Effect of Dietary Magnesium Levels on Some Physiological Parameters of Nile Tilapia *Oreochromis nilotics* (L.).

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Abstract

The effect of magnesium levels on some physiological and histological parameters of Nile tilapia (*Oreochromis niloticus* L.) were studied. Nile tilapia fish (4±1 g/fish) were assigned to seven treatments, with three replicates each. Magnesium was added to the feed at different levels from 100 to 700 mg/kg feed, which contained 25% CP and fed at a rate of 3% of live body weight for 105 days. The obtained results revealed that there was no definite effect of dietary Mg levels on hemoglobin or hematocrit levels. Glucose levels in fish plasma was significantly decreased, while uric acid was increased by increasing dietary Mg levels. Mg levels in the diets were insignificantly affected creatinine. Plasma AST and ALT activities were insignificantly affected by dietary Mg levels in the diet. Condition factor of fish was significantly increased with increasing of Mg levels in the diet and the significantly highest HS index was obtained with fish fed 700 mg Mg/kg diet. Magnesium concentration in plasma was positively correlated with Mg levels in the diet. Some histological changes in studied organs (gills, liver and kidney) occurred although the diet was supplemented with Mg and fish showed good growth.

Key words: Dietary Mg, Nile tilapia, minerals contents, physiological parameters, histology.

Introduction

Magnesium is one of the essential dietary minerals and its dietary deficiency results in increasing the mortality rate, loss of appetite, poor growth, sluggishness and convulsion (Ogino and Chiou, 1976). Magnesium requirements of freshwater fish have been studied by a number of workers. Diets containing 0.05%-0.07% magnesium seemed to meet the requirements of rainbow trout (Ogino *et al.*, 1978; Knox *et al.*, 1981, 1983). The role of magnesium in metabolism and nutrition of fish has been well established. When a mineral deficiency is suspected, it is necessary to examine specific biochemical or compositional indicators of nutritive status. When a deficiency is suspected, a presumptive diagnosis based on clinical signs of deficiency is the first step. This has proven difficult with fish since the signs of most elemental deficiencies i.e. reductions in growth, feed consumption and feed efficiency are nonspecific (Tacon, 1985). The magnesium requirement for tropical fish has not been clarified.

Since blood may provide an index of the physiological status of fish, care must be exercised in the choise of technique for blood sampling (Welles and Tetens, 1984). Haematological tests and analysis of serum constituents have provide useful in detection and diagnosis of metabolic disturbances and disease processes (Aldrin *et al.*, 1982).

The histological changes have been reported in liver, kidneys and gills of fish in response to feeding different ratios of dietary magnesium. The fish liver is an important organ in detoxification that disturbed its structure (Freeman *et al.*, 1983).The kidneys of fish are a compound tubular organ with a massive highly vascular structure containing vast numbers of convoluted tubules separated from one another by haemopoietic reticulo-endothelial and interrenal cells (Al-Zahaby *et al.*, 1985). The importance of gills in respiration, ion regulation and acid-base balance are among the first physiological processes to be disturbed when fish are exposed to different pollutants (Mc Donald, 1983).

The purpose of this study was to determine the effect of dietary magnesium levels on some physiological and histological parameters of Nile tilapia.

Materials and Methods

Healthy fish of Nile tilapia (*Oreochromis niloticus*) weighing 3-5 g/fish were acclimated indoor tank for 2 weeks to laboratory conditions. The fish were distributed randomly into seven groups of 20 fish/aquarium each in triplicates in 150 liter glass aquaria supplied of aerated tap water.

A magnesium free mineral premix was firstly prepared. Diets were used to quantify magnesium requirements by adding different levels of magnesium in the form of powdered magnesium sulphate. Fish were fed purified casein/gelatin basal diets containing 25% C.P. at different levels of magnesium as magnesium sulfate i.e. 100, 200, 300, 400, 500, 600 and 700 mg/kg feed and fed at a

rate of 3% of live body weight twice daily six days a week for 105 days. Fish in each aquarium were biweekly weighed and the feeding rate was adjusted accordingly.

The chemical analysis of surrounding water revealed that pH range was 6.6-7.0, total hardness range was 124 - 132 mg/L as $CaCO_3$, total alkalinity range was 135 - 145 mg/L as $CaCO_3$, phosphorus range was 0.36 - 0.49 mg/L, and magnesium range was 4.52 - 4.70 mg/L. The chemical water analyses showed no apparent fluctuations during the experimental period, however, water quality was found to be within the acceptable range for tilapia growth (Stickney, 1979).

