinus carpio* were described in details. The most common gill changes resulted from the two doses were desquamation and necrosis. Besides to the congestion in secondary lamellae, lifting of the lamellar epithelium, oedema, epithelial hyperplasia and fusion of the secondary lamellae were observed. Histomorphological lesions in liver revealed cytoplasmic and nuclear degeneration and fibrosis in addition to the lymphatic infiltration. Lesions in the kidney tissues of fish fed the humic acid diets were observed and characterized by degeneration in the epithelial cells of renal tubule, degeneration of glomerulus and deposition of hyaline casts with renal tubules. It can be concluded that despite dietary supplementation of humic acid at levels of 180 or 360 mg /kg feed can slightly improve growth and feed utilization efficiency; it causes more potent destructive effect in the gill, liver and kidney tissues of *Cyprinus carpio*.

**KEY WORDS:**

*Cyprinus carpio*; Humic acid; Growth performance, gills, liver, kidney, histology

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**INTRODUCTION:**

Banarescu and Coad (1991) reported that Carps are important fish food distributed throughout the world except in Australia and North America where the fish is considered unpalatable. They also reported that the world catch rate of carp per year exceed 200,000 tons. With the introduction of modern commercial aquaculture in the late 1970s and early 1980s, Egypt built four carp hatcheries and imported brood stock fish from Germany and Hungary. Common carps were also extensively used in the government-financed national rice-cum-fish programs. The total production of carps in 2009 was 73 958 tones or approximately 10.48 percent of the total aquaculture production, most from poly-culture in rice fields (FAO, 2011).

Antimicrobial feed additives are used worldwide, so far, in animal husbandry to improve the economy and ecology of animal production by increasing growth rate, decreasing feed expenditure per gain and diminishing the risk of disease (Hayes, 1981; Gropp *et al.* 1992). However, the unavoidable spread of bacterial resistance and cross-resistance to antibiotics used in veterinary and human therapy were observed (Barton, 1998; Khachatourians, 1998). Among the antimicrobial feed additives, many alternatives humic acids (HA) are described. Humic substances are the most ubiquitous carbon substance on the surface of the earth, found in almost every drop of water and in almost all soils (Avic *et al.*, 2007). Humic acid is resulting from decomposition of organic matters; particularly plants and humus contain humic acid, fulvic acid, ulmic acid and some microelements (Stevenson, 1994).

The use of humic preparations, as part of food supplements, has been fully researched using highly productive broiler

poultry. It was established that the use of humates in broilers' feed activates the synthetic phase of albuminous exchange. As a result, there was a 10% increase in mass growth, and the poultry's immunity rose by 5-7% (Parks *et al.*, 1996; Karaoglu *et al.*, 2004; Kncukersan *et al.*, 2005). Unfortunately, their supplementation as feed additives in fish diets has not been well reported. The present study was designed to elucidate the long term effects of feeding humic acid on growth performance and the subsequent histomorphological alterations of specific organs of common carp, *Cyprinus carpio*.

## MATERIAL AND METHODS:

### Fish and experimental condition:

Apparently healthy common carp (*Cyprinus carpio*) fingerlings with an average body weight of  $4.5 \pm 0.5$  gram were obtained from the Central Laboratory for Aquaculture Research (CLAR), Faculty of Agriculture, Suez Canal University. Fish were transported alive to the laboratory in air-pumped large polyethylene bags. Fish were kept in glass aquaria (100 × 50 × 30 cm each) and maintained in well-aerated and de-chlorinated tap fresh water at  $22.0^{\circ}\text{C} \pm 2.0$  for 14 days as an acclimatization period. The health status of experimental fish under lab conditions was monitored throughout the acclimatization period by visual observation.

### Experimental design:

A total of 72 common carp *Cyprinus carpio* were distributed into 6 glass aquaria and acclimatized to the experimental conditions prior to the start of feeding trail. During this period fish were adapted to feeding on the control diet (without any additives). The water of the aquaria was partially drained every 2 days, with the help of plastic tubing using siphon technique. This method was also helpful in removing the surplus food settled at the bottom along with the fecal matter. One fourth of the water was removed and replaced by overnight kept dechlorinated water for maintaining the constant temperature. Water temperature range and pH were 20-22°C and 7.4, respectively during the experimental period. Each dietary treatment had two replicate aquaria with 12 fish per aquarium. The experimental fish were fed the respective diet pellets or control at a predetermined rate of 5% of live mass for 10 weeks daily with the ration being split into two meals.

### Fish diets:

A basal diet was prepared in the Department of Animal Production, Faculty of Agriculture, Suez Canal University, as shown in table 1. Diets were prepared using locally available ingredients. The ingredients were mixed mechanically and oil was added gradually to ensure even distribution of the

ingredients. Chromic oxide was added to diets to enhance carbohydrate utilization. Two suggested concentrations of humic acid (obtained from Grand Vet Company, Biofarm, Egypt) were calculated for use to be 180 and 360 mg /kg feed, then the proper amounts of humic acid suspension were mixed with the feed using hen eggs (3 eggs/kg feed) as a coating agent to prevent leaching of humic acid. Then, the feed was air dried under sterile conditions for 12 hr and stored at 20°C until use. The basal diet was mixed with the same amount of egg alone and served as the control diet.

Table 1. Composition [g/kg (DW)] of the experimental diets as fed.

