Egyptian J. Nutrition and Feeds (2002) 5 (2) :207-223

EFFECT OF DIETARY PROTEIN LEVEL AND COPPER SUPPLEMENTATION IN PERFORMANCE OF COMMON CARP FISH *C. CARPIO* L.

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SUMMARY

A total number of 180 common carp fry with an average initial weight of 2.5 g were divided into nine experimental groups, 20 fish each. Three protein levels (20.6, 25.7 and 31.5%) within each copper level (0, 50 and 100 mg copper/kg diet) were tested. Fish were individually weighed to the nearest 0.1 gm at the beginning of the experiment and biweekly intervals throughout the experimental period. Live body weight and daily weight gain of common carp fish increased significantly (P<0.01) as affected with increasing dietary protein level. Also, body weight of common carp increased significantly (P<0.01) as affected with copper supplementation to the diets. Average daily weight gain increased by 11.49 and 20.69%, respectively, in fish fed diets supplemented with 50 and 100 mg copper than those fed diets without copper supplementation. Live body weight of common carp fish significantly (P<0.01) affected with the interaction between dietary protein level and copper supplementation after 12 weeks of the experiment. Increasing dietary protein level improved the feed conversion. Copper supplementation to common carp fish diets improved feed conversion. Aspartate amino transferase (ASAT), blood urea-N and creatinine significantly (P<0.01) affected with dietary protein level, while alanine amino transferase (ALAT) and glucose insignificantly affected. ASAT, blood urea-N and creatinine significantly (P<0.01) affected with the supplementation of copper to the fish diet, while ALAT and glucose insignificantly affected. Blood urea-N and creatinine significantly (P<0.05 and 0.01, respectively) affected with the interaction between the dietary protein level and copper supplementation, while ASAT, ALAT and glucose insignificantly affected. Ether extract percentages of the fish were significantly (P<0.05) decreased with increasing the dietary protein level. The percentages of fish protein and moisture increased with the increasing the copper level in fish diets, while ether decreased. Copper accumulation in fish muscles or internal organs increased with increasing copper level in the fish diets. Fish fed the diets supplemented with copper showed that the gills exhibited congestion and edema, the liver revealed vacuolar degeneration of most hepatocytesand the kidney revealed congestion in the interlubular blood vessels, edema in the interstitial tissue and degeneration of renal tubules.

Keywords: *Copper supplementation; dietary protein; growth rate; feed conversion; blood components; copper residues.*

INTRODUCTION

Protein requirements vary based on the type of fish. However, protein is a key element required for good health and growth in all types of fish. For vigorous health growth, young fish require 50% or in their diets. As protein is more expensive than carbohydrates and fat, the amount of protein in the diet should be just enough for growth and tissue repair (Lovell, 1980). The effect of varying levels of dietary copper intake on several fish species have been studied (Ogino and Yang, 1980; Murai *et al.*, 1981; Knox *et al.*, 1982 and Ayyat *et al.*, 2000).

Copper is essential to the growth and development of aquaculture species. It also plays a frequent role in the supply of water that makes fish farming possible. Levels of copper in fresh water and salt water have been found generally low. The role of copper in small quantities is essential to fish life. Copper is a very important component and absolutely essential to the performance of the enzymes. Enzymes are critical to the development of bone tissue and the production of red blood cells. A copper deficiency would contribute to anemia. Conversely, scientists have observed that overly high presence of copper in natural waters, due to pollutants or produced experimentally, may badly damage gills, adversely affect the liver and kidneys of fish or cause some neurological damage (Murai *et al.*, 1981). Fish need small amounts of iron, iodine, magnesium, sodium, potassium, copper, and zinc. The dietary copper requirement of common carp was 3 mg/kg diet (Ogino and Yang, 198). Fish appear to be more tolerant of copper in the diet than of dissolved copper in the water (Friedman and Shibko, 1972). A basal diet containing 0.7 mg Cu/kg diet was reported to suppress growth in carp as compared to a control diet containing 3 mg Cu/kg diet (Ogino and Yang, 1980). Copper promotes iron absorption from the gastrointestinal system, is involved in the transport of iron from tissues into plasma (Sorensen, 1990). Copper toxicity has been experimentally produced in rainbow trout fed 730 mg cobber/kg diet for 24 weeks (Lanno *et al.*, 1985).

The objective of the present study was to investigate effects of dietary protein level and dietary copper supplementation on growth performance, feed efficiency, blood components, histological studies and body composition of common carp (*Cyprinus carpio L.*).

MATERIALS AND METHODS

Common carp fish were reared at the wet laboratory of the Department of Animal Production, Agriculture Faculty, Suez Canal University. The fish were stocked in nine aquaria (75 X 50 X 60 cm), 20 fish. Each aquarium was supplied with fresh water aerated tap water. Fish faces and residues were removed by siphoning by using a plastic tube. About of 70% of the water in each aquarium were daily replaced by aerated fresh tap water. Each aquarium was supplied with air pump to supply fish with oxygen. The fish of the first three aquaria were fed a low protein diet, other three groups were fed on a medium protein diet, while the last three groups were fed on a high protein diet (Table 1). Fish were fed two times daily at a feeding rate of 3% of the body weight. The total duration of the experimental feeding trial was 3 months. Within each dietary protein level, three fish groups were fed diets supplemented with copper sulfate to supply 0, 50 and 100 mg copper per kg diet. The fish were individually weighed to the nearest 0.1 gm at the beginning of the experiment

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Table (1) : **Ingredients and chemical composition of the experimental diets.**

Ingredients %	Dietary protein levels		
	Low	Medium	High
Fish meal	7.0	8.0	12.0
Soybean meal	14.0	28.0	40.0
Corn	21.0	14.0	8.0
Wheat bran	35.0	35.0	30.0
Alfalfa hay	20.0	12.0	7.0
Minerals mix.*	0.5	.0.5	0.5
Vitamin mix.**	1.5	1.5	1.5
Carboxymethyl cellulose	1.0	1.0	1.0
Chemical composition %			
Crude protein	20.6	25.7	31.5
Ether extract	3.1	2.8	2.7
Crude fiber	8.9	8.6	8.2
Gross energy (Kcal/Kg)[1]	3210.0	3348.0	3577.0

* Each one Kg of mineral mixture contained: Zinc 1.23g, manganese 930 mg, Iron 630 mg, Copper 105mg and selnium 2.1mg.

