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## **CRACKED HEAD DISEASE IN AFRICAN CATFISH (*CLARIAS GARIEPINUS*) IN EGYPT**

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### ***Abstract***

Three hundred and sixty apparently healthy *Clarias gariepinus* fingerlings with an average of 6.04 g. were divided into two groups; group1 (Good water quality G1) & group2 (bad water quality G2) with (6 treatments: 0&25&50&75&100&200 mg/k.g. Ascorbic Acid AA) for each one, to be used for nonstressed treatments (G1) and the other as a stressed (G2). Each of the six dietary treatments was fed for 20 weeks in a glass aquarium with a fixed feeding regime of 4% body weight per day. In both groups *C. gariepinus* fed the basal diet and the diet supplemented with 25 mg AA/ kg diet showed signs of Cracked head disease. In both groups, Weights Gain (WG), Specific Growth Rates (SGR) Feed Conversion Rates (FCR) and Feed Efficiency Ratio (FER) were significant between control group and those with varying levels of vitamin C. The vertebral collagen content & liver and plasma Ascorbic Acid concentration of G1 was significantly higher than that of G2. This is the first record to diagnosis of Cracked head disease in Egypt.

### **INTRODUCTION**

*Clarias gariepinus* was popular fresh water fish consumed in our country with production of 463.1 ton and nearly (4.34% of total fish production) in Egypt (General Authority for Fish Recourses Development 2008). Diseases of fish often occur as secondary infections following stress due to environmental disorder conditions such as poor water quality

and nutritional deficiency. African catfish *C. gariepinus* in commercial cultures often suffer from a disease of unknown etiology called (Broken-skull disease); the disease is characterized by destruction of accessory respiratory (arborescent) organs and an inflammation of the skull followed by a lateral skull fracture, parallel to the skull plate joints (Eya and megbenka 1990). Similar condition known as (Crack-head syndrome) has been reported in Asian catfish species *Clarias betrachus* and *Clarias macrocephalus* and has been associated with vitamin C deficiency (Awa and Alegbleye 1991). Vitamin C was shown to exert a powerful antioxidant effect on biological water soluble compartments representing an outstanding antioxidant in plasma. *C. gariepinus* fed Ascorbic Acid deficient diet showed poor growth and deficiency signs such as hemorrhages, tissues erosions on the edges of dorsal fins, operculum and pectoral spine (Eya and megbenka 1990). Poor water quality known to reduce vertebral collagen concentrations in catfish *Ictalurus punctatus*, potentially resulting in broken or deformed back bones (Usnlm 1995). Dip treatment in either 2.0 ppm potassium permanganate or 0.5 ppm formalin (with alcohol) for 30 days resulted in 65.0 and 41.3% full recovery in 7 days respectively (Awa and Alegbleye 1991). Thus the objective of this study was to study the occurrence and treatment of Cracked head disease of cultured *C. gariepinus*.

## MATERIAL AND METHODS

### A) - Experimental diets:

As shown in table (1), the basal experimental diets was formulated according to Eya and Mgbenka (1990), with commonly available ingredients and vitamin C-free premix to meet the predetermined 40% crude protein requirement of *C. gariepinus*. Six graded levels of vitamin C L-Ascorbic Acid, AA at 0, 25, 50, 75, 100 and 200 mg Kg<sup>-1</sup> diets were included in the basal diet. Source of ascorbic acid was ethyl cellulose

coated L-ascorbic acid (El Nasr pharmatherapeutical chemicals Co.Egypt).

### **B) -Experimental fish and feeding regime:**

It was performed according to Ibiyo *et al.* (2007), where *C. gariepinus* fingerlings with an average of 6.04 g. were obtained from Kafr El-Sheikh fish farms and conditioned for 2 weeks. After acclimatization fingerlings were divided into two groups: (Good water quality group G1) & (bad water quality group G2) then weighted into 36 glass aquaria (18 for each), with six treatments: 0&25&50&75&100&200mg/k.g.Ascorbic Acid AA to be used for nonstressed treatments (G1) and the other as a stressed (G2). The stress was achieved by leaving the water with only one siphoning each 7 days. Glasses scrubbed and drained at two days intervals to prevent microbial growth that could possibly alter the experiment in good water group. Each of the six dietary treatments was fed for 20 weeks. A fixed feeding regime of 4% body weight per day divided into two meals and given one in the morning and the other in the afternoon. The growth performance was calculated according to Ibiyo *et al.* (2007) where; Weight Gain (WG) =  $W_t - W_0$  & Specific growth rate (SGR) =  $(\ln W_t - \ln W_0) \times 100 \text{ t}^{-1}$  & Feed efficiency ratio (FER) =  $Wet \text{ WG in (g)} / \text{dry feed fed in (g)}$  & FCR=  $\text{dry weight gain (g)} / \text{Fish weight gain (g)}$ .

**Table (1):** A practical basal diet containing 40% crude protein was supplemented with six different levels of 1-ascorbic acid mg/kg diet.

Food ingredient	T1	T2	T3	T4	T5	T6
Fish Meal	23.5	23.5	23.5	23.5	23.5	23.5
Soya bean meal	49	49	49	49	49	49
YellowMaize	10	10	10	10	10	10
Vegetable oil	3.5	3.5	3.5	3.5	3.5	3.5
Rice Bran	8	8	8	8	8	8
Fish Oil	2	2	2	2	2	2
Vitamin Premix	1	1	1	1	1	1
Mineral Permixon	2	2	2	2	2	2
Cellulose	1	1	1	1	1	1
Ascorbic Acid mg/kg	0	25	50	75	100	200

T: Treatment, (WG) is weight gain, (Wt) and (W0) were final and initial fish weights, respectively and (t) the experimental period in days.