Feed Ingredients	Experimental Diets		
	Control	1	2
Fish meal(FM) <sup>a</sup> (60% CP)	10	10	10
Corn gluten(CG) (60% CP)	12	12	12
Soybean meal (SBM) (44% CP)	22	22	22
Wheat bran	18	18	18
Yellow corn	29.5	29.5	29.5
Soy and fish oil(FO) <sup>b</sup> (1:1)	4	4	4
Vitamin and Mineral Mix <sup>c</sup>	3	3	3
Di Calcium phosphate	1	1	1
Cr <sub>2</sub> O <sub>3</sub> <sup>d</sup>	0.5	0.5	0.5
Humic acid mg / kg	–	180	360

a: (FM) (Cherkha Sharif for export and import, Egypt)

b: (FO) solvent extracted (Cherkha Sharif for export and import, Egypt)

c: Each Kg vitamin and mineral mixture premix contained Vitamin A, 4.8 million IU, D3, 0.8 million IU; E, 4 g; K, 0.8 g; B1, 0.4 g; Riboflavin, 1.6 g; B6, 0.6 g, B12, 4 mg; Pantothenic acid, 4 g; Nicotinic acid, 8 g; Folic acid, 0.4 g Biotin, 20 mg, Mn, 22 g; Zn, 22 g; Fe, 12 g; Cu, 4 g; I, 0.4 g, Selenium, 0.4 g and Co, 4.8 mg.

d: Cr<sub>2</sub>O<sub>3</sub>: Chromic oxide; (CP %): crude protein, (DW) Dry weight

### Data Collection:

The amount of daily diet for the experimental groups was calculated for each tank biweekly as 5.0% of the mean body weight of the fish. All fish from each tank were removed weighed and then returned to their corresponding tanks.

### Diet composition:

Diet was analysed for protein, lipid, and dry matter moisture and ash contents. Protein (N x 6.25) was analysed using the micro-Kjeldahl digestion according to Crooke and Simpson (1971), lipid by petroleum ether extraction (Soxhlet technique), dry matter by drying at 110°C for 24 hours, and ash by incineration in a muffle furnace at 500°C for 12 hr. the composition of diet was: Crude

protein, 30%; Lipid, 15%; Ash, 7.5%; Cellulose, 8.52; and Moisture, 10%.

**Growth performance indices:**

Body weight and feed given data were recorded at bi-weekly intervals as follows:

$$\text{Body weight gain (g/fish)} = BW_f \text{ (g)} - BW_i \text{ (g)} / t$$

$$\text{Specific Growth Rate (\%/ day): SGR (\% / day)} = 100 (\ln BW_f - \ln BW_i)$$

$$\text{Feed conversion ratio (FCR)} = \text{dry feed intake (g/fish)} / \text{live body weight gain (g/fish)}$$

$$\text{Condition factor (K)} = 100 \times \text{body weight (g)} / \text{length}^3 \text{ (cm)}$$

**Histomorphological examination:**

At the end of the experimental period, 6 fish from each dietary group were sacrificed for further analysis. Pieces of gills, kidney and liver were carefully excised, rinsed in physiological saline solution and fixed in aqueous Bouin's solution for 24-30 h. Tissues were dehydrated through a graded series of ethanol, cleared in terpinole, and mounted in paraffin wax. Sections of 4-5 μm were prepared from paraffin blocks by using a rotary microtome. The tissue sections were stained with haematoxylin and eosin (H&E) (Luna, 1968) and 6 sections of each tissue from each fish were examined by light microscopy.

Histopathological changes of all organs were described qualitatively and changes in gills, liver, and kidney were evaluated according to the assessment protocol of Bernet *et al.* (1999). The pathological changes were classified into five reaction patterns (rp): circulatory, regressive, progressive, inflammatory, and neoplastic. According to the degree and extent of lesions an organ index for each organ could be determined. The sum of all organ indices resulted in a total index for each individual fish. Every organ alteration (alt) was assessed using an arbitrary score (a) ranging from 0 to 6, and was estimated according to the following grades: 0 = unchanged, 2 = mild occurrence, 4 = moderate occurrence, 6 = severe occurrence (diffuse lesion), and importance factor (w) which revealed the pathological importance of the lesions and how it affect the ability of fish to survive.

The presence of histological alterations for each organ was evaluated quantitatively by using the following equations

$$\text{(Organ index)} \sum I_{org} = \sum_{rp} \sum_{alt} (a_{org rp alt} \times w_{org rp alt})$$

- $I_{org}$ : Organ index
- rp: reaction pattern
- alt: alterations
- a: the score value
- w: importance factor

This index represents the degree of damage to an organ. It is the sum of the multiplied importance factors and score values of all changes found within the examined organ. A high index indicates a high degree of damage.

This index represents a measure of the overall normal status of organ based on the histological lesions. It is calculated by adding up all organ indices of an individual fish. As the total index is calculated in the same way for every fish, a comparison between individuals is possible. When  $I_{org} = \text{zero}$  means normal morphology.

**Total index (Tot-I) =**

$$\sum_{org} \sum_{rp} \sum_{alt} (a_{org rp alt} \times w_{org rp alt})$$

- $I_{org}$ : Organ index
- rp: reaction pattern
- alt: alterations
- a: the score value
- w: importance factor

**Data analysis:**

All results were expressed as mean values ± Standard Error (SE). Data were analysed by using SPSS program (version 14.0). Mean values obtained for the different dietary groups were compared by one way ANOVA followed by Duncan's multiple range test and significance was defined as  $P < 0.05$  (Field, 2000).