** Each one Kg of vitamin mixture contained: Vit. A 72000IU, Vit. B1 6 mg, Vit. B3 12000 IU, Vit. B6 9 mg, B12 0.06 mg, Vit E 60 mg, Vit. K 12 mg, Pantothonic acid 60 mg, Nicotinic acid 120 mg, Folic acid 6 mg, Biotin 0.3 mg and Choline chlorids 3mg.

1 Calculated according to NRC (1993).

and biweekly intervals throughout the experimental period. Feed conversion was calculated as the quantity of feed required to obtain one unit growth during the experimental period, according to Berger and Halver (1987). Blood samples were taken from the caudal vein from three fish in each group were randomly selected at the end of the experimental period. The blood samples were centrifuged at 3000 rpm for 20 min. to separate the serum. Serum transaminase enzymes (ASAT and ALAT), glucose, urea-N and creatinine were estimated in blood serum by colormetric methods using commercial kits (Boehringer Manmheim kit).

Chemical compositions of the experimental diets and body fish were determined in three fish in each treatment according to AOAC (1980). The lead content in the tissues and eggs were determined by using the atomic absorption spectrophotometric technique.

For histological studies, piece of gills, kidneys, and livers were fixed in Boun's fluid. Afterwards, they were transferred to 70% alcohol, dehydration by using an ascending series of ethyl alcohol, cleared in xylol, embedded in paraffin blocks and sectioned at thickness of 5-7μ. Sections were stained by Haematoxylin-Eosin according to Carleton *et al.* (1967). The slides were then examined microscopically and photographed.

The obtained data were statistical analyses as a 3 X 3 factorial experiment (Sendecor and Cochran, 1982). Differences between treatments were statistically tested by Duncan's multiple range test (Duncan, 1955).

RESULTS

Growth performance:

Live body weight of common carp fish increased significantly ($P<0.001$) as affected with increasing dietary protein level (Table 2). Average daily weight gain increased with 30.59 and 8.24%, respectively, in the fish fed the high protein diet (31.5% crude protein) and the medium protein diet (25.7% crude protein) than those fed the low protein diet (20.6%).

Live body weight of common carp increased significantly ($P<0.01$) as affected with copper supplementation in the diets (Table 2). Average daily gain weight increased with 11.49 and 20.69%, respectively, in the fish fed diets supplemented with 50 and 100 mg copper/kg diet than those fed diets without copper supplementation.

Results of Table 2, revealed a significant interactions among protein and copper levels tested on live body weight at the 12th week. However, the interaction of both factors had insignificant effect on this trait at the 6th week. Within each dietary protein level, copper supplementation increased body weight and daily gain (Table 2). The higher daily weight gain was obtained with the fish fed the high protein diet and supplemented with 100 and 50 mg copper/kg diet. When considering the value of daily weight gain of fish fed low protein diet without copper supplementation as 100%, daily gain weight of fish fed the high protein diet and supplemented with 100 or 50 mg copper were 160.26 and 144.87%, respectively.

Feed efficiency:

Daily feed intake increased with the same ratio of increasing body weight. Increasing dietary protein level improved the feed conversion during the whole experimental period (Table 3). The fish fed the high protein diet recorded the best feed conversion ratio during the whole experimental period (0–12 weeks).

Table (2): Average body weight (g) and daily body gain (g) of Common carp as affected by dietary protein level, copper supplementation and their interaction.

Items	Live body weight (g) at (weeks)			Daily body gain (g) at (weeks)		
	0	6	12	0-6	6-12	0-12
Dietary protein:						
Low protein	2.39±0.05	5.34±0.13a	9.56±0.17a	0.070	0.100	0.085
Medium protein	2.45±0.07	5.14±0.08a	10.16±0.13b	0.064	0.119	0.092
High protein	2.48±0.08	6.29±0.11b	11.77±0.20c	0.091	0.130	0.111
Significance	NS	***	***			
Dietary copper supplementation:						
0 mg Cu/kg	2.47±0.07	5.34±0.12a	9.74±0.15a	0.068	0.105	0.087
50 mg Cu/kg	2.48±0.07	5.82±0.12b	10.58±0.20b	0.080	0.113	0.097
100 mg Cu/kg	2.38±0.06	5.63±0.14a	11.18±0.23c	0.077	0.132	0.105
Significance	NS	**	***			
Interaction between dietary protein and copper levels:						
Low protein:						
0 mg Cu/kg	2.46±0.10	4.97±0.17	9.03±0.15f	0.060	0.097	0.078
50 mg Cu/kg	2.44±0.08	5.69±0.22	9.53±0.31ef	0.078	0.091	0.084
100 mg Cu/kg	2.29±0.09	5.35±0.27	10.06±0.31c	0.073	0.112	0.092
Medium protein:						
0 mg Cu/kg	2.45±0.11	5.03±0.13	9.69±0.14de	0.061	0.111	0.086
50 mg Cu/kg	2.52±0.14	5.36±0.18	10.14±0.20c	0.068	0.114	0.091
100 mg Cu/kg	2.39±0.11	5.06±0.08	10.62±0.25c	0.064	0.132	0.098
High protein:						
0 mg Cu/kg	2.51±0.15	5.97±0.21	10.42±0	0.082	0.106	0.094
50 mg Cu/kg	2.49±0.15	6.39±0.13	12.01±0	0.093	0.134	0.113
100 mg Cu/kg	2.46±0.13	6.53±0.18	12.94±0	0.097	0.153	0.125
Significance	NS	NS	**			