### C) - Collagen content of the vertebral column:

The collagen fraction of the vertebrae was isolated according to the method of Flanagan and Nichols (1962). One g. of ground vertebrae of *C. gariepinus* was extracted with 20 ml of 0.1 N NaOH for 16 hours at room temperature. The fraction extracted by this procedure to contain all of the alkaline-soluble cellular material. Therefore, this fraction was removed by vacuum filtration and the remaining bone chips were further extracted with continuous shaking in three washings of 10% sodium EDTA (pH 7.5) for a total of 48 hours. Such treatment removes 96 to 100% of the ash content. After demineralization, the remaining fraction was washed with water and acetone, and finally reextracted with a 1:1 mixture of ethanol-ether for 1 hour. Following removal of these solvents, the remaining in soluble collagen fraction was dried in a vacuum desiccator to constant weight.

**D) - Tissue and plasma Ascorbic Acid AA concentration:**

Vitamin C concentrations in liver tissue and plasma were determined by dinitrophenylhydrazine (NDPH) spectrophotometric method as described by Bai and Gatlin (1992).

**E) - Treatment trial using formalin and vitamin C for treatment of Cracked head disease:**

For 30-day treatment trial, the affected *C. gariepinus* were divided into three main groups; the first group was dipped in 0.5- ppm formalin alone according to Awa and Alegbeye (1991). The second group was supplemented with 200 mg ascorbic acid /kg diet according to Geogoria and Mohd (2001). The third group was a mixed treatment of both dipping and supplemental ascorbic acid diet.

**F) - The correlation between some environmental factors and Cracked head disease:**

Water quality parameters were measured in the water of aquaria of the experimental occurrence of Cracked head disease.

**G) - Statistical analysis:**

The data obtained from the trial was subjected to one-way ANOVA analysis of variance and T-test analysis of variance to test for effects of dietary treatments according to Zar (1984).

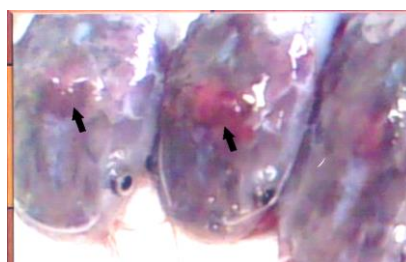
**RESULTS AND DISCUSSION****Clinical signs and postmortem changes:**

In both groups, *C. gariepinus* fed the basal diet (0 mg ascorbic acid /kg diet) and the diet supplemented with 25 mg ascorbic acid /kg diet showed classical signs of Cracked head disease as: an inflammation of the skull followed by a red band appeared on the mid-dorsal area of the skull (Figs.1&2). This was followed by inflammation around the band and after 4 or 5 day, a fissure appeared on the dorsal skull bone along the band

(Fig. 3). In addition, dark skin coloration, short opercula, eye, head and fin hemorrhages, skin and fin erosion, exophthalmia, swollen abdomen, and changes in the head bones (Fig. 4). These signs appeared to be similar to that reported by Awa and Alegbleye (1991) and Eya (1996). The incidence of Cracked head disease and mortalities as shown in (Table 2) was significantly higher in the bad water groups than those of the good water groups. The high mortality rates may be due to secondary bacterial infection of the poor healed Cracked head wound. Impaired healing response showed direct correlation to ascorbate level in the diet. This *C. gariepinus* behaves aggressively has no scales and has pectoral spines that can inflict wounds especially at high stocking densities. Fish given diets with ascorbic acid have more fibrous granulation tissue at the epidermal and dermal layers than fish fed diets without ascorbic acid. This was reported by Russell Ross and Earl (1962).



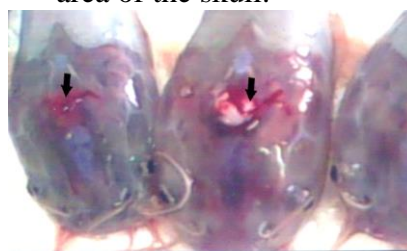
**Figure (1):** Showed *C. gariepinus* in which Cracked head diseases as head inflammation and skin hemorrhages.



**Figure (2):** Showed *C. gariepinus* in which Cracked head disease as a red band appeared on the mid-dorsal area of the skull.



**Figure (3):** Showed *C. gariepinus* in which Cracked head disease as a fissure appeared on the dorsal skull bone along the band with hemorrhages.



**Figure (4):** Showed *C. gariepinus* in which Cracked head diseasea hemorrhages on the edges of the dorsal fins, operculum, pectoral spine and snout

The data shown in (Tables 3-10), in both groups, (WG), (SGR) (FCR) and (FER) was significant ( $P \leq 0.05$ ) between the control group and those with varying levels of vitamin C in both groups. Average WG, SGR, FCR and FER increased significantly ( $P \leq 0.05$ ) with increasing dietary ascorbic acid level and tend to reach a plateau at 75mg AA kg<sup>-1</sup> & beyond which, a significant ( $P \leq 0.05$ ) decline. These results were in agreement with Ibiyo and Chuka (2006) but lower than that recorded by Ibiyo *et al.*, (2007).The good water group had a higher (WG), (SGR), (FCR) and (FER) than the bad water groups. Vitamin C increases the radical scavenging potential for hazardous oxidative agents, thus ensuring



physical integrity, normal growth and survival. Vitamin C also has known important role in resisting infection and enhancing immune responses. Bad water with harmful chemicals, heavy metals, trace elements etc. may suppress the immune system of fish leading to increased susceptibility to diseases, decreased production and increased mortality (Saxena and Saxena 2008).

#### **Collagen content of the vertebral column:**

As shown in table (11) the vertebral collagen content of good water groups were significantly ( $P \leq 0.05$ ) higher than that of the bad water groups. The vertebral collagen contents of fish fed 50mg ascorbic acid kg diet and above were higher than those of fish fed the 0 and 25 mg ascorbic acid kg diets, these results were similar to that reported by Eya (1996). Improper development of skull bony tissues, skeletal malformations common in ascorbic acid devoid or deficient fish caused by impaired collagen synthesis due to vit. C. deficiency, this was evidenced by the low vertebral collagen content of *C. gariepinus* in the treatments fed the diets low or devoid of ascorbic acid. The role of ascorbic acid in the hydroxylation of proline and lysine in collagen synthesis was that, L-ascorbic acid is involved in modulation of important enzymatic reaction such as metabolism of several amino acids which lead to formation of hydroxyproline and hydroxylysine which essential for normal functioning of osteoblast and fibroblast. These enzymes are typically are mono or dioynase that contain transition metal such as iron or cupper at their active sites and require L- ascorbic acid for optimum activity, this was reported by Eya (1996) and Halver (2002).