**RESULTS:**

**Growth performance and Food utilization indices:**

Growth performance indices of common carp (*Cyprinus carpio*) fingerlings which fed diets supplemented with either 180 mg or 360 mg humic acid (HA)/kg diet are shown in table 2. Averages of initial body weight (BW<sub>i</sub>) of common carp fingerlings fed the experimental diets at the start were not significantly ( $P > 0.05$ ) different indicating that dietary groups were initially homogenous and up to 6 weeks of the whole experimental period (10 weeks). Thereafter, the two fish groups fed HA-supplemented diets grew better than fish fed the control diet. Fish fed diets 1 and 2 recorded significantly ( $P < 0.05$ ) higher weight gain (WG), daily weight gain (DWG) and specific growth rate (SGR) than those of control fish. Growth indices showed significant ( $P < 0.05$ ) increase only for fish fed diet 1 after 8 weeks as compared to the control diet. However at the end of the experiment (10 weeks) the fish group fed diet 2 had significantly ( $P < 0.05$ ) higher WG, ADG and SGR than those of control fish. Mean growth indices at the end of feeding trial are summarized in table 3.

Table 2. Effect of dietary humic acid supplementation (mean  $\pm$  S.E) on growth indices, and condition factor of common carp (*Cyprinus carpio*)

Period (Weeks)	Treatment	BWi (g)	WG (g)	DWG (mg/fish/day)	SGR (%/day)	K (%)
0-2	Control	4.72 $\pm 0.39$	0.40 $\pm 0.05$	0.03 $\pm 0.004$	0.64 $\pm 0.08$	3.73 $\pm 0.08$
	Diet 1	4.73 $\pm 0.29$	0.89 $\pm 0.07$	0.06 $\pm 0.005$	1.51 $\pm 0.07$	3.66 $\pm 0.18$
	Diet 2	3.68 $\pm 0.31^*$	0.97 $\pm 0.06$	0.07 $\pm 0.005$	1.71 $\pm 0.07$	4.69 $\pm 0.48$
2-4	Control	5.06 $\pm 0.39$	0.68 $\pm 0.05$	0.05 $\pm 0.004$	0.95 $\pm 0.07$	4.10 $\pm 0.11$
	Diet 1	5.37 $\pm 0.39$	0.92 $\pm 0.05$	0.07 $\pm 0.004$	1.33 $\pm 0.07$	3.63 $\pm 0.18$
	Diet 2	4.65 $\pm 0.36$	0.92 $\pm 0.07$	0.07 $\pm 0.005$	1.32 $\pm 0.08$	4.11 $\pm 0.16$
4-6	Control	5.68 $\pm 0.41$	0.21 $\pm 0.06$	0.02 $\pm 0.005$	0.23 $\pm 0.06$	3.93 $\pm 0.11$
	Diet 1	5.72 $\pm 0.42$	0.37 $\pm 0.04^{*#}$	0.03 $\pm 0.003^{*#}$	0.45 $\pm 0.04$ $^{*#}$	3.52 $\pm 0.16$
	Diet 2	5.57 $\pm 0.40$	0.50 $\pm 0.03^{*#}$	0.04 $\pm 0.002^{*#}$	0.68 $\pm 0.06^{*#}$	4.21 $\pm 0.23$
6-8 weeks	Control	5.89 $\pm 0.44$	0.32 $\pm 0.05$	0.02 $\pm 0.004$	0.38 $\pm 0.053$	3.66 $\pm 0.21$
	Diet 1	6.18 $\pm 0.46$	0.48 $\pm 0.05$	0.03 $\pm 0.003$	0.53 $\pm 0.032$	3.74 $\pm 0.20$
	Diet 2	6.05 $\pm 0.40$	0.35 $\pm 0.05$	0.03 $\pm 0.004$	0.38 $\pm 0.039$	4.11 $\pm 0.17$
8-10	Control	5.83 $\pm 0.54$	0.57 $\pm 0.05$	0.04 $\pm 0.003$	0.68 $\pm 0.064$	3.70 $\pm 0.18$
	Diet 1	6.62 $\pm 0.50$	0.48 $\pm 0.05$	0.03 $\pm 0.003$	0.51 $\pm 0.045$	4.06 $\pm 0.23$
	Diet 2	6.36 $\pm 0.44$	0.39 $\pm 0.050$	0.03 $\pm 0.004$	0.41 $\pm 0.038$	4.10 $\pm 0.12$

DWG: Average daily gain (g /fish /day) = BWf – BWi/t

SGR: Specific growth rate = (Ln BWf – Ln BWi) 100 / t

K: condition factor = 100 x BW (g)/ (length (cm))<sup>3</sup>,

Mean value  $\pm$  (S. E.) for 24 fish / group

\*: indicates a significant difference between the control and treated group ( $P < 0.05$ ).

#: indicates a significant difference between the low and high humic acid treated group ( $P < 0.05$ ).

Table 3. Summary of growth indices (mean  $\pm$  S.E) and condition factor (K) of *Cyprinus carpio* at the end of the experiment (10 weeks)

Parameters	Control	Diet 1	Diet 2
Body weight (g /fish)			
BWi	4.45 $\pm$ 0.43	3.67 $\pm$ 0.29	3.68 $\pm$ 0.31
WG	1.94 $\pm$ 2.95	2.95 $\pm$ 0.22 <sup>*</sup>	3.07 $\pm$ 0.19 <sup>*</sup>
DWG (g / fish/day)	0.03 $\pm$ 0.002	0.04 $\pm$ 0.003 <sup>*</sup>	0.04 $\pm$ 0.003 <sup>*</sup>
SGR (%)/ day	0.52 $\pm$ 0.04	0.82 $\pm$ 0.04 <sup>*</sup>	0.88 $\pm$ 0.03 <sup>*</sup>
FCR	3.57 $\pm$ 0.27	2.12 $\pm$ 0.18 <sup>*</sup>	2.24 $\pm$ 0.20 <sup>*</sup>
K (%)	3.70 $\pm$ 0.18	4.06 $\pm$ 0.23	4.10 $\pm$ 0.12

Mean  $\pm$  S.E. (n=24)

\*: represents a significant difference ( $P < 0.05$ ) between the control and treated groups.