At the end of experiment, the blood samples were taken from caudal vein of the anaesthetized fish by sterile syringe using EDTA solution as anticoagulant. These blood samples were used for determining hematocrite and hemoglobin contents according to Van Kampen and Zijlstra (1961). Plasma were obtained by centrifugation at 3000 rpm for 15 min and nonhaemolyzed plasma was stored in deep freezer for further biochemical analyses. Glucose was determined according to Trinder (1969). Uric acid was measured according to Barham and Trinder (1972). Creatinine was measured colorimetrically as described by Henry (1964). Activities of aspartate amninotransferase (AST) and alanine aminotransferase (ALT) were determined colorimetrically according to Reitman and Frankel (1957). The studied elements in blood samples were determined using flame atomic absorption spectrophotometer (Perkin Elmer model 2280). Sodium and potassium were determined by flame photometry as described by AOAC (1990).

The condition (K) factor was calculated as follows:

K factor = 100 W / L^3, where W and L are fish body weight (g) and length (cm).

The hepato-somatic (HS) index was calculated as following:

HS index = liver wt. / fish body wt.

The tissue specimens of liver, kidneys and gills were fixed in 10% buffered formalin, then washed and dehydrated through a graded series of ethanol, then cleared in xylol, infiltered and embedded in paraffin blocks. The specimens were sectioned at thickness of 6 µm, and the sections were fixed and stained with hematoxylene-eosin after (Carleton *et al.*, 1967). The slides were examined microscopically and photographed.

The obtained data were subjected to analysis of variance according to Snedecor and Cochran (1982). Differences between means were tested at the 5% probability level using Duncan's new multiple range test (Duncan, 1955).

Results and Discussion

The mean final weight was shown in Fig (1). The obtained results showed that fish given low Mg in the diet (100 mg Mg/kg diet) showed the lowest growth, and the maximum growth was obtained at 600 mg Mg/kg diet (14.90 g/fish). The obtained results are similar to those obtained by Cowey *et al.* (1977); Ogino *et al.* (1978); Konx *et al.* (1981) and Gatlin *et al.* (1982). They stated that the maximum body weight occurred at similar dietary Mg concentrations (400-700 mg Mg/kg diet) for some fish species. Also, Shim and Ng (1988) found that Mg level required to obtain the optimal growth of guppy fish was found to be 0.54 g/kg diet. Shearer (1989) found that the maximum growth rate of rainbow trout was observed at 1.3-1.4 g Mg/kg diet.

Condition factor of fish was significantly increased with increasing of Mg levels in the diet up to 600 mg Mg/kg diet. The least K factor was observed with fish fed 200 and 700 mg Mg/kg diet as shown in Table (1). K factor is a measurement used to compare the differences between fish populations related to conditions of food, density and climate (Weatherley, 1976). Hepato-somatic index (HSI) was approximately similar up to 600 mg Mg/kg diet ($P>0.05$), while the highest HSI was significantly obtained with fish fed 700 mg Mg/kg diet ($P<0.05$). This result may be due to liver enlargement due to fats deposition and the low final body weight, these results are in agreement with those of Lee and Putnan, (1973) and Nose and Arai, (1976).

No definite effects of dietary Mg on hemoglobin or hematocrit levels (Fig 2) were observed. Data of glucose, creatinine and uric acid were shown in Table (2). Glucose levels in fish plasma was significantly decreased at high dietary Mg levels. Also, uric acid was increased by increasing dietary Mg levels, and the highest uric acid values were obtained at 300 and 600 mg Mg/kg diet (1.758 and 10574 mg/L, respectively; $P<0.05$). On the other hand, creatinine was insignificantly affected by Mg levels in the diet. Plasma AST and ALT activities were insignificantly affected by dietary Mg levels in the diet (Fig 3).

These results are in agreement with the findings of Dabrowska *et al.* (1989) who found that blood parameters showed a decrease in hemoglobin and hematocrit levels in Nile tilapia fed different Mg sources. Determination of enzymes activities in plasma has proved to have diagnostic application in fish health studies (Gaudat *et al.*, 1975; Bouck *et al.*, 1978). Many metals have been shown to act specially by inhibiting certain enzymes, thus interfering with metabolic processes (Weis *et al.*, 1981). Creatinine and uric acid are considered as good indicators of glomular filtration rate and kidney dysfunction (Lockhart and Metner, 1984; Zaghloul *et al.*, 2000).