#### **Tissue and plasma Ascorbic Acid AA concentration:**

As shown in table (12), there were significant increase ( $P \leq 0.05$ ) in liver and plasma ascorbic acid concentration in fish fed the graded levels of vitamin C in good water groups than bad water group. The 50, 75 and 100 mg AA kg-1 diet were better than the 0 and 25 mg AA kg-1 diet. As

dietary vitamin C increased, ascorbic acid concentration in plasma and liver increased, this was in agreement with that reported by Mengh *et al.*, (1998).

### **Physiochemical parameter of water of the good water group and the bad water group:**

Physico-chemical characteristics of water can be summarized as follows; the water temperature values of ( $18 \pm 0.58$  °C) while ( $20 \pm .57$  °C) in the other groups, D.O values at good water groups were ( $6.00 \pm 0.32$  mg/l) and ( $4.5 \pm .026$  mg/l) in bad water groups, PH values of ( $7.10 \pm 0.23$ ) at the good water group and ( $8.73 \pm 0.14$ ) in the other groups & NH<sub>3</sub> values of ( $0.20 \pm 0.06$  mg/l) in the first groups and ( $1.63 \pm 0.08$  mg/l) in the other and Nitrite values of ( $0.02 \pm 0.008$  mg/l) at the first groups and ( $0.12 \pm 0.01$ mg/l) in the other one. Poor water quality was also likely to contribute to occurrence of Cracked head disease, this was in agreement with Brown and Nunez (1998) who reported that, bad water has led to increase in temperature, pH, hardness, nitrite, ammonia and total solids and reduce vertebral collagen concentrations in catfish, potentially resulting in broken or deformed bones in bad water quality.

Thus, the cause of Cracked head disease may be primary due to vitamin C deficiency indicated by decreased vertebral collagen content, liver & plasma ascorbic acid or secondary due to bad water quality of decreased D.O and increased temperature, pH, nitrite and ammonia level and it was indicated by early appearance in bad water groups, high mortality, poor growth parameters, low vertebral collagen content and serum ascorbic acid rates.

### **Treatment trial:**

A good result had been obtained in the mixed treatment with formalin dipping and diet containing 200 mg ascorbic acid/kg body weight treatment than moderately results obtained by dipping formalin

alone or diet supplementation, indicated by decreased mortality rate of 20% in the first one treatment than 40% mortality rate in the second and third ones. This was in agreement with Awa and Aleghbleye (1991).

**Table (2):** Incidence of Cracked head disease and mean mortality rates of African catfish *C. gariepinus* fed diets with graded levels of vitamin. C.

Dietary levels of ascorbic acid	Incidence of Cracked head disease				Mortality			
	Good water quality groups(G1)		Bad water quality groups (G2)		Good water quality groups (G1)		Bad water quality groups (G2)	
	No	%	No	%	No	%	No	%
0 mg	8	26.67	10	33.33	10	33.33	15	50
25 mg	5	16.76	7	23.33	9	30	14	46.67
50 mg	0	0	1	3.33	2	6.67	13	43.33
75 mg	0	0	1	3.33	2	6.67	14	46.67
100 mg	0	0	0	0	2	6.67	13	43.33
200 mg	0	0	0	0	2	6.67	14	46.67
$\chi^2$ values	30.26**		32.06**		19.73**		0.44 N.S.	

\*\* : There is highly significant differences at  $p \leq 0.05$ . N.S: Non Significant.

**Table (3):** Effects of graded levels of vitamin C (0 – 20 weeks) on weight gain (WG) of *C. gariepinus* fingerlings (G1).

weeks	T1	T2	T3	T4	T5	T6	Total
2 <sup>nd</sup> week	0.75±0.02 <sup>e</sup>	1.04±0.05 <sup>d</sup>	1.68±0.01 <sup>b</sup>	2.39±0.08 <sup>a</sup>	1.34±0.04 <sup>c</sup>	1.26±0.02 <sup>c</sup>	1.41±0.13
4 <sup>th</sup> week	0.79±0.03 <sup>d</sup>	1.29±0.15 <sup>c</sup>	2.06±0.19 <sup>b</sup>	2.52± 0.12 <sup>a</sup>	1.59±0.06 <sup>c</sup>	1.34±0.05 <sup>c</sup>	1.76±0.22
6 <sup>th</sup> week	0.83±0.03 <sup>d</sup>	1.61±0.11 <sup>c</sup>	2.53±0.37 <sup>b</sup>	4.79 ± 0.17 <sup>a</sup>	1.95±0.07 <sup>c</sup>	1.55±0.09 <sup>c</sup>	2.21±0.31
8 <sup>th</sup> week	0.87±0.03 <sup>d</sup>	1.89±0.11 <sup>c</sup>	3.19±0.37 <sup>b</sup>	6.19 ± 0.27 <sup>a</sup>	2.45±0.11 <sup>c</sup>	1.85±0.12 <sup>c</sup>	2.74±0.42
10 <sup>th</sup> week	0.92±0.03 <sup>e</sup>	2.12±0.12 <sup>d</sup>	3.87±0.36 <sup>b</sup>	7.72 ±0.36 <sup>a</sup>	2.99±0.05 <sup>c</sup>	2.21±0.22 <sup>d</sup>	3.31±0.53
12 <sup>th</sup> week	1.10±0.15 <sup>e</sup>	2.39±0.16 <sup>d</sup>	4.62±0.33 <sup>b</sup>	9.45 ± 0.40 <sup>a</sup>	3.67±0.07 <sup>c</sup>	2.61±0.56 <sup>d</sup>	3.97±0.66
14 <sup>th</sup> week	1.29±0.26 <sup>e</sup>	2.49±0.12 <sup>d</sup>	5.41±0.31 <sup>b</sup>	11.45±0.48 <sup>a</sup>	4.17±0.46 <sup>c</sup>	3.10±0.46 <sup>cd</sup>	4.65±0.81
16 <sup>th</sup> week	1.53±0.32 <sup>d</sup>	2.87±0.19 <sup>cd</sup>	6.32±0.3 <sup>9d</sup>	13.51±0.62 <sup>a</sup>	5.21±0.57 <sup>b</sup>	3.56±0.56 <sup>c</sup>	5.50±0.96
18 <sup>th</sup> week	1.87±0.34 <sup>d</sup>	3.11±0.16 <sup>cd</sup>	7.19±0.53 <sup>b</sup>	15.99±0.73 <sup>a</sup>	6.13±0.43 <sup>b</sup>	4.02±0.07 <sup>c</sup>	6.39±1.14
20 <sup>th</sup> week	2.00±0.29 <sup>d</sup>	3.40±0.21 <sup>cd</sup>	8.15±0.75 <sup>b</sup>	19.21±0.75 <sup>a</sup>	7.48±0.41 <sup>b</sup>	4.41±0.91 <sup>c</sup>	7.44±1.39