A total weight gain values of 1.94 (control diet), 2.95 and 3.07 g/fish for diets 1 and 2 (containing 180 mg and 360 mg humic acid / kg) respectively were obtained and were significantly different ( $P < 0.05$ ). Also, similar to WG, DWG and SGR followed the same trend from control (Table 3). Therefore, Feeding common carp HA-supplemented diets

had resulted to significant ( $P < 0.05$ ) higher growth indices: WG, DWG and SGR than those of control fish.

In the mean time, results showed better FCR values; 2.12 and 2.24 for HA-fed fish groups, respectively than those recorded for control fish (3.57), meaning improved feed utilization for the two dietary groups. Condition factor showed significance difference in fish fed diet 2 after 2, 4, and 6 weeks as compared of corresponding control.

Table 4 summarizes the estimated mean organ indices (gill, liver and kidney) for the 2 dietary groups fed diets 1 or 2.

Table 4. Organ indices (mean  $\pm$  S.E) for HA-fed fish for 10 weeks

Parameters	Control	Diet 1	Diet 2
gill index (IG)	ND	15.0 $\pm 2.86$ <sup>*</sup>	22.2 $\pm 1.37$ <sup>*</sup>
liver index (IL)	ND	20.0 $\pm 5.29$ <sup>*</sup>	23.0 $\pm 2.56$ <sup>*</sup>
kidney index (IK)	ND	16.0 $\pm 3.57$ <sup>*</sup>	20.0 $\pm 1.26$ <sup>*</sup>
Total index (Tot – I)	ND	51.0 $\pm 11.5$ <sup>*</sup>	65.0 $\pm 4.7$ <sup>*</sup>

Data represent the mean  $\pm$  S. E. (n=6)

\*: represents a significant difference between the control and treated groups,

ND: not determined.

The mean organ indices for gills (IG), liver (IL) and kidney (IK) for fish fed diet 1 were 15, 20 and 16 vs 22.2, 23 and 20 for fish fed diet 2 which in turn indicating that the hepatic lesions showed potent damage than gill and kidney. The organ index and total index values were significantly ( $P < 0.05$ ) higher in fish fed either diet 1 or 2 as compared to the corresponding for those of control fish.

Figures 1-3 show tissue examination of the selected organs from fish fed diet 1 and 2.

#### Gills:

Histomorphological alterations were noticed among dietary groups. No recognizable changes were observed in the gills of control fish. Each gill consisted of a primary filament and secondary lamellae (Fig 1A).

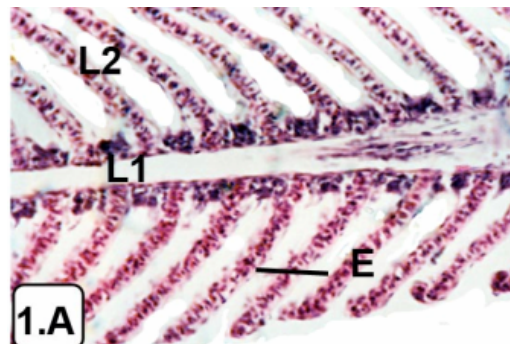


Fig. 1A. T.S in Gills of control fish showing primary lamella (L1), secondary lamellae (L2) and arising from these filaments, parallel with them and perpendicular to the filament axis and Epithelial layer (E) x 400.

The primary lamellar epithelium (L1) consisted of one or two thick cell layers. Chloride cells were visible along the primary lamellar epithelium. Fish fed the lower dose of

humic acid supplemented diet (Diet 1) showed gill-cognition in primary lamellae and, the thickness of primary lamellar epithelium was noticeably irregular (Fig 1B).

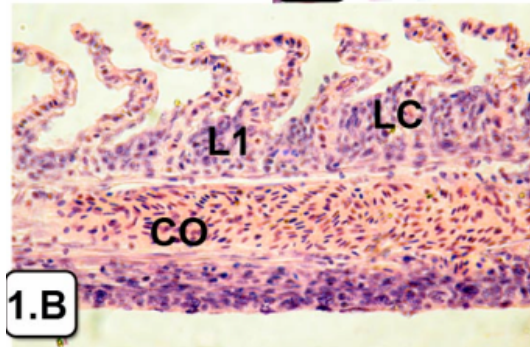


Fig. 1B. T.S. in gills of fish fed diet 1, showing irregular thickening of primary lamellar epithelium (L1), cognition (CO) and a slight infiltration of leukocytes (LC) x400.

Edema with lifting of secondary lamellar epithelium (L2) and leukocyte infiltration were also observed (Fig 1C).

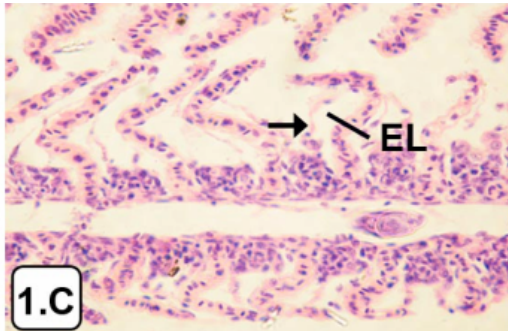


Fig. 1C. T.S. in gills of fish fed diet 1, showing Lifting of Epithelial lining in the base and tips of secondary lamella (arrow). Edematous separation layer (EL) x 400.

Similar changes were observed in fish fed the higher concentration (360 mg HA) with more pronounced thickness of primary lamellar epithelium and partial lamellar fusion (Fig 1D).