Means in the same column within each classification by the different litters were differ significantly (P<0.05).
NS = Not significance, ** P<0.01 and *** P<0.001.

Table (3) : Daily feed intake (g) and feed conversion (g food/g gain) of Common carp fish as affected by dietary protein, copper supplementation and their interaction.

Items	Daily feed intake at (week)			Feed conversion at (week)		
	0-6	6-12	0-12	0-6	6-12	0-12
Dietary protein:						
Low protein	0.116	0.224	0.170	1.657	2.240	2.000
Medium protein	0.114	0.230	0.172	1.781	1.933	1.870
High protein	0.132	0.271	0.202	1.451	2.085	1.820
Dietary copper supplementation:						
0 mg Cu/kg	0.117	0.226	0.172	1.721	2.152	1.977
50 mg Cu/kg	0.125	0.246	0.186	1.563	2.177	1.918
100 mg Cu/kg	0.120	0.252	0.186	1.558	1.909	1.771
Interaction between dietary protein and copper levels:						
Low protein:						
0 mg Cu/kg	0.111	0.210	0.161	1.850	2.165	2.064
50 mg Cu/kg	0.122	0.228	0.175	1.564	2.505	2.083
100 mg Cu/kg	0.115	0.231	0.173	1.575	2.063	1.880
Medium protein:						
0 mg Cu/kg	0.112	0.221	0.167	1.836	1.991	1.942
50 mg Cu/kg	0.118	0.233	0.176	1.735	2.044	1.934
100 mg Cu/kg	0.112	0.235	0.174	1.750	1.780	1.776
High protein:						
0 mg Cu/kg	0.127	0.246	0.187	1.549	2.321	1.989
50 mg Cu/kg	0.133	0.276	0.205	1.430	2.060	1.814
100 mg Cu/kg	0.135	0.292	0.214	1.392	1.908	1.712

Copper supplementation in common carp fish diets improved feed conversion during the whole experimental periods. Common carp fed the diets supplemented with 100 mg copper/kg diet recorded the best feed conversion during the whole experimental period (Table 3).

Within each dietary protein level, copper supplementation improved the feed conversion. Fish fed the high protein diet and supplemented with 100 mg copper/kg diet consumed lower feed to produce one unit of body gain (Table 3).

Blood components:

Blood urea-N, ASAT and creatinine significantly ($P<0.001$) affected by dietary protein level, while ALAT and glucose insignificantly affected (Table 4). Increasing the dietary protein level significantly increased the concentration of ASAT (as indicator of liver function), urea-N and creatinine (as indicator of kidney function).

Blood urea-N, ASAT and creatinine significantly ($P<0.01$ and 0.001) affected by the supplementation of copper in fish diet, while ALAT and glucose insignificantly affected (Table 4). Copper supplementation significantly increased the concentrations of urea-N and creatinine, while the activity of ASAT decreased.

Blood urea-N and creatinine significantly ($P<0.05$ and 0.01) affected by the interaction between the dietary protein level and copper supplementation, while ALST, ALAT and glucose insignificantly affected (Table 4). Within each dietary protein level, the copper supplementation increased the concentrations of urea-N and creatinine.

Body composition:

Fish body composition was not affected significantly by dietary protein level, except ether extract contents affected significantly ($P<0.01$). Ether extract percentages significantly ($P<0.05$) decreased with increasing dietary protein level. Fish body composition affected significantly with the dietary copper supplementation, except ash percentage. The percentages of protein and moisture increased with increasing copper level in fish diets, while ether decreased. Fish body composition did not affected significantly with the interaction between dietary protein level and copper supplementation (Table 5).

Copper residues in fish body:

Copper residues in fish muscles or internal organs did not affected significantly with dietary protein level or the interaction between dietary protein level and copper supplementation, while significantly ($P<0.01$) affected by the copper supplementation (Table 6). Copper accumulation in fish muscles or internal organs increased with increasing copper level in the fish diets.

Histological studies:

Fish fed diets supplemented with 50 mg copper/kg diet showed that the gills exhibited congestion and edema. The lumen of secondary lamellae was expands and infiltrated with erythrocytes (Figures 1, 2 and 3). Also, the fish fed 100 mg copper/kg diet showed focal hyperplasia of the secondary lamella together with congestion and infiltration of the primary lamellae. The gill arch exhibited edema and congestion with few erythrocytes. The interlamellar cells proliferated in excess filling the spaces between secondary lamella. The gills lamella has the shape of a club (Figures 4 and 5).

The liver of fish fed diets containing 50 mg copper/kg diet showed focal areas of vacuolar degeneration and other cells were apparently normal (Figures 6 and7). In fish fed diets containing 100 mg copper/kg diet, the liver revealed

Table (4) : Blood parameters of Common carp fish as affected by dietary protein, copper supplementation and their interaction.