Means within the same column carrying different litters are similar at ( $P \leq 0.05$ ).

T. = Treatment.

**Table (4):** Effects of graded levels of vitamin C (0 – 20 weeks) on feed conversion rate (FCR) of *C. gariepinus* fingerlings (G1).

weeks	T1	T2	T3	T4	T5	T6	Total
2 <sup>nd</sup> week	4.03± 0.10 <sup>a</sup>	2.78±0.24 <sup>b</sup>	1.75±0.08 <sup>d</sup>	1.22±0.07 <sup>e</sup>	2.16±0.03 <sup>c</sup>	2.32±0.07 <sup>c</sup>	2.38±0.22
4 <sup>th</sup> week	4.29± 0.15 <sup>a</sup>	2.74±0.45 <sup>b</sup>	1.84±0.15 <sup>c</sup>	1.15±0.04 <sup>d</sup>	2.23±0.04 <sup>bc</sup>	2.62±0.11 <sup>b</sup>	2.48±0.25
6 <sup>th</sup> week	4.54± 0.13 <sup>a</sup>	2.52±0.19 <sup>b</sup>	1.93±0.23 <sup>c</sup>	1.20±0.03 <sup>d</sup>	2.21±0.042 <sup>bc</sup>	2.70±0.18 <sup>b</sup>	2.52±0.02
8 <sup>th</sup> week	4.70± 0.18 <sup>a</sup>	2.54±0.13 <sup>b</sup>	1.88±0.12 <sup>c</sup>	1.300±0.03 <sup>d</sup>	2.13± 0.05 <sup>c</sup>	2.67±0.16 <sup>b</sup>	2.54±0.26
10 <sup>th</sup> week	4.96± 0.19 <sup>a</sup>	2.690.11 <sup>b</sup>	1.94±0.09 <sup>c</sup>	1.43± 0.03 <sup>d</sup>	2.14± 0.03 <sup>c</sup>	2.66±0.19 <sup>b</sup>	2.74±0.28
12 <sup>th</sup> week	4.74± 0.53 <sup>a</sup>	2.82±0.13 <sup>b</sup>	2.02±0.10 <sup>bc</sup>	1.56± 0.03 <sup>c</sup>	2.20± 0.10 <sup>bc</sup>	2.68±0.23 <sup>b</sup>	2.67±0.26
14 <sup>th</sup> week	4.600±0.77 <sup>a</sup>	3.16±0.16 <sup>b</sup>	2.11±0.13 <sup>bc</sup>	1.67± 0.01 <sup>c</sup>	2.41± 0.37 <sup>bc</sup>	2.70±0.28 <sup>bc</sup>	2.78±0.26
16 <sup>th</sup> week	4.35± 0.89 <sup>a</sup>	3.20±0.12 <sup>ab</sup>	2.25± 0.18 <sup>b</sup>	1.81± 0.03 <sup>b</sup>	2.31± 0.28 <sup>b</sup>	2.76± 0.33 <sup>b</sup>	2.78±0.25
18 <sup>th</sup> week	3.89± 0.60 <sup>a</sup>	3.54± 0.23 <sup>a</sup>	2.41± 0.21 <sup>b</sup>	1.96± 0.02 <sup>b</sup>	2.34± 0.15 <sup>b</sup>	2.96±0.39 <sup>ab</sup>	2.85±0.20
20 <sup>th</sup> week	4.00± 0.40 <sup>a</sup>	3.54± 0.09 <sup>a</sup>	2.57±0.24 <sup>bc</sup>	2.02± 0.03 <sup>c</sup>	2.26± 0.10 <sup>c</sup>	3.16±0.46 <sup>ab</sup>	2.92±0.20

Means within the same column carrying different litters are similar at ( $P \leq 0.05$ ).

**Table (5):** Effects of graded levels of vitamin C (0 – 20 weeks) on feed efficiency rate (FER) of *C. gariepinus* fingerlings (G1).