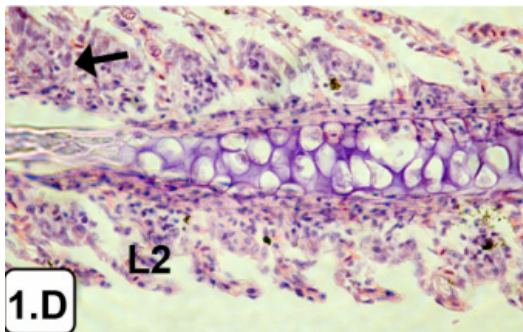


Fig. 1D. T.S. in gills of fish fed diet 2, showing Shortening of the secondary lamellae (L2) partial fusion of secondary lamella (arrow) x 400.

It is clear that the epithelium is completely disrupted as a result of cell-lysis (necrosis) (Fig 1 E).

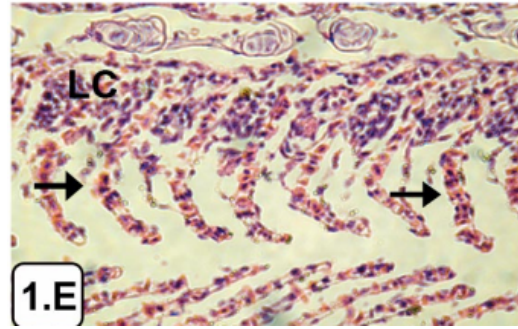


Fig. 1E. T.S. in gills of fish fed diet 2, showing Necrosis of epithelium lining secondary lamellae (arrow) and a slight infiltration of leukocytes (LC) x 400.

**Liver:**

Examination of liver hepatocytes morphology of control fish revealed a typical parenchymatous appearance. At the light microscopic level, the liver was divided into irregularly shaped lobules separated by the hepatopancreas and bile duct (Fig. 2A).

Normal hepatocytes were polygonal with a central spherical nucleus and a densely stained nucleolus. Hepatic tissue of fish fed diet 1 showed varied degrees of cirrhosis as evidenced in the density of fibrous connective tissue within and around the hepatic parenchyma (Fig. 2B), in addition to congestion and hemorrhage (Fig 2C).

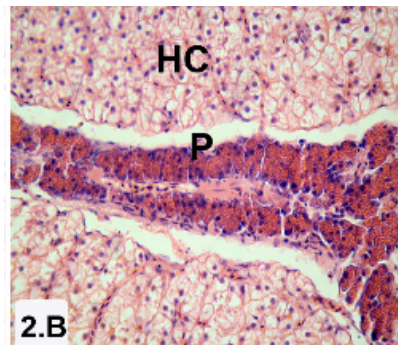
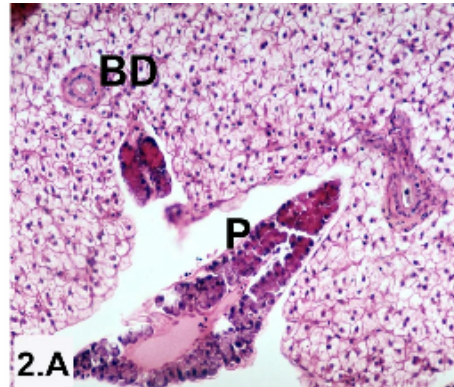


Fig. 2A&B. T.S in liver of control fish, showing the exocrine pancreas (P) around the blood vessels, Parenchymatous distribution of the hepatocytes (HC) in cords around the sinusoids and bile ducts (BD) x 200 & x 400.

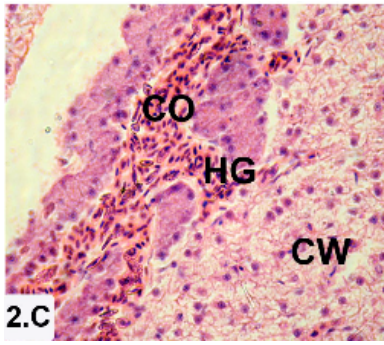


Fig. 2C. T.S. in liver of fish fed diet 1, showing Congestion (CO) hemorrhage (HG) cloudy swelling of hepatocytes (HC)

Meanwhile, hepatocytes of fish fed diet 2 began to swell and slight infiltration of leukocytes was observed indicating a progressive increase of fibroconnective tissue (Fig. 2E).

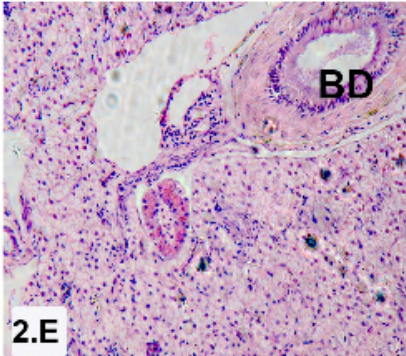


Fig. 2E. T.S. in liver of fish fed diet 1, showing hyperplasia of bile duct epithelium (BD) X 200.

As a result, signs of congestion were noticed at the sinusoid (Fig. 2C).