Item	ASAT	ALAT	Glucose	Urea	Creatinine
	(U/l)	(U/l)	(g/100 ml)	(mg/100 ml)	(mg/100 ml)
Dietary protein:					
Low protein	57.7±1.5a	9.0±0.5	143.0±2.4	10.6±0.8a	7.5±0.3a
Medium protein	67.0±0.8b	10.6±0.5	145.9±4.0	13.3±1.3b	8.2±0.6b
High protein	64.3±1.4b	10.2±0.4	144.3±3.8	14.0±1.1b	8.9±0.6c
Significance	***	NS	NS	***	***
Dietary copper supplementation:					
0 mg Cu/kg	65.9±1.6a	9.6±0.6	148.6±3.8	9.4±0.5a	6.5±0.2a
50 mg Cu/kg	61.4±2.1b	10.2±0.6	143.3±3.3	12.1±0.4b	8.2±0.3b
100 mg Cu/kg	61.7±1.5b	10.0±0.3	141.3±2.8	16.3±0.9c	9.8±0.4c
Significance	**	NS	NS	***	***
Interaction between dietary protein and copper levels:					
Low protein:					
0 mg Cu/kg	61.3±3.0	8.3±0.9	147.3±2.8	7.8±0.6d	6.6±0.3d
50 mg Cu/kg	53.7±1.2	9.0±1.2	138.3±3.2	11.0±0.3bc	7.7±0.6c
100 mg Cu/kg	58.0±1.5	9.7±0.3	143.3±5.5	12.8±0.5b	8.1±0.1c
Medium protein:					
0 mg Cu/kg	67.7±1.2	9.7±1.3	148.3±8.1	9.5±0.7cd	6.1±0.1d
50 mg Cu/kg	66.7±1.9	11.3±0.9	150.0±5.7	12.4±0.6b	8.0±0.1c
100 mg Cu/kg	66.7±1.9	10.7±0.3	139.3±4.8	17.9±0.9a	10.4±0.3a
High protein:					
0 mg Cu/kg	68.7±2.0	10.7±0.3	150.0±7.6	10.9±0.1cb	6.7±0.3d
50 mg Cu/kg	64.0±1.5	10.3±0.9	141.7±7.3	12.9±0.9b	9.1±0.3b
100 mg Cu/kg	60.3±0.7	9.7±0.9	141.3±5.9	18.1±0.8a	10.8±0.4a
Significance	NS	NS	NS	*	***

Means in the same column within each classification by the different litters were differ significantly (P<0.05).
NS = Not significance, * P<0.05, ** P<0.01 and *** P<0.001.

Table (5) : Body composition (%) of Common carp fish as affected by dietary protein, copper supplementation and their interaction.

Items	Moisture	Protein[1]	Ether extract[1]	Ash[1]
Dietary protein:				
Low protein	77.07±0.24	66.08±0.27	12.90±0.24a	15.37±0.26
Medium protein	77.93±0.24	66.31±0.23	11.89±0.24b	15.04±0.07
High protein	77.30±0.43	65.86±0.22	11.46±0.14c	15.18±0.12
Significance	NS	NS	***	NS
Dietary copper supplementation:				
0 mg Cu/kg	76.66±0.31a	65.35±0.17a	12.77±0.29a	15.31±0.13
50 mg Cu/kg	77.43±0.22b	66.30±0.18b	11.90±0.25b	15.20±0.18
100 mg Cu/kg	78.21±0.23c	66.60±0.12b	11.58±0.19b	15.08±0.21
Significance	**	***	***	NS
Interaction between dietary protein and copper levels:				
Low protein:				
0 mg Cu/kg	76.47±0.32	65.20±0.40	13.67±0.33	15.63±0.22
50 mg Cu/kg	76.87±0.20	66.40±0.26	12.77±0.26	15.40±0.55
100 mg Cu/kg	77.87±0.20	66.63±0.22	12.27±0.15	15.07±0.64
Medium protein:				
0 mg Cu/kg	77.20±0.40	65.57±0.32	12.80±0.15	15.07±0.12
50 mg Cu/kg	78.13±0.19	66.73±0.12	11.57±0.18	15.00±0.15
100 mg Cu/kg	78.45±0.28	66.63±0.30	11.31±0.17	15.07±0.12
High protein:				
0 mg Cu/kg	76.30±0.82	65.28±0.23	11.83±0.12	15.23±0.20
50 mg Cu/kg	77.30±0.32	65.77±0.26	11.37±0.23	15.20±0.21
100 mg Cu/kg	78.30±0.67	66.53±0.20	11.17±0.22	15.10±0.31
Significance	NS	NS	NS	NS

1, Calculated as dry matter base.
Means in the same column within each classification having different litters differ significantly ($P<0.05$).
NS = Not significance, ** $P<0.01$ and *** $P<0.001$.

Table (6) : Copper residues (ppm in dry matter bases) in muscle and internal organs of Common carp fish as affected by dietary protein, copper supplementation and their interaction.

