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DIETARY ZINC REQUIREMENT OF COMMON CARP *(CYPRINUS CARPIO)* FRY

Mohamed M. Zenhom

Fish Nutrition Department, Central Laboratory for Aquaculture Research, Agriculture Research Center, Ministry of Agriculture, Egypt

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ABSTRACT

The present experiment was conducted to estimate the optimum requirement of dietary zinc (Zn) for Common carp, *(Cyprinus carpio)* fry with an average 1.0 ± 0.01g, by using purified diets (35% crude protein) supplemented with increasing zinc levels. The basal diet was supplemented with 5, 10, 15, 20, 25, 30 and 35 mg zinc sulfate mono hydrate/kg^1. The experiment was conducted in 21 aquariums with three replications per treatment, each aquarium supplied with well-aerated and dechlorinated tap water for 12 weeks.

The results revealed that the optimum fish live body weight and weight gain, were obtained at Zn levels from 20–30 mg/kg^1 diet. While the optimum specific growth rate, condition factor and Survival rate were obtained at Zn levels from 15–30 mg/kg^1diet. Feed consumed was significantly ($P < 0.05$) higher with fish fed Zn from 20-30 mg/kg^1diet ($P < 0.05$). Feed conversion ratio (FCR), Feed efficiency ratio (FER) and Protein efficiency ratio (PER) were significantly ($P < 0.05$) improved with fish fed Zn levels from 10-30 mg/kg^1diet. Dry matter and crude protein of fish body increased significantly ($P < 0.05$) with increasing Zn up to 30 mg/kg^1diet. Body fat was decreased significantly ($P < 0.05$) with increased Zn level, while ash appear no significant different. Fish fed dietary Zn levels up to 25 mg/$kg^{–1}$ was significantly increased Ca, P and Fe contents ($P < 0.05$), Zinc concentration in whole fish body was positively correlated with Zn levels in the diet.

It could be concluded that the common carp fry require 15-30 mg Zn/kg^{-1} dry diet to enhance the growth performance, feed utilization efficiency and body composition as well as minerals contents in fish muscle.

Key words: Common carp, zinc requirement, growth performance, feed utilization and minerals contents.

INTRODUCTION

Carp species are widely cultivated freshwater fish family in worldwide. They are second only to tilapia as the most widely farmed freshwater fish in Egypt (GAFRD, 2014). Carp species have become important species in fish culture systems because of their tolerance of wide differences in pond temperature and water quality, ease of management, reproduction, high growth rates and resistance to disease (Tapia and Zambrano, 2003). Common carp, *Cyprinus carpio*, is one of the most widely cultured carp species.

Zinc (Zn) is an important inorganic trace metal required for various physiological functions including growth performance, immune system and defense mechanism against free ions and radicals (Watanabe *et al*., 1997). It is an essential trace element required in more than 1000 structural, regulatory and catalytic proteins necessary for normal physiology, growth, reproduction and development in all fish species and animals (Eide, 2006; Maret & Krezel, 2007 and Zhang *et al* ., 2020).

Fish accumulate zinc from both water and dietary sources; however, ambient water-borne Zn concentration in most aquatic environments is not adequate to supply the requirements of fish and a diet-borne Zn concentration is mostly requested (Moazenzadeh *et al*., 2018).

Deficiency of zinc renders poor appetite, poor growth, less synthesis of hemoglobin, erythrocyte maturation, and anemia (Hazell, 1985 and Stickney, 1994). increased mortality, eye cataracts, short body dwarfism and low tissue Zn (Lall, 1989). Dietary Zn levels were found to affect not only the appetite, growth rate and mortality of the carp, but also the levels of Zn, Fe, Mg, Ca and P in the tissues (Ogino & Yang, 1979 and Musharraf & Khan, 2019). Broodstock diets low in zinc reduced egg production and hatchability (Takeuchi *et al*., 1981)

On the other hand, zinc may become toxic if accumulated at high levels (Abdel-Tawwab *et al*., 2013 and Elaiyaraja *et al*., 2018). And, because the presence of excessive levels of dietary Zn can disrupt transport and absorption systems, thus affecting the nutritional status of other metals including Fe, Cu and Cd, it is desirable to avoid unnecessarily high supplementation of Zn which also may limit the loading of minerals in the aquatic environment (Buentello *et al*., 2009). Accordingly, the diets offered to aquatic animals have to be contained a balance between fulfilling the Zn requirement and avoiding Zn deficiency and toxicity (Luo *et al*., 2011).

