



## **Effect of different levels of folic acid on the growth and some physiological aspects of Nile tilapia "*Oreochromis niloticus*"**

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### **ABSTRACT**

Folic acid is an essential vitamin in fish nutrition, and its lack or excessive intake may cause physiological disorders that reduce growth and production. Therefore, this study was conducted to evaluate the effects of different levels of folic acid (0.0, 10, 20, 40, 80 and 160 mg/kg of dried rations) on the specific growth rate (SGR), weight gain (WG) and some physiological measurements in Nile tilapia; *Oreochromis niloticus* fingerlings.

The experiment was designed in six groups in glass aquaria, each group in three replicates each with 10 fingerlings (20-30 g) after acclimation in laboratory conditions. Fish in each replicate was weighed and each group was fed with the processed feed to the extent of satiation twice a day for 8 weeks. At the end of the 8<sup>th</sup> week, fish weights were taken in each replicate separately to calculate the specific growth rate and weight gain. Blood samples were taken for physiological examinations.

Results showed clear improvement in the specific growth rate, weight gain and physiological measurements in groups fed on diet with folic acid compared to the control group (Free of folic acid). The improvement in these parameters was increased in groups fed diets with 10 to 20 mg folic acid/kg diet. While, fish in groups fed concentrations of folic acid from 40 to 160 mg/kg showed gradual decrease in the specific

growth rates, weight gain and other blood components with increase the folic acid level.

Accordingly, it could be concluded that feeding Nile tilapia with diets containing levels of 10-20 mg folic acid/kg diets improves the growth and health status of the Nile tilapia and therefore improves their production.

## INTRODUCTION

The improvement of different agricultural and livestock farming, such as increase in dense fish production efficiency per liter are the possible ways to meet the demands. In turn, since feeding is a critical factor for production of healthy and high-qualified fishes, dense fishery operation is closely depended to the fish nutrition (**FAO, 2011; Brown and Gratzek, 1980**). Although nutrition is one of main fish farming expenses (40 to 50%), the qualification and quantification of many of the food components, including vitamins, has not yet precisely determined for many farmed fish species (**Craig and Helfrich, 2002**).

Folic acid is a form of the water-soluble vitamin B9 and it is important in metabolism of amino acids and nucleotides, growth and health in most aquatic animals (**Shiau & Huang 2001b; Lin *et al.* 2011; Miao *et al.* 2013**). It is an indispensable micro-nutrient which plays important roles in various biochemical and physiological processes, including improving growth performance (**Kocabas and Gatlin, 1999**), enhancing immunity (**Trushenski and Kohler, 2007; Verlhac-Trichet, 2010**). Typical symptoms of its deficiency include anorexia, anemia, slow growth and poor feed conversion in some aquatic animals (**NRC, 2011**). **Halver (1989)** recorded that folic acid is essential nutrient for fish as it is for other vertebrates. Deficiency of folic acid is consistently resulted in megaloblastic anemia together with anorexia and associated low weight gain. **Duncan *et al.* (1993)** and **Cowey and Woodward (1993)** signified the important role of folic acid in growth of Channel catfish and rainbow trout. In addition, the same results were recorded by **Shiau and Huang (2001 a, b)** in case of tilapia and Grass shrimp. It has been generally believed that intestinal microorganisms may contribute a considerable quantity of folic acid to the host. **Duncan *et al.* (1993)** demonstrated that intestinal microorganisms are a significant source of folic acid for channel catfish. **Kashiwada *et al.* (1971)** isolated folic acid-synthesizing bacteria from the intestine of common carp.

Thus, this study was conducted to evaluate the effect of different levels of folic acid on the specific growth rate (SGR%), weight gain (g), and physiological parameters of Nile tilapia, *O. niloticus*.

## MATERIALS AND METHODS

### Diet preparation

Folic acid was added to the basal diet at the levels of 0.00 (T<sub>1</sub> control), 10 (T<sub>2</sub>), 20 (T<sub>3</sub>), 40 (T<sub>4</sub>), 80 (T<sub>5</sub>) and 160 (T<sub>6</sub>) mg folic acid/kg diet with a corresponding decrease of dietary cellulose. The preparation of experimental diets was carried out following the procedure described by **Chen *et al.* (2005)**.