Items	Muscle	Internal organs
Dietary protein:		
Low protein	11.579±0.904	14.654±1.094
Medium protein	12.389±1.141	14.948±1.078
High protein	12.500±1.115	15.891±0.979
Significance	NS	NS
Dietary copper supplementation:		
0 mg Cu/kg	8.664±0.256a	11.758±0.372a
50 mg Cu/kg	12.031±0.284b	15.064±0.430b
100 mg Cu/kg	15.772±0.345c	18.671±0.331c
Significance	***	***
Interaction between dietary protein and copper levels:		
Low protein:		
0 mg Cu/kg	8.543±0.638	11.280±0.689
50 mg Cu/kg	11.610±0.381	14.093±0.271
100 mg Cu/kg	14.583±0.363	18.590±0.459
Medium protein:		
0 mg Cu/kg	8.733±0.421	11.510±0.789
50 mg Cu/kg	11.983±0.606	14.833±0.882
100 mg Cu/kg	16.450±0.275	18.500±0.577
High protein:		
0 mg Cu/kg	8.717±0.434	12.483±0.393
50 mg Cu/kg	12.500±0.507	16.267±0.377
100 mg Cu/kg	16.283±0.404	18.923±0.850
Significance	NS	NS

Means in the same column within each classification having the different litters were differ significantly ($P<0.05$).
NS = Not significance and *** $P<0.001$.

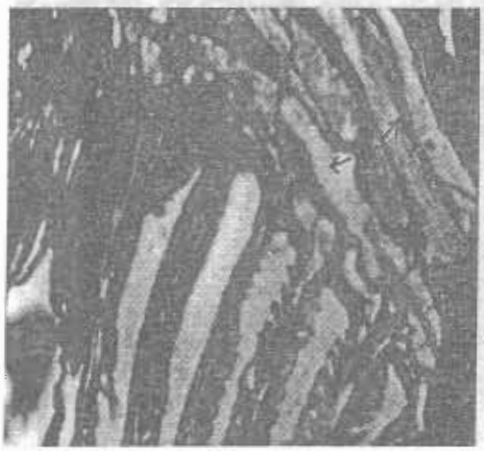

Fig.1: T.S. in gills supplemented with 50 mg Cu; (100x)

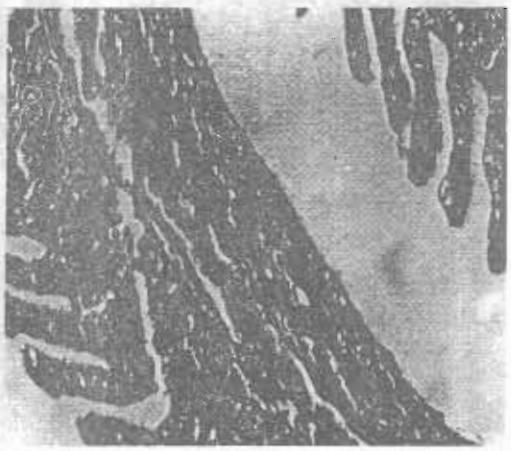

Fig.2: T.S. in gills supplemented with 50 mg Cu; (100x)

Fig.3: T.S. in gills supplemented with 100 mg Cu; (250x)

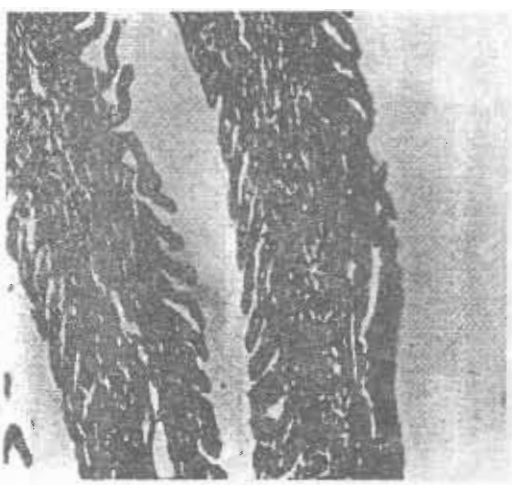

Fig.4: T.S. in gills supplemented with 100 mg Cu; (200x)

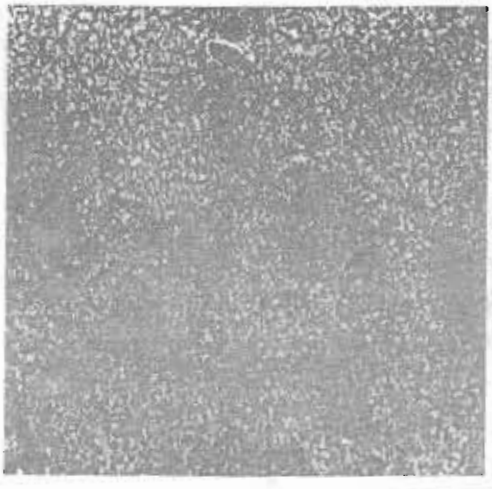

Fig.5: T.S. in liver supplemented with 50 mg Cu; (250x)

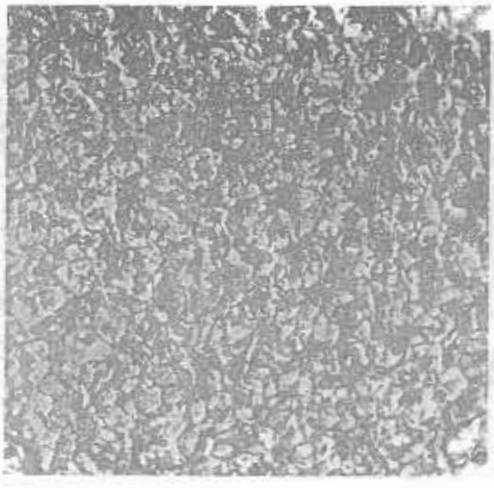

Fig.6: T.S. in liver supplemented with 100 mg Cu; (250x)