A number of studies have determined dietary zinc values requirement for Common carp in some age stage, the requirement values which obtained were 24 for Fingerling, 16 for Grower, 8 for Table fish and 25 mg/kg^{-1} for Brood fish (Csengeri and Majoros, 2004). Also Ogina & Yang, 1979, Satoh, 1991 and Elalakawy *et al*., 2017, found that, the requirement values were 15 to 30 mg Zn/kg^{-1} diets for common carp, Cyprinus *carpio*. In addition Tan *et al*. (2011) noted that, 48.7 mg/kg^{-1} diet for juvenile *Cyprinus carpio*. Liang *et al*. (2012) reported that Quantitative dietary Zn requirements for several species of freshwater fish were in ranged from 15 to 80 mg/kg^{1}.

Recently, nutrition of fish fry and fingerlings are being used complete artificial feeds to take the place of live food organisms, this complete artificial feeds must be made to provide all nutritional requirement of fish.

The present study was conducted to determine the dietary zinc requirement of Common carp fry.

MATERIALS AND METHODS

Fish experimental:

Common carp, *(Cyprinus carpio)* with an average 1.0 ± 0.01g was obtained from the fish hatchery, Central Laboratory for Aquaculture Research, Abbassa. They kept for 2 weeks in indoor tank as an acclimation period to the laboratory conditions. Fish fed on prepared diet containing 35% crude protein.

Feed preparation:

The experimental diet was prepared in the laboratory by using purified ingredients. Zinc (zinc sulfate monohydrate) was adding graded levels 5, 10, 15, 20, 25, 30 and 35 mg zinc/kg^{-1}. The ingredients of each diet were separately blended with additional 100 ml of water to make a paste. The pastes were separately passed through a grinder, and pelleted in a modified paste extruder to form the tested diets. The pellets were dried in a drying oven (Fisher oven 13 – 261 – 28A) at 85^{o}C for 24 hours and stored in plastic bags and finally kept in a refrigerator at -2^{o}C for further use. Experimental diets were formulated to meet the nutritional requirement of fish (NRC, 1993).

Feeding experiment:

After 15 days of acclimation period in the stock culture tanks, clinically healthy common carp, *C . carpio,* were divided into seven equal groups at a rate of 20 fish/tank (75 – 60 – 45cm in diameters). Each tank was filled with dechlorinated tap water devoid of zinc, supplied with continuous aeration via air-stones using aquarium air pumps and a natural photo-period. About half of the water was changed daily in all experimental aquaria. Fecal matters were siphoned out once daily. The biomass of fish in each tank was measured at the beginning of experiment and after each sampling; thereby the daily ration was adjusted. Fish were fed with their respective diets at the rate of 5% of their body weight per day for the period of the experiment. The daily ration was subdivided into three feeds.

Chemical analysis of diets and fish:

The tested diets and whole-fish body from each group at the beginning and at the end of the experiment will be analyzed for dry matter, crude protein ether extract ash and crude fibers, according to the methods of (AOAC, 1990 and NRC, 1993). Composition and proximate analysis of the experimental diet are shown in Table (1).

Table 1. Composition and proximate chemical analyses (% on dry matter basis) of the experimental diet containing 35% crude protein.

Ingredients	**%**
Casein	32
Egg white	9
Gelatin	8
Dextrin	21
Corn starch	12
L-cellulose	3
Corn oil	3
Fish oil	3
Vitamin mixture (1)	1
Zinc-free mineral mixture (2)	4
Carboxymethyl cellulose	2
Potasium phosphate	0.5
Calcium carbonate	1
Soddium carbonate	0.5
Chemical analysis (%)	
Dry matter	90.3
Crude protein	35.0
Ether extract	6.3
Ash	5.2
Crude fibers	49.0
Nitrogen free extract(NFE) (3)	4.5
Gross energy (GE)(Kcal/100g) (4)	458.415

(1) Each one kg of vitamin mixture contains: vitamin A 72000 IU; E 60 mg; B_1 6 mg; B_3 12000 IU; B_6 9 mg; B_{12} 0.06 mg; C 12 mg; Pantothenic acid 60 mg; Nicotinic acid 120 mg; Folic acid 6 mg; Biotin 0.3 mg; Choline chloride 3 mg.