week	T1	T2	T3	T4	T5	T6	Total
2 <sup>nd</sup> week	0.25±0.06 <sup>e</sup>	0.36±0.03 <sup>d</sup>	0.58±0.03 <sup>c</sup>	0.83±0.04 <sup>a</sup>	0.4±60.07 <sup>c</sup>	0.43±0.01 <sup>cd</sup>	0.49± 0.04
4 <sup>th</sup> week	0.23±0.09 <sup>d</sup>	0.38±0.05 <sup>c</sup>	0.55±0.04 <sup>b</sup>	0.87±0.03 <sup>a</sup>	0.50±0.05 <sup>bc</sup>	0.38± 0.02 <sup>c</sup>	0.49± 0.05
6 <sup>th</sup> week	0.22±0.06 <sup>d</sup>	0.40±0.03 <sup>c</sup>	0.53±0.06 <sup>b</sup>	0.84±0.03 <sup>a</sup>	0.46±0.07 <sup>bc</sup>	0.37± 0.02 <sup>c</sup>	0.47±0.05
8 <sup>th</sup> week	0.21±0.09 <sup>e</sup>	0.40±0.02 <sup>d</sup>	0.54±0.03 <sup>c</sup>	0.77±0.02 <sup>a</sup>	0.47± 0.01 <sup>c</sup>	0.38± 0.02 <sup>d</sup>	0.46± 0.04
10 <sup>th</sup> week	0.20±0.07 <sup>e</sup>	0.37±0.02 <sup>d</sup>	0.52± 0.2 <sup>b</sup>	0.70±0.01 <sup>a</sup>	0.47± 0.06 <sup>c</sup>	0.37±0.02 <sup>d</sup>	0.44± 0.04
12 <sup>th</sup> week	0.22±0.03 <sup>d</sup>	0.36±0.2 <sup>cd</sup>	0.66±0.14 <sup>a</sup>	0.64±0.01 <sup>ab</sup>	0.45±0.02 <sup>bc</sup>	0.38±0.03 <sup>cd</sup>	0.45± 0.04
14 <sup>th</sup> week	0.23±0.04 <sup>c</sup>	0.32±0.02 <sup>c</sup>	0.65±0.14 <sup>a</sup>	0.60±0.09 <sup>ab</sup>	0.43±0.06 <sup>abc</sup>	0.38± .04 <sup>bc</sup>	0.43± 0.04
16 <sup>th</sup> week	0.25±0.05 <sup>c</sup>	0.30±0.09 <sup>c</sup>	0.62±0.14 <sup>a</sup>	0.55±0.07 <sup>ab</sup>	0.45±0.05 <sup>abc</sup>	0.36±0.04 <sup>bc</sup>	0.42± 0.04
18 <sup>th</sup> week	0.27±0.04 <sup>c</sup>	0.30±0.07 <sup>c</sup>	0.42±0.04 <sup>ab</sup>	0.51±0.03 <sup>a</sup>	0.43± 0.03 <sup>ab</sup>	0.35±0.04 <sup>bc</sup>	0.38± 0.02
20 <sup>th</sup> week	0.25±0.02 <sup>d</sup>	0.28±0.07 <sup>d</sup>	0.40±0.04 <sup>bc</sup>	0.49± 0.09 <sup>a</sup>	0.44±0.01 <sup>ab</sup>	0.33±0.04 <sup>cd</sup>	0.37± 0.02

Means within the same column carrying different litters are similar at ( $P \leq 0.05$ ).

**Table (6):** Effects of graded levels of vitamin C (0 – 20 weeks) on specific growth rate (SGR) of *C. gariepinus* fingerlings (G1).

week	T1	T2	T3	T4	T5	T6	Total
2 <sup>nd</sup> week	0.33±0.02 <sup>d</sup>	0.50±0.04 <sup>c</sup>	0.75±0.03 <sup>b</sup>	1.03±0.05 <sup>a</sup>	0.60±0.03 <sup>c</sup>	0.59±0.01 <sup>c</sup>	0.63±0.05
4 <sup>th</sup> week	0.30±0.02 <sup>d</sup>	0.52±0.07 <sup>c</sup>	0.73±0.05 <sup>b</sup>	1.09±0.03 <sup>a</sup>	0.60±0.09 <sup>c</sup>	0.55±0.01 <sup>c</sup>	0.63±0.06
6 <sup>th</sup> week	0.28±0.03 <sup>c</sup>	0.55±0.04 <sup>b</sup>	0.69±0.08 <sup>b</sup>	1.04±0.02 <sup>a</sup>	0.61±0.01 <sup>b</sup>	0.68±0.14 <sup>b</sup>	0.64±0.06
8 <sup>th</sup> week	0.27±0.02 <sup>e</sup>	0.54±0.02 <sup>cd</sup>	0.72±0.06 <sup>b</sup>	0.97±0.02 <sup>a</sup>	0.62±0.01 <sup>c</sup>	0.51±0.03 <sup>d</sup>	0.61±0.05
10 <sup>th</sup> week	0.29±0.09 <sup>d</sup>	0.51±0.02 <sup>c</sup>	0.68±0.02 <sup>b</sup>	0.90±0.02 <sup>a</sup>	0.68±0.04 <sup>b</sup>	0.52±0.04 <sup>c</sup>	0.60±0.05
12 <sup>th</sup> week	0.31±0.04 <sup>d</sup>	0.53±0.05 <sup>c</sup>	0.66±0.03 <sup>b</sup>	0.83±0.01 <sup>a</sup>	0.61±0.03 <sup>bc</sup>	0.52±0.04 <sup>c</sup>	0.58±0.04
14 <sup>th</sup> week	0.32±0.06 <sup>d</sup>	0.47±0.02 <sup>c</sup>	0.63±0.03 <sup>b</sup>	0.78±0.01 <sup>a</sup>	0.58±0.07 <sup>bc</sup>	0.51±0.05 <sup>bc</sup>	0.55±0.04
16 <sup>th</sup> week	0.35±0.06 <sup>d</sup>	0.44±0.01 <sup>cd</sup>	0.61±0.05 <sup>ab</sup>	0.72±0.06 <sup>a</sup>	0.60±0.06 <sup>ab</sup>	0.50±0.05 <sup>bc</sup>	0.54±0.03
18 <sup>th</sup> week	0.37±0.05 <sup>c</sup>	0.41±0.09 <sup>c</sup>	0.57±0.04 <sup>ab</sup>	0.68±0.03 <sup>a</sup>	0.58±0.03 <sup>ab</sup>	0.49±0.06 <sup>bc</sup>	0.52±0.03
20 <sup>th</sup> week	0.36±0.03 <sup>d</sup>	0.40±0.09 <sup>d</sup>	0.54±0.05 <sup>bc</sup>	0.66±0.01 <sup>a</sup>	0.85±0.02 <sup>ab</sup>	0.45±0.06 <sup>cd</sup>	0.50±0.03

Means within the same column carrying different litters are similar at ( $P \leq 0.05$ ).