Mineral contents in plasma are shown in Table (3). Zinc and copper concentrations are

insignificantly changed. Sodium concentration was significantly higher at 600 mg Mg/kg diet, and potassium was only decreased at 400 mg Mg/kg diet. Manganese concentration was significantly increased up to 0.5 g Mg/kg diet, and sharply decreased at 600 and 700 mg Mg/kg diet (22.43 and 23.10 ppm, respectively). Magnesium concentration in plasma was positively correlated with Mg levels in the diet. The highest Mg concentration in plasma was obtained in fish fed 500, 600 and 700 mg Mg/kg diet (7702.7, 8420.0 and 8548.7 ppm, respectively). These results are in agreement with those of Knox *et al.* (1981), (1983) and Dabrowska *et al.* (1989) who found positive correlation between Mg concentration in the diet and Mg concentration in plasma of rainbow trout.

Nile tilapia which was fed on (700 mg/kg) Mg supplemented diet showed deformation of the gill cartilage, and gill filament degeneration (Fig. 4 and 5). Examination of the liver cleared the presence of atrophy and pycnotic nucleus of the hepatocytes with the previous concentration of Mg (Fig. 6). As for the kidney, marked dilation of the tubule lumen was cleared (Fig. 7) with (400 mg/kg) Mg diet. Histological results revealed also that increasing the concentration of Mg to 700 mg/kg in the diet affect the kidney and dilation of glomerulus was noticed (Fig. 8). The histological altrations in the studied organs (gills, liver and kidney) in the current work occurred although the diet was supplemented with Mg and fish showed normal growth which is in agreement with Ayyat *et al.* (2002) who showed that feeding a diet containing high protein level supplemented with 50 and 100 mg copper/kg diet released histological changes in gills, liver and kidneys of Nile tilapia.. The low concentrations of Mg (100, 200 and 300 mg/kg) did not affect the structure of the different organs. Changes were evident with both 400 and 700 mg/kg Mg diet, Steniford *et al.* (2003) reported some changes in the gill, liver and kidney of three studied fish species (*P. slesus*, *P. minutus* and *Z. viviparus*) living in contaminated localities.

Table (1): Changes in condition factor, hepato-somatic index and survival rate of Nile tilapia (*O. niloticus*) fed diets containing different levels of Mg.

Items	Mg levels (mg/kg feed)						
	100	200	300	400	500	600	700
Condition (K) factor	1.62 ± 0.17^{b}	1.66 ± 0.02^{b}	1.72 ± 0.03^{a}	1.69 ± 0.02^{b}	1.71 ± 0.02^{b}	1.69 ± 0.01^{ab}	1.65 ± 0.05^{b}
Hepato-somatic index	1.93 ± 0.36^{b}	1.97 ± 0.47^{b}	2.31 ± 0.52^{b}	2.25 ± 0.39^{b}	2.29 ± 0.22^{b}	2.14 ± 0.16^{b}	4.16 ± 0.36^{a}
Survival rate (%)	90.9	91.7	96.7	98.3	100	98.3	86.7

A, b and c averages with the same letter in the same row is not significantly different at P<0.05.

Table (2): Changes in glucose, creatinine and uric acid concentrations in plasma of Nile tilapia (*O. niloticus*) fed diets containing different levels of Mg.

Items	Mg levels (mg/kg feed)						
	100	200	300	400	500	600	700
Glucose (mg/100 ml)	91.26 ± 12.36^{b}	96.12 ± 4.120^{b}	151.79 ± 18.02^{a}	147.16 ± 11.12^{a}	86.99 ± 17.61^{b}	101.07 ± 8.688^{b}	87.94 ± 14.07^{b}
Creatinine (mg/100ml)	2.64 ± 0.33	2.713 ± 0.249	2.925 ± 0.699	2.456 ± 0.175	3.062 ± 0.186	3.219 ± 0.687	2.604 ± 0.510
Uric acid (mg/100ml)	0.55 ± 0.16^{c}	0.572 ± 0.173^{c}	1.758 ± 0.154^{a}	0.624 ± 0.032^{c}	1.574 ± 0.129^{b}	0.509 ± 0.023^{c}	0.976 ± 0.047^{b}

A, b and c averages with the same letter in the same row is not significantly different at P<0.05.

Table (3): Changes in some major and minor elements (ppm) in plasma of Nile tilapia (*O.niloticus*) fed diets containing different levels of Mg.