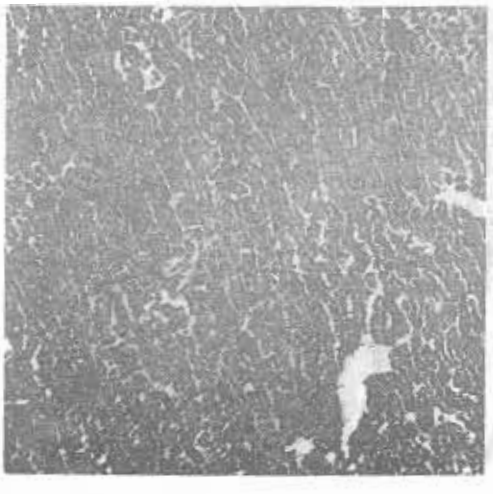

Fig.7: T.S. in kidney supplemented with 50 mg Cu; (250x)

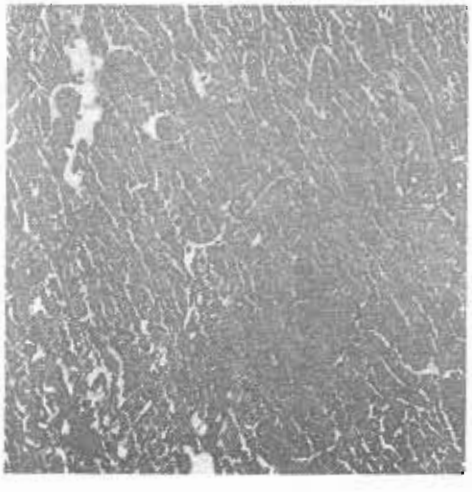

Fig.8: T.S. in kidney supplemented with 100 mg Cu; (250x)

vacuolar degeneration of most hepatocytes. It appeared as small to large vacuoles within the cells where the nucleus was pycnotic (condensation of the chromatin materials of the nucleus, Figure 8).

In fish fed diets supplemented with 50 mg copper per 1 kg diet, the kidney showed degeneration of renal tubules lumin (Figures 9 and 10). However, in 100 mg copper groups, the kidney revealed congestion in the interlubular blood vessels, edema in the interstitial tissue and degeneration of renal tubules (Figure 11).

DISCSSION

Fish fed the 31.5% crude protein diet recorded higher body weight and daily gain than those fed the diets with 25.7 and 20.6% crude protein. Lochman and Phillips (1994) found that weight gain was the best in fish fed on diets containing 28.9% protein than the diets contained 21.2, 25.3, 31.1, or 34.5% crude protein levels. However, Tidwell *et al.* (1992) reported that fish fed diet with 37% protein had significantly ($P<0.05$) higher body gain and specific growth rate than fish fed on diets with 26 and 31% protein levels. On the other hand, Watanabe *et al.* (1990) found that final body weight and body gain were higher for tilapia fish given diets with 28% crude protein than these fed diets with 32%. Fish fed the diet supplemented with 100 mg copper/kg diet recorded higher body weight and daily gain than those fed the diets supplemented with 50 or 0 mg copper/kg diet. Daily body weight gain also increased with increasing dietary protein level and copper supplementation in fish diets. The high copper level (100 mg copper/kg diet) was effective within medium and high dietary protein levels. The obtained results indicated that fish fed 35% protein diet and supplemented with 100 mg copper recorded higher growth rate. Similar results were reported by Ayyat *et al.* (2000). Mount *et al.* (1994) reported that dietary copper concentrations up to 350 mg copper/kg diet did not reduce fish body weight and survival or growth rate. Fish fed copper concentrations higher than 660 or 800 mg copper/kg diet showed about 30% mortality with no effect on body weight and growth rate. The copper requirement depends on the physiological state of the fish, copper concentration in the water and probably the level of elements that are metabolic antagonists of copper, such as iron, zinc, cadmium and molybdenum (Lall, 1989). Copper is currently classified as a trace mineral and is generally recognized as safe when added at levels consistent with good feeding practice (Ayyat *et al.*, 2000). Won *et al.* (1995) reported that lowering the dietary protein content from 32% to 28 and 24%, significantly improved ($P<0.05$) the protein efficiency ratio from 1.10 to 1.21 and 1.47. However, there were no differences in feed conversion ratio. In the present study, improvement in feed conversion rate was achieved when dietary protein increased ($P<0.05$). On the other hand, protein efficiency ratio increased as dietary protein decreased (El-Dahhar and Lovell. 1995).

Fish fed the high protein diet (31.5%) recorded the best feed conversion than the other dietary protein level (25.7 and 20.6%). Feed conversion improved with copper supplementation in fish diets. The best-feed conversion was obtained in the fish fed diets supplemented with 100 mg copper/kg diet. Fish fed the high protein diet and supplemented with 100 mg copper recorded the best feed conversion than the other fish groups. The obtained results are in agreement with those obtained with Steffens *et al.* (1988) and

Ayyat *et al.* (2000), while the opposite trend was reported with Murai *et al.* (1981) and Julshamn *et al.* (1988).

The results obtained from the blood components indicated that the kidney function (urea-N and creatinine) increased as affected by the increasing dietary protein, and the obtained results may indicate that the protein synthesis increased in fish fed the high protein diet. Copper level in fish diets recorded significant ($P<0.01$) increase of serum urea-N and creatinine and decreased in ASAT activity. Increasing the level of ASAT in the serum of fish fed the high protein diet or diet supplemented with copper may be related to the increase of protein synthesis in the liver. These results are in agreement with those obtained by McKim *et al.* (1970). Lauren and McDonald (1985) showed that copper exposure increased plasma levels of ammonia and glucose.