(2) Each one kg of mineral mixture contains: magnesium sulphate 0.335 g; cuprous chloride 0.10 g; calcium phosphate monobasic 135.8 g; calcium lactate 327.0 g; ferric citrate 29.7 g; potassium phosphate dibasic anhydrous 239.8 g; sodium phosphate monobasic 87.2; sodium chloride 43.6 g; aluminium chloride anhydrous 0.15 g; potassium iodide 0.15 g; cobalt chloride 1.0 g; manganese sulphate, 0.05 g; sodium selenite 11 mg and L-cellulose 135.114 g.

(3) Nitrogen free extract (NFE) = 100 – (protein + lipid + ash + fiber)

(4) Gross energy (GE): Calculated according to NRC (1993) as 5.64, 9.44 and 4.11 Kcal/g for protein, lipid and carbohydrates, respectively.

Data calculation:

Fish growth and feed utilization parameters were calculated as follows:

Growth performance.

Weight gain = Final weight – initial weight.

Specific growth rate (%/day) = Ln final weight – Ln initial weight/time (days)×100.

Condition factor = weight / length 3 x 100.

Feed utilization.

Feed conversion ratio (FCR) = feed intake (g) / weight gain.

Feed efficiency ratio (FER) = body weight gain (g)/ feed intake(g) × 100.

Protein efficiency ratio (PER) = gain in weight (g)/ protein intake in feed (g).

Minerals contents.

Minerals Contents in whole fish were determined by atomic absorbance spectrophotometer Thermo 6600 (Thermo Electron Corporation, Cambridge, UK).

Statistical Analysis:

One- way analysis of variance was used to test the effects of the treatments on growth and feed utilization parameter. Duncan's Multiple Range Test (Duncan, 1955) was applied to compare the significance of the various parameters among the tested treatments.

RESULTS AND DISCUSSION

Results in (Table 2) indicated that, Common carp fry fed diet containing zinc level up to 30 mg/kg^{-1} exhibited the highest growth performance in terms of final weight (g/fish), weight gain (g/fish), final length, SGR (%/day), condition factor, and survival rate. These results indicate that, supplemental zinc could improve growth performance of Common carp fry, which is consistent with the results obtained on common carp (Ogino & Yang, 1979, Satoh, 1991, Tan *et al*., 2011 and Elalakawy *et al*., 2017), juvenile grass carp (liang *et al*., 2012) and fingerling Indian major carp (Msharraf and Khan, 2019). The lowest growth was obtained when Common carp fed basal diet 5 mg Zn/kg^{-1}. These results are in agreement with (Ogino & Yang, 1979 and Tan *et al*., 2011).

Fish fed diets in which different Zn levels had higher feed utilization efficiencies in terms of FCR, PER and FER to fish fed 10-30 mg Zn/kg^{-1} diet than that fed diet contain 5 and 35 mg Zn/kg^{-1} diet (Table 3). Also feed Intake was increased with increasing dietary zinc concentrations up to 30 mg/kg^{-1}diet. Feed utilization efficiency has close relationship with growth performance (He *et al*., 2009). In the present study (FCR, FER and PER) enhanced with increasing dietary zinc levels up to a certain point. A similar trend is found in Common carp (Tan *et al*., 2011 and Elalakawy *et al*., 2017), fingerling Indian major carp (Msharraf and Khan, 2019) and *Labeo rohita* juvenil Akram *et al*. (2019).

Table 2. Growth performance of Common carp *Cyprinus carpio* fed diets containing different levels of dietary Zn.