Items	Mg levels (mg/kg feed)						
	100	200	300	400	500	600	700
Na	4.76 ± 0.29^{b}	3.530 ± 0.468^{b}	4.633 ± 1.067^{b}	4.447 ± 0.439^{b}	5.137 ± 0.291^{ab}	7.173 ± 0.688^{a}	4.820 ± 0.811^{b}
K	31003 ± 21.62^{b}	350.0 ± 15.27^{ab}	514.3 ± 49.94^{a}	268.7 ± 22.51^{b}	516. 7 ± 14.65^{a}	390.0 ± 20.20^{ab}	390.1 ± 40.42^{ab}
Mg	3290.0 ± 360.2^{d}	3468.0 ± 483.1^{d}	4180.3 ± 236.6^{cd}	6010.3 ± 229.8^{bc}	7702.7 ± 818.7^{ab}	8420.0 ± 586.9^{ab}	8548.7 ± 825.2^{a}
Zn	78.60 ± 9.67	74.90 ± 10.48	77.00 ± 7.59	66.73 ± 13.07	60.63 ± 5.28	63.80 ± 3.93	61.03 ± 4.37
Mn	31.16 ± 2.62^{c}	33.40 ± 2.13^{bc}	31.80 ± 1.59^{c}	39.60 ± 1.71^{b}	48.00 ± 4.46^{a}	22.433 ± 2.26^{d}	23.10 ± 1.34^{d}
Cu	76.16 ± 4.62	82.95 ± 7.14	79.07 ± 14.02	71.01 ± 8.61	85.53 ± 2.82	86.47 ± 3.70	79.80 ± 2.81

A, b and c averages with the same letter in the same row is not significantly different at P<0.05.

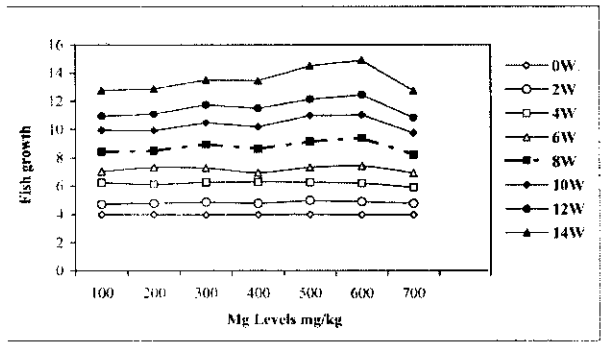


Fig. (1): Changes in live body weight (g/fish) of Nile tilapia (*O.niloticus L.*) fed diets eontaining different dietary magnesium levels.

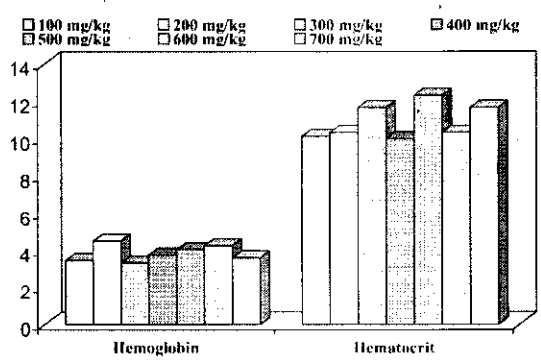


Fig. (2): Changes in hemoglobin (g/100ml) and hematocrit (%) of Nile tilapia (*O.niloticus L.*) fed diets containing different dietary magnesium levels.

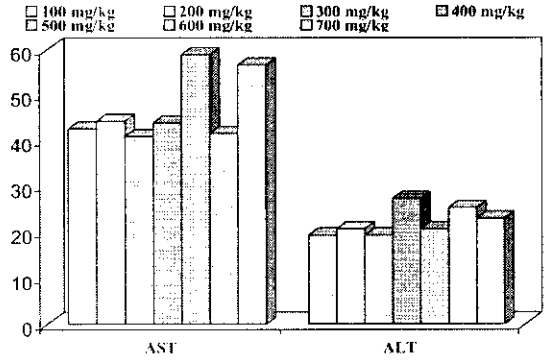
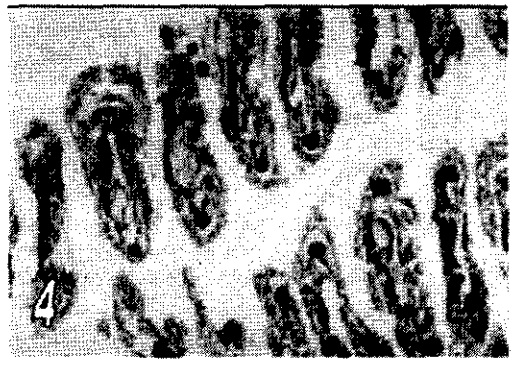
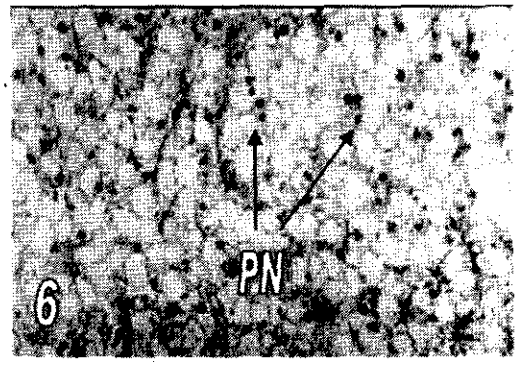