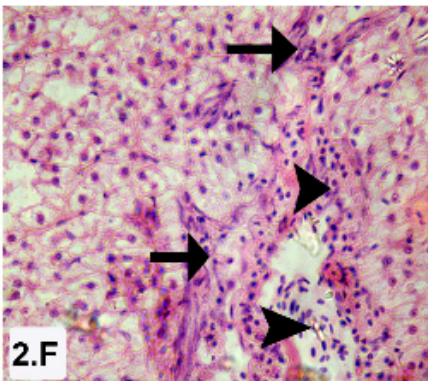


Fig. 2F. T.S. in liver of fish fed diet 2, showing lymphatic infiltration (Head arrow), fibrosis (arrows)

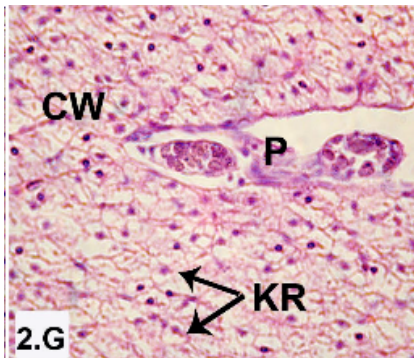
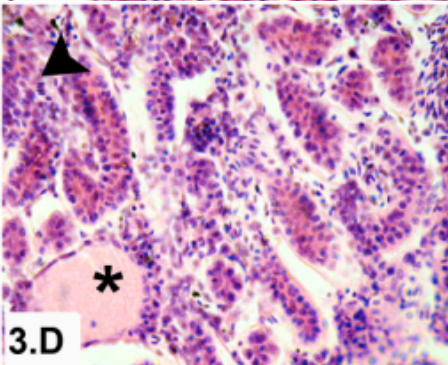
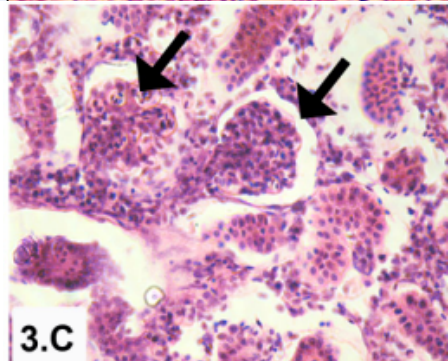
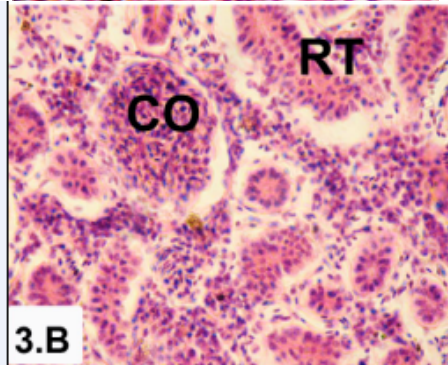
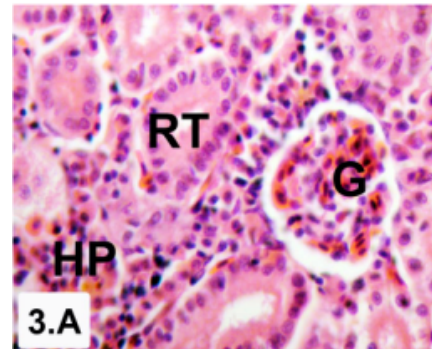


Fig. 2G. T.S. in liver of fish fed diet 2, showing atrophied pancreatic tissue, some hepatocytes r showing karyolitic nuclei (KR) x 400.

**Kidney:**

Histomorphological examination of kidney from control fish revealed a normal structure with no signs of any alterations. However, kidney of diet 2 fed fish illustrated extensive alterations including sever degenerative changes in tubular epithelium and congestion, in addition to tubular necrosis and atrophied glomeruli. In comparison, kidney of diet 1 fed fish revealed slight morphological changes such as moderate congestion areas between renal tubules, mild atrophied glomerulus, deposition of hyaline cast and cellular infiltrations comprised mostly of mononuclear cells in the interstitium (Fig. 3).



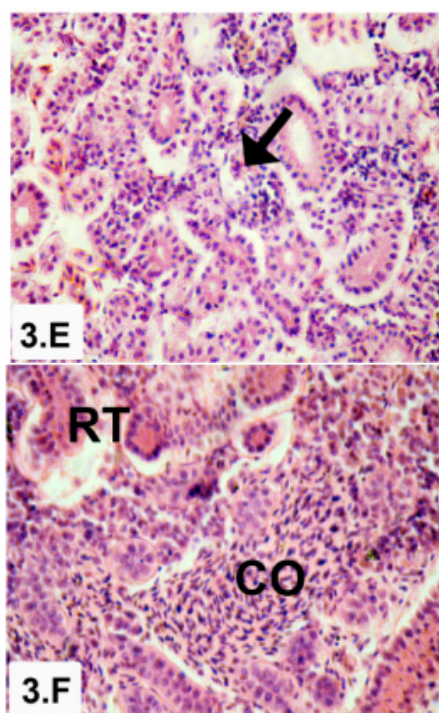


Fig. 3.

- A. T.S in kidney of control fish, showing glomerulus (G) renal tubules (RT) and hematopoietic tissue (HP) x 400.
- B. T.S. in kidney of fish fed diet 1, showing congestion (CO) and necrotic renal tubules (RT) x 400.
- C. T.S. in kidney of fish fed diet 1, showing shrinkage of glomerulus and expansion of space inside the Bowman's capsule (arrows x 400).
- D. T.S. in kidney of fish fed diet 1, showing Hyaline casts (asterisk) and lymphatic infiltration (head arrow x 400).
- E. T.S. in kidney of fish fed diet 2, showing Atrophied glomerulus (arrow) in 360 mg humic acid treated fish. X 400.
- F. T.S. in kidney of fish fed diet 2, showing Congestion (CO) between renal tubules and necrotic renal tubules (RT). X 400.