Items	Zn levels (mg kg^{-1} diet)						
	5mg	10mg	15 mg	20 mg	25 mg	30 mg	35 mg
Initial weight (g/fish)	1.03± 0.01	1.01± 0.01	0.98± 0.03	0.97± 0.01	1.0± 0.01	1.01± 0.04	1.0± 0.01
Final weight (g/fish)	24.34^{e} ±0.62	27.31^{cd} ±0.65	28.47^{b} ±0.59	29.94^{ab} ±0.28	30.91^{a} ±0.42	31.13^{a} ±0.43	26.79^{d} ±1.23
Weight gain[1] (g/fish)	23.31^{e} ±0.62	26.30^{cd} ±0.65	27.49^{bc} ±0.59	28.97^{ab} ±0.28	29.91^{a} ±0.42	30.12^{a} ±0.44	25.79^{d} ±0.24
Final length (cm)	12.12^{d} ±0.031	12.38^{c} ±0.043	12.53^{bc} ±0.036	12.70^{ab} ±0.041	12.90^{a} ±0.054	13.00^{a} ±0.055	12.50^{c} ±0.045
SGR[2] (%/day)	3.51^{c} ± 0.03	3.66^{b} ±0.03	3.74^{ab} ±0.02	3.81^{a} ±0.01	3.81^{a} ±0.02	3.81^{a} ±0.02	3.65^{b} ±0.09
Condition factor[3]	1.37^{b} ±0.02	1.44^{a} ±0.02	1.45^{a} ±0.02	1.46^{a} ±0.03	1.44^{a} ±0.01	1.42^{a} ±0.01	1.37^{b} ±0.02
Survival rate (%)	85.77^{c} ±0.50	88.2^{bc} ±0.92	94.5^{a} ±1.33	95.51^{a} ±1.32	96.22^{a} ±1.54	95.16^{a} ±0.73	90.55^{b} ±1.23

[a,b,c] Means with superscripts in the same row are significantly different at P<0.05.

Table 3. Feed utilization efficiency parameters of Common carp *Cyprinus carpio* fed formulated diets containing different levels of dietary Zn.

Items	Zn levels (mg kg^{-1} diet)						
	5mg	10mg	15 mg	20 mg	25 mg	30 mg	35 mg
Feed Intake (g /fish)	43.13^{d} ±1.15	45.18^{cd} ±1.11	46.33^{bcd} ±0.99	48.50^{abc} ±0.50	49.79^{ab} ±0.71	51.20^{a} ±0.74	49.16^{ab} ±2.13
FCR[1]	1.85^{a} ±0.09	1.72^{b} ±0.13	1.69^{b} ±0.10	1.67^{b} ±0.05	1.66^{b} ± 0.11	1.70^{b} ±0.12	1.91^{a} ±0.12
FER[2]	54.05^{b} ±1.55	58.21^{a} ±1.28	59.34^{a} ±1.17	59.73^{a} ±0.37	60.07^{a} ±1.46	58.83^{a} ±1.58	52.46^{b} ±1.44
PER[3]	1.54^{b} ±0.03	1.66^{a} ±0.04	1.70^{a} ±0.02	1.71^{a} ±0.01	1.72^{a} ±0.01	1.68^{a} ±0.01	1.50^{b} ±0.03

[a,b,c] Means with superscripts in the same row are significantly different at P<0.05.
Feed conversion ratio (FCR) = feed intake (g) / weight gain.
Feed efficiency ratio (FER) = body weight gain (g)/ feed intake(g) × 100.
Protein efficiency ratio (PER) = gain in weight (g)/ protein intake in feed (g).

At the end of the study, the results of proximate analysis of whole body common carp fry fed different levels of Zn for dry matter, protein, fat and ash are shown in Table 4. Dry matter and protein contents were increased with the increasing of Zn levels up to 30 mg kg^{-1} in diets (P< 0.05). The protein and dry matter contents were significantly lower in fish group fed 5 mg Zn/kg^{-1}. Fat content in whole fish body was slightly decreased significantly but ash was increased insignificantly with increasing the dietary Zn levels (P< 0.05). These results are in agreement with those obtained by (Elalakawy *et al*., 2017) who found that, body protein and dry matter content increased, with increased in zinc levels up to 30 mg Zn/kg^{-1} diet of fingerlings Common carp, while fat and ash content were not significantly difference**.** In the same trend liang *et al*. (2012) found that, body protein content increased, while fat content decreased significantly with increment in zinc levels up to 34 mg Zn/kg^{-1} diet of Juvenile grass carp. Moazenzadeh *et al*. (2018) found that, body protein content increased and fat content of juvenile Siberian sturgeon decreased significantly with increment in zinc levels up to 27.3 mg Zn/kg^{-1} diet. While ash and dry matter were increased insignificantly with increment in zinc levels up to 27.3 mg Zn/kg^{-1} diet. On other hand, Musharraf and Khan (2019) reported that, no significant differences in the body protein and moisture contents of fingerling

Indian major carp were noted. While, body fat content decreased and ash content increased significantly with increment in zinc levels up to 51.42 mg Zn/kg^{-1} diet.