Fig. (3): Changes in AST and ALT (IU/L) of Nile tilapia (*O.niloticus L.*) fed diets containing different dietary magnesium levels.





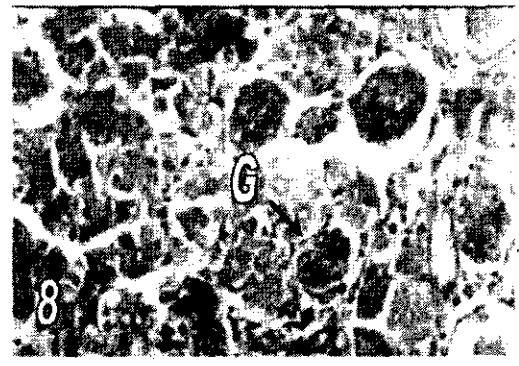


Fig. (4):Photomicrograph of a section of fish supplemented with Mg (700 mg/kg) diet showing deformation of the gill structure. (HE,X100).

Fig. (5): Photomicrograph of a section of fish supplemented with Mg (700 mg/kg) diet showing deformation of the gill cartilage and degenerating gill filaments. (HE, X400).

Fig. (6): Photomicrograph of a section of fish supplemented with Mg (700 mg/kg) diet showing atrophy and pycnotic nucleus (PN) of liver cells (HE,X400).

Fig. (7): Photomicrograph of a section of fish supplemented with Mg (400 mg/kg) diet showing dilation of the tubule lument (TL) of the kidney. (HE,X400).

Fig. (8): Photomicrograph of a section of fish supplemented with Mg (700 mg/kg) diet showing dilation of kidney glomerulus (G). (HE, X400).

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تأثير مستويات الماغنسيوم فى العليقة على بعض الصفات الفسيولوجية لأسماك البلطي النيلي

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تمت دراسة تأثير مستويات مختلفة من الماغنسيوم (١٠٠ ، ٢٠٠ ، ٣٠٠ ، ٤٠٠ ، ٥٠٠ ، ٦٠٠ ، ٧٠٠ ملجم/كجم عليقة) على بعض القياسات الفسيولوجية والهستولوجية على أسماك البلطي النيلي. وزعت الأسماك التى تزن (٤جم تقريبا) على سبعة معاملات بثلاثة مكررات لكل معاملة.

وتم اختبار عليقة تحتوى على ٢٥% بروتين بمستويات الماغنسيوم المدروسة وغذيت العليقة بمعدل ٣% من وزن الكتلة الحية لمدة ١٠٥ يوم. أظهرت النتائج انه لا يوجد تأثير ملحوظ على كل من الهيموجلوبين والهيماتوكريت بينما قلت بشكل ملحوظ نسبة الجلوكوز فى الدم فى حين زاد مستويات حامض اليوريك وذلك بزيادة مستويات الماغنسيوم.

أيضا لم تتأثر مستويات أنزيمات ALT, AST وذلك بزيادة مستويات الماغنسيوم. واظهر كلا من معامل الحالة والدليل الكبدى المنسلى HSI, K تأثيرا ملحوظ بزيادة نسبة الماغنسيوم فى العليقة واظهر تقدير الماغنسيوم فى البلازما ارتباط قوى مع تركيز الماغنسيوم فى العليقة. وبالرغم من بعض التغيرات التى حدثت فى القطاعات الهستولوجية لكل من الخياشيم والكلية والكبد إلا إن الأسماك أظهرت نموا جيدا عند تغذيتها على العلائق المحتوية على مستويات ماغنسيوم ٥٠٠ ، ٦٠٠ مجم/كجم من العليقة.