## DISCUSSION:

The present study indicated that diet supplemented with humic-acid significantly ( $P < 0.05$ ) increased growth indices. The results concerning the improvement effect of the addition of humic acid to fish feed conversion rates are similar to these obtained from earlier studies carried out on broiler (Eren and Gezen, 2000; Kocabağlı *et al.*, 2002; Tancho, 2003). Moreover, Humin Tech (2004) reported that replacing antibiotic with HA as a growth promoter in fish feed does not cause any loss in the performance of animals. The use of HA in animal feed excludes, of course, the possibility of antibiotic residue or microbial resistance (Humin Tech, 2004). It was also concluded by Ceylan and Ciftci (2002) that HA would be an alternative to antibiotic growth promoters in broiler diets.

Yasar *et al.* (2002) concluded that humic acids induced an increase in weight gain in rats and they suggested that the improved weight gain was associated with increased feed intake and improved gain:feed ratio. Stepchenko *et al.* (1991) demonstrated that after the feeding diets

containing humic substances (HS) broilers evidenced an improvement in ADG. The HS can form a protective film on the mucus epithelium of the gastrointestinal tract against infections and toxins, thus ensuring an improved utilization of nutrients in animal feed (Islam *et al.*, 2005). Another study has compared the effects of dietary humate supplementation at 1.5 and 2.5 g/kg feed on broiler performance from 0 to 42 d (Eren and Gezen, 2000). Although there was no performance difference at 21 days, the authors found that dietary supplementation of humate at 2.5 g kg<sup>-1</sup> significantly improved the live weights of broilers at 42 days.

The present results showed slight increase in weight gain (total and daily) for common carp fed diet supplemented with HA, similar findings were assessed for rats (Yasar *et al.*, 2002) who recorded improved feed intake and consequently WG, and for broilers (Stepchenko *et al.*, 1991). In contrast, Rath *et al.* (2006) suggested that humic acid exerted a negative effect on the growth performance of broiler chickens. The Inconsistent results observed in different studies may be attributable principally to the composition of different HS preparations and addition levels, as well as the different animal species and ages used in these different studies and the variability between birds, rats, and fish nature of feeding, as well as digestive system.

Huck *et al.* (1991) reported that HA when fed may influence, in particular, the metabolism of proteins and carbohydrates in microbes and this results in a direct devastation of bacterial cells or viral particles, which should result in improved growth performance. Humic acids are prebiotics which direct inhibition of bacterial and virus growth, thus reducing mycotoxin levels. They improve gut health, nutrient absorption and nutritional status in fed animals. In the current study, the improvement of growth was only observed during the entirety of the experimental period, whereas no significant effects were observed in subsequent periods. This indicated that the effects of HA may also be associated with the administration period, and long-term administration may exert a more prominent effect. Our results were in agreement with those of Wang *et al.* (2008) who reported the same observation when fed pigs with humic acid supplemented diet.

A further approach to reveal the safety of humic acid as an antibiotic alternative we evaluated the morphohistological alteration which can be used as a biomarkers to effect of exposure to environmental stressors. This category of biomarkers has the advantage of allowing one to examine specific target organs and cells as they are affected under *in vivo* conditions. Histopathology is the most rapid method of detecting adverse acute and chronic effects of exposure in the various tissues and

organs comparing an individual finfish (Kumar and Pant, 1984; Hinton *et al.*, 1992).

The gills, which participate in many important functions in fish, such as respiration, osmoregulation and excretion, remain in close contact with the external environment, and particularly sensitive to changes in the quality of the water, are considered the primary target of the contaminants (Mazon *et al.*, 2002; Fernandes and Mazon, 2003). Alterations like epithelial lifting, hyperplasia and hypertrophy of the epithelial cells, besides partial fusion of some secondary lamellae are examples of defense mechanisms, since, in general, these result in the increase of the distance between the external environment and the blood and thus serve as a barrier to the entrance of contaminants (Mallatt, 1985; Hinton and Laurén, 1990; Fernandes and Mazon, 2003). In the present study, the histomorphological changes were more evidenced in fish fed humic acid added diets but not observed in the control fish. Common gill abnormalities were observed in *Cyprinus carpio* fed humic acid supplemented diets were described as congestion in primary and secondary lamellae desquamation of secondary lamellar epithelium, hypertrophy of epithelial cells, intraepithelial oedema and epithelial necrosis and lifting of the lamellar epithelium and lamellar fusion which could be protective as it diminishes the amount of vulnerable gill surface area.

The results of gill alterations could be considered as a defense mechanism against feeding HA rather than as an irreversible toxic effect. In general Au (2004) and Cengiz (2006) suggested that gill histopathology appears to be a promising biomarker for general environmental contamination.

The present study showed also that the histomorphological changes in liver parenchyma of fish fed humic acid supplemented diets revealed signs of degeneration (cytoplasmic, nuclear degeneration and fibrosis) besides the lymphatic infiltration that was noticed in diet 2 fed fish. Teleost liver is the primary organ for biotransformation of organic xenobiotics, and probably also for the excretion of harmful trace metals, food digestion and storage, and metabolism of sex hormones (Health, 1995; Hinton *et al.*, 2001). There have been numerous reports of histo-cytopathological changes in livers of fish exposed to a wide range of organic compounds and heavy metals (Hinton and Lauren, 1990; Hinton *et al.*, 1992; Vandenberghe, 1996; GlobalTox, 1997; Braunbeck, 1998). Livers of fish are sensitive to environmental contaminants because many contaminants tend to accumulate in the liver, making this organ exposed to a much higher levels (several orders of magnitude) than in the environment, or in other organs (Health, 1995).

The present study has recorded the presence of a remarkable fibrosis in the liver of humic acid- fed fish. Sokol (2002) reported that

cytokinase and reactive oxygen species induce the activation and proliferation of hepatic stellate cells. These cells are the major players in the development of liver fibrosis (Hendricks *et al.*, 1981; Kent *et al.*, 1988).