Carcass fat content (ether extract) decreased significantly with increasing the dietary protein level, while the other components did not affected significantly. Lochmann and Phillips (1994) and El-Dahhar and Lovell (1995) found that dietary protein content in the diet had no effect on the body composition. On the other hand, Mohantly and Samantaray (1996) reported that there was a significant increase in carcass protein and a significant decrease in ash content with progressive dietary protein content. Copper supplementation in fish diet significantly increased carcass protein and moisture and significantly decreased fat content.

The present results indicated that the copper accumulation in muscle or internal organs increased with increasing dietary copper level. The present results are in agreement with those reported with Daramola and Oladimeji (1989), Murai *et al.* (1981), Ogino and Yang (1980) and Ayyat *et al.* (2000). Wild and cultured salmonids accumulate exceptionally high copper level in the liver without being exposed to elevated waterborne or dietary copper (Poppe *et al.*, 1986). The copper accumulation in fish muscles was in the normal range. In other words, copper may used to stimulate the growth performance of common carp fish without any harmful effect on human which consumed the fish. This work cleared that the increasing of dietary protein level and the high copper level increased the growth rate and improved feed conversion ratio.

Despite of the high growth rate recorded as a result of high protein diet supplemented with 50 and 100 mg copper/kg diet, the microscopic study of gills, livers and kidneys showed changes in these organs. More histological changes were observed with the increasing copper supplementation in fish diets, such as hyperplasia of the secondary lamella, congestion and infiltration of the primary lamellae and increasing of interlamellar cells filling the spaces between the secondary lamellae of the hills. The kidney showed congestion in the interlubular blood vessels and edema in the interstitial tissues (Hibiya, 1982).

REFERENCES

AOAC. (1980). Official Methods of Analysis, 13th Edition. Association of Official Analytical Chemists, Virginia.

Ayyat, S.M.; F.A. Hafez.; S.M. Sharaf and F.S. Abbas (2000). Copper-protein nutrition of the Nile tilapia, Oreochromis niloticus. Egypt. J. Aquat. Biol. And Fish. 4: 313-335.

Berger A. and J.E. Halver (1987). Effect of dietary protein, lipid and

carbohydrate content on the growth, feed efficiency and carcass composition of striped bass (*Morone saxatitis*) fingerlings. Aquaculture, 18: 345-356.

Carleton, M.; Y. Drary.; E.A. Wallington, and H. Cammeron (1967). Carleton's histological technique, 4th ed., Oxford Univ. Press, New York, Toronto.

Daramola, J.A. and A.A. Oladimeji (1989). Accumulation of copper in *Clarias anguillaris* L. and *Oreochromis niloticus* L. Water, Air, and Soil Pollution, 48: 457-461.

Duncan, D.B. (1995). Multiple range and multiple F-test. Biometrics, 11:1-42.

El-Dahhar, A.A. and R.T. Lovell (1995). Effect of protein to energy ratio in purified diets on growth performance, feed utilization and body composition of mozambique tilapia, *Oreochromis mossambicus* (peters). Aquaculture Researche, 26:451-457.

Friedman L. and S.I. Shibko (1972). Nonnutrient components of the diet. In *Fish Nutrition*. Edited by Halver J.E. Academic Press, New York, pp. 182-255.

Hibiya T. (1982). An Atlas Of Fish Histology Normal And Pathological Features. Kodansha Ltd. Tokyo.

Julshamn, K.; K.J. Anderson; O. Ringdal and J. Brenna (1988). Effect of dietary copper on the hepatic concentration and subcellular distribution of copper and zinc in the rainbow trout *Samo gairdneri*. Aquaculture, 73:143-155.

Knox, D.; C.B. Cowey and J.W. Adron, (1982). Effects of dietary copper and copper: zinc ratio on rainbow trout, *Salom gairdneri*. Aquaculture, 27: 111-119.

Lall, S.P. (1989). The minerals. In Fish Nutrition. Edited by Halver J.E. 2nd Edition, Academic Press, INC. Harcourt Brace Jovanovich, Publishers, San Diego, New york, Boston, London, Sydney, Tokyo, Toronto, pp.219-257.

Lanno, R.P.; S.J. Slinger and J.W. Hilton (1985). Maximum tolerable and toxicity levels of dietary copper in rainbow trout *Salmo gairdneri* Richardson. Aquaculture, 49: 257-268.

Lauren, D.J. and D.G. McDonald, (1985). Effects of copper on brachial ionoregulation in the rainbow trout, *Salmo gairdneri* Richardson. Journal of Comparative Physiology B, 155:635.

Lochmann, R.T. and H. Phillips (1994). Dietary protein requirement of juvenile golden shiners *Notemigonus crysoleucas* and goldfish *Carassius auratus* in aquaria. Aquaculture, 128:277-285.

Lovell T.R. (1980). Nutrition and feeding. *Fish Farming Handbook, Food, Bait, Tropicals and Goldfish.* AVI Pub. Co. INC, Westport, Connecticut, USA.

Mckim, J.M.; G.M. Christensen and E.P. Hunt (1970). Changes in the blood of brook trout *Salvelinus fontinalis* after short-term and long-term exposure to copper. Journal of Fish Research Bd. Canada, 27:1883.

Mohantly, S.S. and K. Samantaray (1996). Effect of varying levels of dietary protein on the growth performance and feed conversion efficiency of snakehead *Chana strata* fry. Aquaculture Nutrition, 2: 89-94.

Mount, D.R.; A.K. Barth; T.D. Garrison; K.A. Barten and J.R. Hockett (1994). Dietary and waterborne exposure of rainbow *trout Oncorhynchus mykiss* to copper, cadmium, lead and zinc using a live diet. Environmental Toxicology and Chemistry, 13(12): 2031-2041.