Table 4. Proximate composition (% on dry matter basis) of whole body of common carp fed experimental diets containing different levels of Zn.

Items	Zn levels (mg/kg^{-1} diet)						
	5mg	10mg	15 mg	20 mg	25 mg	30 mg	35 mg
Dry matter	27.99^{d} ±0.55	28.90^{cd} ±0.45	29.78^{bc} ±0.18	30.05^{abc} ±0.58	31.21^{a} ±0.15	31.46^{a} ±0.37	30.79^{ab} ±0.50
Protein	53.12^{c} ±0.19	54.33^{b} ±0.39	55.50^{a} ±0.34	56.33^{a} ±0.33	56.55^{a} ±0.45	56.64^{a} ±0.23	56.12^{a} ±0.59
Fat	26.49^{a} ±0.22	25.79^{a} ±0.23	23.12^{b} ±0.17	22.21^{c} ±0.21	22.15^{c} ±0.24	22.97^{bc} ± 0.29	23.00^{bc} ±0.36
Ash	18.78^{a} ±0.17	18.91^{a} ±0.24	19.35^{a} ±0.18	19.56^{a} ±0.20	19.44^{a} ±0.21	19.77^{a} ±0.21	19.15^{a} ±0.27

[a,b,c] Means with superscripts in the same row are significantly different at P<0.05.

Minerals concentration in fish body is shown in Table (5). Contents of zinc, calcium, phosphorus and ferric in fish body were significantly changed, however Zn content in fish body was increased by increasing dietary Zn levels. In this regard, Abdel-Tawwab *et al.*, 2013 and Musharraf & Khan, 2019, reported that the incorporation of high amount of Zn resulted in an increase in whole body and bone Zn content. Liang *et al*. (2012) found that, Zn contents in whole body, scales, vertebrae and plasma were linearly increased with increased dietary Zn level. Also Moazenzadeh *et al*. (2018) found that, Zn contents in muscle, serum, Liver and cartilage were increased with increased dietary Zn level up to 37.7 mg/kg^{-1}.

In the present study Common carp fry fed with dietary Zn levels up to 25 mg/kg^{-1} was significantly increased Ca, P and Fe contents ($P < 0.05$). While dietary Zn level 5 mg/kg1 was significantly decreasing the Ca, P and Fe contents in whole body. In the same trend (liang *et al.*, 2012) found that, Juvenile grass carp fed with dietary Zn levels up to 34 mg kg^{-1} was significantly increased Ca, P and Fe contents ($P < 0.05$).

Musharraf and Khan (2019), reported that, the magnesium and iron contents of fingerling Indian major carp in the whole body, liver, scales and vertebrae were significantly affected by the increasing levels of dietary zinc, but no change ($P > .05$) was noted in calcium and phosphorus concentrations.

In conclusion, the study clearly indicated that the common carp fry require 15-30 mg Ze/kg^{-1} dry diet to maintain the optimum growth performance and feed utilization efficiency. These results revealed that the dietary Zn requirement of Common carp fry was lower than that ranges reported for the other fish species (Houng-Yung *et al*., 2014 and Liang *et al*., 2012). This inconsistency may be explained by the fact that fish Zn requirement varies as a function of age, growing stage, season and physiological conditions such as maturation stage and health (Lall, 2002; Lall and Dumas, 2015 and Prabhu *et al*., 2014). Different supplemental Zn sources may also influence its requirement for normal growth, wound healing and more immune parameters of Common carp.

Table 5. Changes in some elements in whole fish body (ppm) of common carp fed diets containing different levels of Zn.