**Table (7):** Effects of graded levels of vitamin C (0 – 20 weeks) on body weight gain (BWG) of *C. gariepinus* fingerlings (G2).

week	T1	T2	T3	T4	T5	T6	Total
2 <sup>nd</sup> week	0.42±0.08 <sup>c</sup>	0.32±0.02 <sup>c</sup>	0.063±0.04 <sup>b</sup>	1.3±30.12 <sup>a</sup>	0.77±0.04 <sup>b</sup>	0.70±0.04 <sup>b</sup>	0.70±0.08
4 <sup>th</sup> week	0.45±0.08 <sup>d</sup>	0.35±0.02 <sup>d</sup>	0.73±0.03 <sup>bc</sup>	1.52±0.10 <sup>a</sup>	0.86±0.05 <sup>b</sup>	0.97±0.22 <sup>bc</sup>	0.82±0.10
6 <sup>th</sup> week	0.48±0.08 <sup>c</sup>	0.42±0.04 <sup>c</sup>	0.93±0.03 <sup>b</sup>	1.91±0.07 <sup>a</sup>	0.99±0.05 <sup>b</sup>	1.08±0.23 <sup>b</sup>	0.97±0.12
8 <sup>th</sup> week	0.54±0.06 <sup>c</sup>	0.51±0.05 <sup>c</sup>	1.22±0.06 <sup>b</sup>	2.53±0.03 <sup>a</sup>	1.20±0.05 <sup>b</sup>	1.30±0.26 <sup>b</sup>	1.22±0.17
10 <sup>th</sup> week	0.47±0.15 <sup>c</sup>	0.65±0.06 <sup>c</sup>	1.69±0.12 <sup>b</sup>	3.30±0.03 <sup>a</sup>	1.63±0.07 <sup>b</sup>	1.78±0.30 <sup>b</sup>	1.59±0.23
12 <sup>th</sup> week	0.70±0.04 <sup>c</sup>	0.70±0.01 <sup>c</sup>	2.25±0.23 <sup>b</sup>	3.46±0.33 <sup>a</sup>	2.00±0.22 <sup>b</sup>	2.41±0.37 <sup>b</sup>	1.92±0.25
14 <sup>th</sup> week	0.60±0.03 <sup>c</sup>	0.67±0.01 <sup>c</sup>	1.87±0.16 <sup>b</sup>	3.24±0.36 <sup>a</sup>	2.16±0.38 <sup>b</sup>	2.31±0.32 <sup>b</sup>	1.80±0.24
16 <sup>th</sup> week	0.54±0.06 <sup>c</sup>	0.61±0.02 <sup>c</sup>	1.52±0.05 <sup>b</sup>	1.96±0.46 <sup>a</sup>	1.85±0.27 <sup>b</sup>	1.17±0.28 <sup>b</sup>	1.61±0.22
18 <sup>th</sup> week	0.48±0.06 <sup>c</sup>	0.52±0.02 <sup>c</sup>	1.17±0.08 <sup>bc</sup>	2.79±0.47 <sup>a</sup>	1.55±0.19 <sup>b</sup>	1.78±0.10 <sup>b</sup>	1.38±0.21
20 <sup>th</sup> week	0.44±0.05 <sup>c</sup>	0.44±0.07 <sup>c</sup>	0.81±0.14 <sup>bc</sup>	2.62±0.53 <sup>a</sup>	1.26±0.08 <sup>b</sup>	1.33±0.04 <sup>b</sup>	1.15±0.20

Means within the same column carrying different litters are similar at ( $P \leq 0.05$ ).

**Table (8):** Effects of graded levels of vitamin C (0 – 20 weeks) on feed efficiency rate (FER) of *C. gariepinus* fingerlings (G2).

Week	T1	T2	T3	T4	T5	T6	Total
2 <sup>nd</sup> week	0.17±0.05 <sup>c</sup>	0.11±0.01 <sup>c</sup>	0.22±0.02 <sup>bc</sup>	0.45±0.05 <sup>a</sup>	0.26 ±0.09 <sup>b</sup>	0.23± 0.02 <sup>b</sup>	0.24±0.03
4 <sup>th</sup> week	0.15±0.02 <sup>d</sup>	0.12±.09 <sup>cd</sup>	0.23±0.02 <sup>bc</sup>	0.42±0.03 <sup>a</sup>	0.26± 0.09 <sup>b</sup>	0.30 ±0.07 <sup>b</sup>	0.24±0.03
6 <sup>th</sup> week	0.15±0.01 <sup>c</sup>	0.13±0.01 <sup>c</sup>	0.25 ±0.01 <sup>b</sup>	0.44±0.01 <sup>a</sup>	0.27±0.07 <sup>b</sup>	0.29± 0.05 <sup>b</sup>	0.26±0.03
8 <sup>th</sup> week	0.15±0.09 <sup>c</sup>	0.15±0.01 <sup>c</sup>	0.33 ±0.2 <sup>b</sup>	0.48±0.03 <sup>a</sup>	0.29 ±0.09 <sup>b</sup>	0.30± 0.04 <sup>b</sup>	0.29±0.03
10 <sup>th</sup> week	0.17±0.01 <sup>c</sup>	0.18±0.02 <sup>c</sup>	0.37± 0.02 <sup>b</sup>	0.50±0.03 <sup>a</sup>	0.35 ±0.02 <sup>b</sup>	0.36±0.04 <sup>b</sup>	0.32±0.02
12 <sup>th</sup> week	0.17±0.02 <sup>b</sup>	0.18±0.03 <sup>b</sup>	0.42± 0.03 <sup>a</sup>	0.43±0.04 <sup>a</sup>	0.36± 0.04 <sup>a</sup>	0.42± 0.02 <sup>a</sup>	0.33±0.03
14 <sup>th</sup> week	0.14±0.01 <sup>b</sup>	0.15±0.03 <sup>b</sup>	0.29 ±0.02 <sup>a</sup>	0.33±0.03 <sup>a</sup>	0.33± 0.05 <sup>a</sup>	0.34± 0.02 <sup>a</sup>	0.26±0.02
16 <sup>th</sup> week	0.11±.01 <sup>c</sup>	0.15±0.02 <sup>c</sup>	0.21± 0.09 <sup>b</sup>	0.26±0.02 <sup>a</sup>	0.25±0.02 <sup>ab</sup>	0.27± 0.06 <sup>a</sup>	0.21±0.02
18 <sup>th</sup> week	0.10±0.01 <sup>c</sup>	1.10±0.06 <sup>c</sup>	0.14±0.01 <sup>c</sup>	0.22±0.03 <sup>a</sup>	0.18±0.09 <sup>ab</sup>	0.20± 0.01 <sup>a</sup>	0.15 ±0.01
20 <sup>th</sup> week	0.08±0.07 <sup>d</sup>	0.09±0.03 <sup>d</sup>	0.09±0.02 <sup>cd</sup>	0.18±0.02 <sup>a</sup>	0.14±0.09 <sup>b</sup>	0.13±0.07 <sup>bc</sup>	0.11±0.10

Means within the same column carrying different litters are similar at ( $P \leq 0.05$ ).

