COPPER CHELATING COMPLEX AS IMMUNSTIMULANT OF AFRICAN CATFISH (*CLARIAS GARIEPINUS*)

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ABSTRACT

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The present study was planned to investigate the effect of dietary supplementation with copper Chelating complex as immunstimulant of catfish *Claris gariepinus*. Forty acclimated African sharp tooth catfish, were divided into two groups. The first group (control group G I) received the basic diet, for 30 days. The second group received the basal diet mixed with 60 mg copper albumin chelating complex/kg basal diet; (G II). At the end of 30 days feeding, the fish of each group were, weight, blood film and serum sample were collected and tested. Fish of each group were challenged by local strain of *Aeromonas hydrophila,* for challenge. The clinical signs, P.M. lesions and mortalities were also monitored for 7 days post challenge. The obtained results were indicated that copper chelating complex improve the growth rate, and enhance the protective effect against challenge with *A. hydrophila* and potentiate the non-specific immune response through increase the percent of basophile, the lymphocytes and increase the total protein and the globulin in the blood of the treated fish. In conclusion use of copper chelating complex as feed additive for Clariasgariepinus at a dose of 60mg / kg diet has shown to be an activator of non-specificimmune response.

Keywords: *Immunstimulant, Copper chelating complex, African catfish, Clarias gariepinus.*

INTRODUCTION

During the last decade there was an increasing interest in the modulation of the nonspecific immune response of fish to elevate the general defense barriers and hence increase resistance against diseases through use of immunostimulants (Raa, 2000: and Sahoo and Mukherjee, 2002). Immunostimulants represents an emerging class of chemicals that are designed to amplify immune responses against infectious diseases and tumor cells. The field of essential metalloelement complexes has attracted many authors in the last decade. El-Ashmawy, *et al.* (2007) and Saad *et al.* (2013) concluded that, fish supplemented with copper (I) nicotinate complex (either 30 or 60 mg/kg feed) showed a significant increase in the percentages of most hematological and biochemical parameters. They also aded that both concentrations of copper (1) nicotinate complex had no adverse effect on liver functions, induced immunostimulant effect in tested Big head carp fish and increased the relative level of protection agains injected virulent strain of *Yersinia ruckeri.* Al-MullaHummadi *et al.* (2005 and 2006) reported that, an organic complex of copper chloride, ascorbic acid and nicotinamide has an immunomodulating effect. Copper (I) nicotinate complex reduced the adverse effects of 5-fluorouracil on patients with hepatocellular carcinoma and enhanced defense mechanisms against oxidative stress (El-Saadani, 2004) and produced antiulcerogenic activity in rates (El-Saadani *et al.,* 1993). The medical benefitsparticularly the immunostimulant effect of the different coppercomplexes were previously reported by Franklinand Richardson, (1980), El-Ashmawy *et al.* (2007), Eduardo *et al.* (2009), and Saad et al. (2013).Therefore, the present study was planned to investigate the effect of dietary supplementation with copper Chelating complex as immunstimulant of catfish *Clariasgariepinus*.

MATERIALS and METHODS

Fish:

Forty African sharptooth catfish with a range of body weight 100-120 g, and length 20- 25 cm were obtained from a private fish farm at Assiut Governorate and transported alive to laboratory at the farm of the Faculty of Agriculture, Assiut University, Assiut, Egypt. Diets and feed additives were formulated to be used for feeding of fish. A basic diet (control) was formulated of grounded yellow corn

(34.9%), soya bean meal (28.6%), fish meal (17.0%), wheat bran (9.3%), vegetable oils (6.5%), ground lime stone (0.70%), bone meal (0.30%), mineral mixture (1.7%) and vitamin mixture (1.0%), in the form of dry pellets, and the fish were fed with feeding rate 3% from body weight twice daily.

Copper chelating complex mixed diet:
Copper albumin chelating complex was obtained from Prof. Nassar--Dept of biochemistry, Faculty of Medicine, Assiut University, as patent cooperation treaty (PCT) in the international Bureau of World Intellectual Property Organization (WIPO), Geneva, Switzerland World Organization (WO) 2008 028497, and mixed with the basal diet as 60 mg/kg diet.

Aquaria
Fish were kept for 20 days in the recirculation system in the metallic tank for acclimation to the laboratory conditions, these Fish were to fully prepared aquaria for another 4 days for further acclimation.

Experimental design
The forty acclimated African catfish, *Clariasgariepinus*, were used to conduct this study in 2 groups. Fish were randomly divided into 2 groups (20 fish in each group) were used for evaluating the immune blood parameters of fish (blood set), and the challenge experiment. the first group (control group G I)received the basic diet, for 30 days, and the second group received the basal diet mixed with 60 mg copper albumin chelating complex/kg basal diet; (G II).