Items	Zn levels (mg/kg^{-1} diet)						
	5mg	10mg	15 mg	20 mg	25 mg	30 mg	35 mg
Zn	15.79^{f} ±0.19	16.22^{f} ±0.32	17.69^{e} ±0.33	18.9^{d} ±0.30	20.01^{c} ±0.35	23.22^{b} ±0.12	24.44^{a} ±0.23
Ca	2922^{e} ±62.7	3441^{d} ±50.2	3886^{bc} ±46.1	3960^{ab} ±50.0	4121^{a} ±76.6	3810^{bc} ±49.3	3712^{c} ±47.4
P	415.55^{c} ±16.75	688.53^{b} ±8.66	702^{ab} ±26.59	725.07^{ab} ±10	753.03^{a} ±5.77	700.1^{ab} ±15.76	680.05^{b} ±20.20
Fe	45.01^{b} ±0.58	45.20^{b} ± 1.17	46.00^{ab} ±0.59	48.67^{a} ±0.33	48.33^{a} ±0.88	48.16^{a} ±1.17	48.00^{a} ±1.00

[a,b,c] Means with superscripts in the same row are significantly different at $P<0.05$.

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الاحتياجات الغذائية من الزنك لزريعة المبروك العادى

محمد محمد زينهم

قسم بحوث تغذية الأسماك، المعمل المركزى لبحوث الثروة السمكية ، مركز البحوث الزراعية ، وزارة الزراعة ، مصر.

الملخـــــــص العربـــــي

تمت هذه الدراسة لمعرفة الاحتياجات الغذائية لزريعة أسماك المبروك العادى متوسط وزن ١,٠±٠,٠١جم. تم استخدام العليقة النقية المحتوية ٣٥% بروتين خام والمضاف اليها مستويات متزايدة من كبريتات الزنك الأحادية بمعدل ٥، ١٠، ١٥، ٢٠، ٢٥، ٣٠، ٣٥ مجم زنك /كجم علف. وتمت التجربة فى ٢١ حوض زجاجي وزعت فى ٣ مكررات ، كل حوض تم تزويده بماء الصنبور الخالى من الكلور والمحتوى على الاكسجين الازم طوال فترة التجربة.

مدة التجربة ١٢ اسبوع واستخدم ٢١ حوض زجاجى ذات ابعاد ٤٥×٦٠×٧٥سم، وزعت فى ٣مكررات لإنتاج ٧ علائق نقية تحتوى ٣٥% بروتين. اضيف مستويات من تركيز اكسيد الزنك ٥، ١٠، ١٥، ٢٠، ٢٥، ٣٠، ٣٥ مجم زنك /كجم عليقه.

كانت اهم النتائج أن أفضل وزن كلى حى ومعدل نمو يومى فى المستويات من ٢٠ الى ٣٠ مجم زنك/كجم عليقه، بينما كان افضل معدل نمو نوعى ومعامل حاله ومعدل بقاء فى المستويات من ١٥ الى ٣٠ مجم زنك/كجم عليقه. وجد أن أعلى معدل معنوى من استهلاك الغذاء كان فى المعاملات من ٢٠ الى ٣٠ مجم زنك/كجم عليقه، تحسن معنويا معدل التحويل الغذائى ومعدل كفاءة الغذاء ومعدل كفاءة البروتين فى المستويات من ١٠ الى ٣٠ مجم زنك/كجم. زاد معنويا مستوي جسم الأسماك من المادة الجافة ومحتوى البروتين بزياده مستوى العليقة من الزنك حتى ٣٠ مجم زنك/كجم، ايضا انخفض معنويا محتوى الجسم من الدهن بينما لم يتأثر معنويا محتوى الجسم من الرماد. زاد معنويا محتوى جسم الأسماك من والكالسيوم والفوسفور والحديد بزيادة مستوى زنك العليقة حتى ٢٥ مجم زنك/كجم عليقه، بينما محتوى الجسم من الزنك ارتبط معنويا بمحتوى العليقة من الزنك.

يمكننا استنتاج ان مستوى زنك العليقة من ١٥ الى ٣٠ مجم زنك/كجم عليقه يعطى أفضل معدل نمو وكفاءه غذائية وتركيب جسم ومحتوى الجسم من العناصر المعدنية.