**Table (9):** Effects of graded levels of vitamin C (0 – 20 weeks) on specific growth rate SGR of *C. gariepinus* fingerlings (G2).

Week	T1	T2	T3	T4	T5	T6	Total
2 <sup>nd</sup> week	0.21±0.03 <sup>cd</sup>	0.16±0.02 <sup>d</sup>	0.31±0.03 <sup>bc</sup>	0.61±0.06 <sup>a</sup>	0.37± 0.01 <sup>b</sup>	0.34 0.0 <sup>3b</sup>	0.33±0.04
4 <sup>th</sup> week	0.21±0.03 <sup>cd</sup>	0.17±0.01 <sup>d</sup>	0.33±0.01 <sup>bc</sup>	0.57±0.04 <sup>a</sup>	0.37±0.09 <sup>b</sup>	0.41±0.09 <sup>b</sup>	0.34±0.04
6 <sup>th</sup> week	0.21± 0.02 <sup>c</sup>	0.19±0.02 <sup>c</sup>	0.37± 0.02 <sup>b</sup>	0.59±0.01 <sup>a</sup>	0.37±0.01 <sup>b</sup>	0.40±0.07 <sup>b</sup>	0.36±0.03
8 <sup>th</sup> week	0.23 ±0.07 <sup>c</sup>	0.22±0.02 <sup>c</sup>	0.42±0.02 <sup>b</sup>	0.64±0.03 <sup>a</sup>	0.41±0.01 <sup>b</sup>	0.40±0.07 <sup>b</sup>	0.39±0.03
10 <sup>th</sup> week	0.22 ±0.06 <sup>c</sup>	0.25±0.02 <sup>c</sup>	0.50± 0.03 <sup>b</sup>	0.68±0.01 <sup>a</sup>	0.47±0.03 <sup>b</sup>	0.49±0.05 <sup>b</sup>	0.44±0.04
12 <sup>th</sup> week	0.25± 0.03 <sup>b</sup>	0.25±0.07 <sup>b</sup>	0.56± 0.04 <sup>a</sup>	0.58±0.05 <sup>a</sup>	0.50±0.04 <sup>a</sup>	0.57±0.03 <sup>a</sup>	0.45±.04
14 <sup>th</sup> week	0.20± 0.02 <sup>b</sup>	0.22±0.09 <sup>b</sup>	0.41± 0.03 <sup>a</sup>	0.46±0.04 <sup>a</sup>	0.45±0.06 <sup>a</sup>	0.46±0.02 <sup>a</sup>	0.37±0.03
16 <sup>th</sup> week	0.17± 0.02 <sup>c</sup>	0.19±0.06 <sup>c</sup>	0.30± 0.09 <sup>b</sup>	0.36±0.04 <sup>ab</sup>	0.34±0.03 <sup>ab</sup>	0.40±0.09 <sup>a</sup>	0.29±0.02
18 <sup>th</sup> week	0.14± 0.01 <sup>b</sup>	0.15±0.06 <sup>b</sup>	0.18± 0.03 <sup>b</sup>	0.30± 0.03 <sup>a</sup>	0.26± 0.02 <sup>a</sup>	0.29±0.01 <sup>a</sup>	0.22±0.02
20 <sup>th</sup> week	0.13±0.09 <sup>c</sup>	0.13±0.03 <sup>c</sup>	0.13±0.02 <sup>bc</sup>	0.26± 0.03 <sup>a</sup>	0.18±0.01 <sup>bc</sup>	0.20±0.01 <sup>b</sup>	0.17±0.01

Means within the same column carrying different litters are similar at ( $P \leq 0.05$ ).

**Table (10):** Effects of graded levels of vitamin C (0 – 20 weeks) on feed conversion rate (FCR) of *C. gariepinus* fingerlings (G2).

Week	T1	T2	T3	T4	T5	T6	Total
2 <sup>nd</sup> week	7.27± 1.27 <sup>a</sup>	9.11±0.83 <sup>a</sup>	6.62 ±0.40 <sup>b</sup>	2.28± 0.27 <sup>c</sup>	3.80±0.14 <sup>bc</sup>	4.26±0.47 <sup>bc</sup>	5.23±0.60
4 <sup>th</sup> week	7.26 ± 1.16 <sup>a</sup>	8.66±0.63 <sup>a</sup>	4.34± 0.27 <sup>b</sup>	2.39± 0.18 <sup>b</sup>	3.83± 0.13 <sup>b</sup>	3.69±0.76 <sup>b</sup>	5.03±0.57
6 <sup>th</sup> week	7.00 ± 0.80 <sup>a</sup>	7.63±0.76 <sup>a</sup>	3.90 ±0.19 <sup>b</sup>	2.17± 0.16 <sup>b</sup>	3.76± 0.11 <sup>b</sup>	3.69±0.61 <sup>b</sup>	4.69±0.51
8 <sup>th</sup> week	6.55± .34 <sup>a</sup>	6.78±0.60 <sup>a</sup>	3.27± 0.15 <sup>b</sup>	4.07±2.00 <sup>ab</sup>	3.47 ±0.12 <sup>b</sup>	3.40±0.42 <sup>b</sup>	4.59±0.47
10 <sup>th</sup> week	5.68 ± 036 <sup>a</sup>	5.73±0.49 <sup>a</sup>	5.72±0.16 <sup>bc</sup>	1.96±0.04 <sup>c</sup>	2.92± 0.17 <sup>b</sup>	3.19±0.09 <sup>b</sup>	3.70±0.37
12 <sup>th</sup> week	5.86± 0.68 <sup>a</sup>	5.68±0.09 <sup>a</sup>	2.42± 0.20 <sup>b</sup>	2.36±0.18 <sup>b</sup>	2.83 ±0.31 <sup>b</sup>	2.41±0.16 <sup>b</sup>	3.59±0.39
14 <sup>th</sup> week	8.10 ± 0.71 <sup>a</sup>	6.44±0.19 <sup>b</sup>	3.46 ± 0.24 <sup>c</sup>	3.05±0.24 <sup>c</sup>	3.19 ±0.50 <sup>c</sup>	2.98±0.14 <sup>c</sup>	4.54±0.50
16 <sup>th</sup> week	8.8±1 0.87 <sup>a</sup>	7.57 0.26 <sup>a</sup>	4.83 0.15 <sup>b</sup>	4.10 0.28 <sup>b</sup>	4.20 0.47 <sup>b</sup>	3.70 0.08 <sup>b</sup>	5.54±0.50
18 <sup>th</sup> week	10.43±0.97 <sup>a</sup>	9.40±0.33 <sup>a</sup>	6.92± 0.57 <sup>b</sup>	4.71±0.49 <sup>c</sup>	5.52±0.36 <sup>bc</sup>	5.01 0.26 <sup>c</sup>	7.00±0.57
20 <sup>th</sup> week	11.91±0.84 <sup>a</sup>	11.80±43 <sup>a</sup>	11.43±2.45 <sup>a</sup>	5.62±0.70 <sup>b</sup>	7.27±0.40 <sup>b</sup>	7.55±0.46 <sup>b</sup>	9.26±0.72