Sample collection
Blood smears
Blood samples were collected from the caudal vein of 5 fish of each group. Giemsa stained blood smears were prepared. Leucocytes were distinguished microscopically on each blood smear and a mean relative percent was calculated.

Serum
Blood-sera of the examined fish were collected and stored at -80 until used.

Total protein, albumin, globulin in the serum
Serum protein, albumin, and globulin were estimated spectrophotometrically by using kits, Qualigens Diagnostic, Division of Glaxo Smithkline Pharmaceutical Ltd.

a) **Total proteins:** Assay of total proteins was carried spectrophotometric at 540 nm, according to Beirut method. Total proteins were expressed in g/dl
b) **Albumin:** Serum samples from all experimental groups were estimated spectrophotometrically at 630nm
c) **Globulin:** Globulin was calculated by mathematical subtraction of albumin value from total proteins. Globulin was expressed in g/dl

Bacterial strain used for challenge
Aeromonase hydrophila, (a localized strain, Elkamel and Thune, 2003).

Bacterial strain was kept in Brain Heart gnfusion broth with 15% glycerol (EL- Gomhurrhia, Cairo, Egypt) at – 20C. The *A. hydrophila* strain was passed three times in Cat-fish through intraperitoneal injection before using for experimental challenge. Colony forming unit (CFU) counts in bacterial suspension were determined using spectrophotometry optical density values at wavelength 600 nm and standard-plate count method with tenfold serial-dilution.

Challenge test.
At the end of 30 days feeding, the fish of each group were injected intra peritoneal (I/P) with 0.5ml sterile saline containing (1.5×10^8cfu / ml) pathogenic strain of *A.hydrophila.* The clinical signs, P.M. lesions and mortalities were monitored for 7 days post challenge.

RESULTS

Table No 1 show the body weight of all fish in the control group as well as well as the treated group. There is no significant differences in the initial body weight (IBW) before treatment at the beginning of the experiment, while the final body weight (FBW), was significantly high in fish of the group (II)fed on copper albumin chelate mixed diet.

Table 1: The Mean Body weight (BW) in Grams of 20 *ClariasGariepinus*fed with copper chelating complex supplemented diet (GII) compared with the control group (GI) fed with basal diet.

Time / *Group*	Type of feed	Before treatment		After 30 days feeding	
		Mean BW	SEM	Mean BW	SEM
GI	Basal diet	123.650	2.028	156.750	2.496
GII	copper chelating complex supplemented diet	120.600	1.792	163.950	2.037

SEM: Slandered Error Mean

Differential leucocytes indicated the non-significant higher in the percent of lymphocytes in fish of the GII (67.600±1,680%) and 66.0±1,520%. in (GI), while the percent of Basophiles (4.00±1,049%) was two times higher than that in the control group (2.00±0,577%). No significant difference in the mean % of neutrophils, monocytes and eosinophil between the control group (GI) and the treated group (GII) as shown in Table. 2.

Table 2: Differential Leucocytes percent *ClariasGariepinus*30 days fed, copper chelating complex mixed diet (GII) compared with the control group fed on basal diet (GI).

Group	Lymphocytes		Monocytes		Neutrophils		Basophil		Eosinophil	
	Mean %	SEM	Mean %	SEM	Mean %	SEM	Mean %	SEM	Mean %	SEM
GI	66.000	1,520	18.00	3,512	12,00	2,082	2.000	0,577	2,000	0,577
GII	67.600	1,680	13,60	1,364	9,800	0,860	4.000	1,049	2,000	0,632

SEM: Slandered Error Mean

Results of the serum biochemistry were presented in Table 3 show a higher total protein in the serum collected from the fish (GII), (3,567± 0,239 g/dl) and GI (3,117± 0,4080g/dl) as well as a high serum globulin % in fish of GII (2,100g/dl) control group G I (1,698g/dl).

Table 3: Protein profile in serum of *Clariasgariepinus* groups fed on copper chelating complex mixed diet (GII) for 30 days compared with on fish fed on the basal diet (GI).

Group	GI		GII	
	Mean g/dl	SEM	Mean g/dl	SEM
Total proteins	3,117	0,408	3,567	0,239
Albumins	1,420	0,172	1,477	1,100
Globulins	1,698	0,268	2,100	0,287

Table 4: Mortality % of *Clariasgariepinus* fed with copper chelating complex mixed diet (GII) for 30 days compared with the fish fed with the basal diet (GI) and challenged with *A.hydrophila.*

Fish group	Total number	Number of dead fish	% of mortality
G I	20	7	35
G II	20	0	0

The results of artificial challenge with *A. hydrophila* presented in Table 4, showed initial mortality of infected fish beginning at 48 h after injection and resulting in a final mortality of 35% at 7 days in fish of the control group. Died fish manifested external signs associated with *A. hydrophila* infection including redness of the eyes, ecchymosis and exophthalmia in fish of the GI and there were no apparent clinical signs and no mortalities were observed in fish of the group II

DISCUSSION

The significant higher body weight were observed in fish of the group treated with copper chelating complex mixed diet than that fed on the basal diet in the control group this may attributed to that copper is an essential mineral required for proper bone growth and development as well as enzyme function (McDowell, 1992).