Murai, T.; J.W. Andrews and R.G. Jr Smith, (1981). Effects of dietary copper on channel catfish. Aquaculture, 22:353-357.

NRC (1993). Nutrient Requirements of Fish. National Academy Press, Washington, D.C.

Ogino, C. and G.Y. Yang, G.Y. (1980). Requirements of carp and rainbow trout for dietary manganese and copper. Nippon Suisan, Gakkaishi, 46:455-458.

Poppe T.T.; Hastein A. Froslie; N. Koppang and G. Norheim G. (1986). Nutritional aspects of hemorrhagic syndrome in farmed Atlantic salmon *Salmo salar*. Dis Aquat. Organisms 1: 153-162.

Snedecor, G.W. and G.W. Cochran (1982). Statistical methods. 6th edition. The Iowa State University, Press Ames, USA.

Sorensen, E.M. (1990). Metal Poisoning in Fish. CRC Press, Inc, Boca Raton, Ann Arbor, Boston, USA, p. 234-284.

Steffens, W.; B. Rennert; H. Gurtler; E. Renner; G. Hilgenfeld and R. Stolze (1988). Improving feed efficiency by adding mineral supplements to dry feed mixture for carp. Zeitschrift fur die Binnenfischerei der (DDR), 35(5):171-174.

Tidwell, J.H.; C.D. Webster and J.A. Clark (1992). Growth, feed conversion and protein utilization of female green sunfish X male bluegill hybrids fed isocaloric diets with different protrin levels. Progressive Fish Culturist, 54:234-239.

Watanabe, W.O.; J.H. Clark; J.B. Dunham; R.I. Wicklund, and B.L. Olla (1990). Culture of Florida tilapia in marine cages. The effect of stocking density and dietary protein on growth. Aquaculture, 92:123-134.

Won, T.H.; I.K. Han and K.S. Chu, (1995). Effects of dietary energy, protein level and feed processing method on the growth rate, digestibility, body composition and pollution load of Israeli carp fed low protein diet. Korean Journal of Animal Nutrition and Feed Stuffs, 19:1-11.

تأثير مستويات من البروتين فى العليقة مع اضافة النحاس على مظاهر النمـــو فـــى أسماك المبروك

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تمت الدراسة على ١٨٠سمكة من يرقات أسماك المبروك العادى (متوسط الوزن فى بدايـــة التجربـــة ٢,٥جم) وقسمت الى ٩ مجاميع، ٢٠ سمكة فى كل مجموعة على التوالى. تم اختبار ثلاث مستويات من البروتين ٢٠,٦و ٢٥,٧ و ٣١,٥% مع ثلاث مستويات من النحاس (صفر و٥٠ و ١٠٠مجم/كجم عليقـــة). الاســـماك تـــم وزنها فى بداية التجربة و كل اسبوعين خلال فترة التجربة. لوحظ زيادة وزن الجسم الحى وزيادة الوزن اليومي لاسماك المبروك معنويا (P < ٠,٠١) مع زيادة مستويات البروتين فى العليقة. كذلك زاد وزن الجسم معنويا(P < ٠,٠١) تحت تأثير اضافة النحاس فى العليقة. متوسط زيادة الوزن اليـــومى زادت ١١,٤٩ و٢٠,٦٩% علـــى التوالى فى الاسماك التى تغذت على علائق مضافا اليها ٥٠ ، ١٠٠ مجم نحاس مقارنة بالاسماك التى تغذت على علائق غير مضاف لها النحاس. تأثر وزن الجسم الحى لاسماك المبروك معنويا (P < ٠,٠١) مع التداخل بـــين مستوى البروتين فى العليقة و اضافة النحاس بعد ١٢ أسبوع من بداية التجربة. زيادة مستوى البروتين فى العليقة عمل على تحسين معامل التحويل الغذائى، كذلك اضافة النحاس فى العلائق أعطى معامل تحويل غذائى أفضل. و بدراسة مستوى البروتين على الدم، تأثر كل من AST، اليوريا والكرياتينين معنويا (P < ٠,٠١) بينمـــا ALT والجلوكوز كان تأثيرهم غير معنوى. كان تأثير AST، اليوريا و الكريـــاتينين معنويـــا (P < ٠,٠١) باضـــافة النحاس للعليقة، بينما AST والجلوكوز كان تأثيرهم غير معنوى. مع التداخل بين مستوى البروتين فـــى العليقـــة واضافة النحاس كان تأثير اليوريا و الكرياتينين معنويا (P < ٠,٠٥ ، (P < ٠,٠١ على التوالى، بينمـــا AST والجلوكوز كان تأثيرهم غير معنويا. قلت النسبة المئوية لمستوى الدهن معنويا (P < ٠,٠٥) مع زيادة مستوى البروتين فى العليقة. زادت النسبة المئوية للبروتين والرطوبة مع زيادة مستوى النحاس فى العليقة بينمـــا نقـــص لمستوى الدهن. كذلك زاد تراكم النحاس فى عضلات الاسماك أو الاعضاء الداخلية بزيادة مستوى النحـــاس فـــى علائق الاسماك. وبدراسة تأثير اضافة النحاس للعليقة على الخياشيم والكلية والكبد فقد لوحظ احتقان فى الشعيرات الدموية واستسقاء فى الخياشيم، كذلك ظهر احتقان وتحلل لبعض خلايا الكبد، أما فى الكلية فوجـــد احتقـــان فـــى الاوعية الدموية واستسقاء فى الانسجة البينية وتحلل فى الانابيب البولية.