Most common alterations found in the kidney of fishes exposed to water contamination are tubule degeneration (cloudy swelling and hyaline droplets) and changes in the corpuscle, such as dilation of capillaries in the glomerulus and reduction of Bowman's space or atrophied glomerulus (Takashima and Hibiya, 1995). Similar alterations were found in fishes exposed to organic contaminants (Veiga *et al.*, 2002) and mixed environmental contaminants (Schwaiger *et al.*, 1997; Pacheco and Santos, 2002). These reports suggested that the histopathological changes in the kidney, like in gills, could not be considered specific to the stressors. In the present study, kidney of the fish often showed congestion, hyaline degeneration, characterized by the presence of large eosinophilic granules inside the cells and between renal tubules. These granules may be formed inside the cells or by the reabsorption of plasma proteins lost in the urine, indicating damage in the corpuscle (Hinton and Laurén, 1990; Takashima and Hibiya, 1995). In more severe cases, the degenerative process can lead to tissue necrosis (Takashima and Hibiya, 1995). The presence of tubule degeneration in the kidney of humic acid fed fish for long period in the present study indicated that the kidney suffered damage after fed humic acid supplemented diet which enhanced the establishment of necrosis in this organ.

The presence of histological alterations for each organ was evaluated using standardized assessment method described by Bernet *et al.* (1999) which allows the quantification of organ damage. Different indices were calculated which characterize a histology-based health status at different levels: indices of the organism (Tot-I) and organ (Iorg.). The present results indicated significance elevation in histopathological score for gills, kidney and liver which showed the highest score in tissue damage for the 360 mg humic acid fed fish.

### Conclusion:

According to the results of current study, it is concluded that although dietary supplementation with humic acid can slightly improve feed conversion ratio, and weight gain the histomorphological alterations indicated that feeding fish the higher dose of humic acid caused more potent destructive effect in the gill, liver and kidney tissues of *Cyprinus carpio*. Generally, the findings of the present histological investigations demonstrate a direct correlation between humic acid administration and histopathological disorders observed in several tissues depending on the given concentration.



**RECOMMENDATIONS:**

Future studies will be needed to test lower concentrations of HA in fish aquaculture

to determine if it can serve as safer alternative for antibiotics.

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## أثر استخدام حمض الدوبال (كمحفز للنمو) فى علائق أسماك المبروك العادي على النمو والتركيب النسيجي للخياشيم والكبد والكلية

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و 360 ملجم / كجم) احتقان داخل الصفائح الخيشومية وانفصال للطبقة المبطنية للخياشيم بالإضافة إلى استسقاء وكذلك زيادة فى عدد خلايا الصفائح الخيشومية واندماجها مع بعضها البعض. أشتمل الأثر فى الكبد على إضمحلال فى السيبتولازم والأنوية مع وجود ألياف وخلايا لمفاوية. أما التغيرات فى الكلية تميزت بوجود تحلل للخلايا الطلائية المبطنية لأجزاء من الأنبيبات البولية مع وجود ترسيبات داخلها. من خلال ما تقدم فأنه يتضح أن إضافة حمض الدوبال (HA) إلى العلائق السميكة بجرعات 180 ، 360 ملجم / كجم أدى إلى تحسين طفيف لمعايير كفاءة الاستفادة من الغذاء فى تغذية الأسماك بهذه العلائق، إلا إن الجرعة الأعلى (360 ملجم / كجم عليقة) كان لها تأثير ضار على أنسجة كل من الخياشيم والكبد والكلية لأسماك المبروك. ويوصى بإجراء مزيد من الدراسات لتحديد التركيز الأمثل من حمض الدوبال فى علائق أسماك المبروك والفترة الزمنية المناسبة لذلك حيث ثبت ضررها على الأعضاء الداخلية وبالتالى صحة الأسماك عامة.

### المحكمون:

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تعتبر المواد الدبالية نواتج طبيعية توجد فى كل مكان حيث انها تنتج من تحلل المواد العضوية الميتة ولقد تم اختيار حمض الدبال (HA) فى هذه الدراسة . قسمت إثنان وسبعون من أسماك المبروك إلى مجموعة ضابطة ومجموعتان من المعالجات الغذائية (1) و(2) . كل مجموعة من الثلاثة مجموعات احتوت على 24 من الأسماك. تم إجراء التجربة لمدة 10 أسابيع، حيث تم تجهيز جرعتان (180ملجم/كجم من عليقة و 360 ملجم / كجم) من حمض الدوبال (HA). أظهرت النتائج زيادة طفيفة ولكن معنوية ( $P<0.05$ ) فى وزن الجسم للأسماك المغذاة على عليقة(1) و(2) وكذلك المتوسط اليومي للوزن ومعدل النمو وذلك بالمقارنة بالمجموعة الضابطة . بعد إنتهاء الأسابيع العشرة وجد ان هناك زيادة معنوية فى قيم نسبة التحول الغذائى (FCR) من المجموعة (2) ثم المجموعة (1). وأيضاً أظهرت النتائج فى نهاية هذه الدراسة عدم وجود اختلاف معنوى فى معامل الحالة (K) بين مجموعات الأسماك . أظهر الفحص المجهرى الضوئى بعض التغيرات الظاهرية فى تركيب بعض الأعضاء الناتجة من أثر تناول حمض الدوبال (HA) على أنسجة الخياشيم والكبد والكلية. ومن التغيرات الشائعة فى الخياشيم والناتجة من استخدام الجرعتين(180ملجم/كجم