The increase in total protein and globulin of fish administered copper chelating complex mixed diet

may attributed to the role of copper in protein biosynthesis as it is vitally concerned in the growth process (Minganti *et al.,* 2010). In addition, copper is involved in the formation of disulphide linkage of collagen and elastin proteins (Shalaby *et al.,* 2006). Moreover, the significant increase of serum globulin may indicated the immunostimulant effect of copper chelating complex

The relationship between copper and host immunity was reported by Radostits *et al.* (2000). Copper deficiency inducealteration in humeral response (Prohaska and Failla, 1993; Radostits *et al.,* 2000; and Gatesoupe 2007). Moreover, the increase of serum globulin may indicated the immunostimulant effect of copper chelating complex.

Copper chelating complex may stimulates the haemopiotic tissues and subsequently led to production of extensive number of effective functional cells like lymphocytes and basophiles as a defense mechanism (El-Ashmawy, 2007). On the other hand the *Clariasgariepinus* fed on diet supplemented with copper chelating complex show 0% mortality within one week post challenge in comparison with 35% mortalities in the non-treated control group suggesting the Immunoptentiator effect of copper chelating complex. Such suggestion was supported by the high level of the serum total protein and globulin and it's increase of the leucocytes that reflected the protective effect against the infection.

It's concluded that, from the presented results, use of copper chelating complex as feed additive for *Clariasgariepinus* at a dosc of 60mg / kg of diet for one month has shown to be an activator of nonspecific immune response.

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معقد النحاس كمحفز للمناعة في سمك القرموط الافريقى (كلارس جاربينس)

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أجريت هذه الدراسة لتقييم كفاءة معقد النحاس مع الالبيومين كإضافات للأعلاف في تحسين الاستجابة المناعية ومقاومة الأمراض في سمك القرموط الافريقى. وتمت هذه الدراسة على ٤٠ سمكة وزنها من ١٠٠-١٢٠ جرام ، وطولها ٢٠-٢٥ سم من محافظة أسيوط نقلت حية إلى معمل إنتاج الأسماك مزرعة كلية الزراعة ، جامعة أسيوط وقسمت إلى مجموعتان بعد تركها للتأقلم كل مجموعة ٢٠ سمكة, تلقت المجموعة الأولى (الضابطة) عليقة أساسية، لمدة ٣٠ يوما. وتلقت المجموعة الثانية العليقة الأساسية مخلوطة بمعقد النحاس بمعدل ٦٠ مجم/كجم عليقة وتم وزن الأسماك قبل بداية التجربة وفي اليوم الثلاثين بعد التغذية ثم أخذت عينات الدم وتم عمل مسحات للفحص الميكرسكوبى وتحديد نسب خلايا الدم البيضاء في كل عينة وتم تجميع المصل لتقدير نسبة البروتين الكلى والالبيومين والجلوبيلين. ثم حقنت اسماك المجموعتين ببكتريا الايروموناس هيدروفيلا في الغشاء البريتونى ولوحظت الأسماك لمدة أسبوع بعد الحقن لتسجيل الأعراض ونسبة النفوق في كل مجموعة. وكانت النتائج التي حصلنا عليها هى وجود فرق معنوى بالزيادة في أوزان اسماك المجموعة المغذاة على عليقة تحتوى على معقد النحاس مقارنة بالمجموعة الضابطة ,كما لوحظان البروتين الكلى والجلوبيولين الموجود في مصل الدم لأسماك المجموعة المغذاة على عليقة تحتوى على معقد النحاس اعلي منه فى اسماك المجموعة الضابطة وكانت هناك زيادة في نسب خلايا الدم البيضاء في المجموعة المغذاة على المحفزات وخاصة الليمفوسيت والبازوفيل أما باقي الخلايا البيضاء فلا يوجد فرق معنوي يذكر بين المجموعتين كما لوحظ أن معدل الحماية ضد العدوى في اختبار تحدى المناعة في المجموعة المغذاة على عليقة تحتوى على معقد النحاس بلغت ١٠٠% ولم تسجل أية وفيات بعد الحقن مقارنة بنسبة نفوق بلغت ٣٥% في المجموعة الضابطة. ومن النتائج المقدمة نستخلص انه يمكن استخدام معقد النحاس في علائق اسماك القرموط الافريقى كمحفز.