Means within the same column carrying different litters are similar at ( $P \leq 0.05$ ).

**Table (11):** Mean collagen content of the vertebrae of *C. gariepinus* fed graded levels of ascorbic acid for 20 wk.

Dietry A.A	Good water group (G1)	Bad water group (G2)	T-test
0 mg	30.53±0.92	16.74± 1.03	*
25 mg	33.46±1.35	21.72± 1.33	**
50 mg	61.43±1.85	21.01± 3.08	*
75 mg	64.67±1.90	22.21± 1.57	**
100 mg	60.18±1.31	21.24± 1.22	**
200 mg	60.08±2.44	21.46± 1.87	**

\* There is a significant difference at  $p \leq 0.05$ .

\*\*There is highly significant differences at  $p \leq 0.05$ .

**Table (12):** Mean liver ascorbates (mg/g) & mean plasma ascorbates (mg/ ml) of *C. gariepinus* fed different dietary levels of ascorbic acid for 20 wk.

Dietry levels of ascorbic acid	Tissue ascorbic acid content Liver (mg/gram)			Plasma ascorbate mg/ml		
	Good water quality groups (G1)	Bad water quality groups (G2)	T- test	Good water quality groups (G1)	Bad water quality groups (G2)	T-test
0 mg	3.56±0.5	3.37±0.78	*	1.63± 0.34	0.31± 0.07	*
25 mg	37.92±2.54	7.97± 0.73	**	10.8±1.47	3.05± 1.00	*
50 mg	60.19±6.77	26.04±3.01	*	13.95±1.29	5.4 ± 0.79	**
75mg	63.46±2.15	35.18±2.05	**	15.15±1.98	6.31± 0.69	*
100mg	58.42±1.37	30.58±0.73	**	13.55±1.35	5.96± 0.75	**
200mg	53.77±2.20	30.23±0.82	**	12.95±1.55	5.85± 0.54	*

\* There is a significant difference at  $p \leq 0.05$ .

\*\*There is highly significant differences at  $p \leq 0.05$ .

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## مرض الرأس المتشققة في القرموط الأفريقي (كلارياس جارينيس) المستزرع في مصر

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تم فحص عدد (360) سمكة من القرموط الأفريقي المستزرع (كلارياس جارينيس) من مزارع كفر الشيخ لتحديد أسباب حدوث مرض الرأس المتشققة. وقد تلخصت النتائج في الآتي:-

١- مرض الرأس المتشققة نوع من الأمراض الغير معروفة السبب، وأعراضه التهاب في منطقة الرأس يليه شق جانبي موازي. وبدراسة كيفية حدوث هذا المرض خلال تجربة من معاملات تدريجية لفيثامين (ج) فقد وجد حدوثه في نسبة ٢٥ مجم/ حمض اسكوربيك لكل كجم عليقة في المجموعة الأولى (المياه عالية الجودة) إما بالنسبة للمجموعة الثانية (المياه سيئة الجودة) فقد ظهرت في نسبة ٠، ٢٥، ٥٠ مجم اسكوربيك /كجم عليقة.

٢- يلاحظ من خلال هذه النتائج أن هذا المرض من الممكن أن يكون بسبب منع تكوين الكولاجين نتيجة لنقص فيتامين (ج) وقد استدل على ذلك خلال نقص نسبة الكولاجين في العمود الفقري ونقص فيتامين (ج) في بلازما الدم وأنسجة الكبد ، كذلك من الممكن نتيجة لتلوث الماء (سوء جودة المياه) وقد استدل على ذلك خلال ظهور هذا المرض في معاملة ٥٠ مجم اسكوربيك /كجم عليقة في المجموعة الثانية .

٣- نتيجة للعلاقة بين حدوث المرض وفيتامين (ج) فقد وجد أن ٧٥ مجم اسكوربيك /كجم عليقة نسبة كافية ولازمة لنمو الأسماك .

٤- بالنسبة لعلاج هذا المرض فقد وجد أن إضافة الفورمالين عند ٠.٥ في الماء إضافة وفيتامين (ج) مجم اسكوربيك /كجم عليقة عند ٢٠٠ معا يعطي نتائج جيدة.

٥- أوضحت النتائج أن هناك علاقة وثيقة بين وجود خلل في مقاييس جودة الماء (انخفاض نسبة الأكسجين الذائب في الماء ، ارتفاع نسبة الامونيا والنيريت والأس الهيدروجيني) و حدوث مرض الرأس المتشققة في القرموط الأفريقي المستزرع (كلارياس جاريبينس).