### Experimental fish

Nile tilapia; *O. niloticus* (mono sex) was obtained from Abbassa Fish Farm (Abu-Hammad, Sharkia, Egypt). Prior to the experiment, fish were acclimated to the laboratory conditions for 2 weeks in a fiber glass tanks (0.70 x 3.00 x 0.50 m) in the Central Laboratory for Aquaculture Research (CLAR) in Abbassa, Abu-Hammad, Sharkia, Egypt and fed with the commercial diet (35% protein). After acclimation, healthy fish (23.0±2.0 g) were randomly distributed in 6 groups involving six diet treatments. Each group was in 3 replicates, each with 10 fish in glass aquarium with 90 liter capacity.

During the feeding trial, fish in each group were fed to satiation twice daily at 9:00 am and 2:00 pm, respectively. Before the next feeding, remaining diets and feces were removed with a syphon tube. One-third of water in each aquarium was daily renewed with aerated fresh water. The water was continuously aerated and the dissolved oxygen was 5.5 –6.5 mg/l, and other water quality variables were maintained at temperature 25.0–28.0 °C, pH 7.2–8.2 and ammonia and nitrite ≤ 0.01 mg/l.

At the end of the feeding trial (60 day), tilapia was deprived of feed for 1 day, and all were counted and weighed. Growth performance was measured by specific growth rate (SGR%) according to the formula ( $\ln$  final weight -  $\ln$  initial weight/time in days x 100) and weight gain was calculated. Gonado-somatic index (GSI) and hepato-somatic index (HSI) were calculated according to the equation (Weight of gonads or liver/Total body weight x100).

Fish blood samples were collected with a hypodermic syringe from the caudal vessels. The extracted blood was divided in two sets of Eppendorf tubes contained sodium heparin, used as anticoagulant. One set, for hematology (hemoglobin, hematocrit and red blood cells counting). The second set for biochemical analyses was left to clot at 4 °C and centrifuged at 5000 rpm for 5 min at room temperature.

Red blood cells (RBCs) were counted under the light microscope using a Neubauer hemocytometer after blood dilution with phosphate-buffered

saline (pH, 7.2). Hematocrit values (Hct) were immediately determined after sampling by placing fresh blood in glass capillary tubes and centrifuging for 5 min in a microhematocrit centrifuge and measuring the packed cell volume (**Rehulka, 2000**). Hemoglobin levels (Hb) were determined colorimetrically according to **Jian (1993)**. While, mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) were calculated as previously described by **Haney *et al.* (1992)** as follow:-

$$\text{MCV \{Femto-liter (fL}=10^{-15}\text{L)\}} = \text{Hct\%} \times 10 / \text{RBCs} \times 10^6 \text{ cell/mm}^3$$

$$\text{MCH \{Pico-gram (pg} = 10^{-12} \text{ g)\}} = \text{Hb (g/dl)} \times 10 / \text{RBCs} \times 10^6 \text{ cell/mm}^3$$

$$\text{MCHC (g/dl)} = \text{Hb (g/dl)} \times 100 / \text{Hct\%}$$

Plasma glucose, total proteins, total lipids and transaminases (AST & ALT) were determined colorimetrically according to **Trinder (1969)**, **Henry (1964)**, **Joseph *et al.* (1972)** and **Reitman and Frankel (1957)**, respectively.

### Statistical analysis

All the results were subjected to analysis of variance (ANOVA). Duncan multiple range test (**Duncan, 1955**) was further used to evaluate the mean differences at 0.05 significant levels.

## RESULTS

Data in Table 1 showed that the specific growth rate (SGR) and weight gain (WG) were affected by addition of different levels of the folic acid to the diets of treated fish. The higher specific growth rate ( $1.12 \pm 0.10\%$ ) was obtained in T<sub>3</sub> (20 mg/kg diet) followed by  $1.03 \pm 0.15$  in T<sub>2</sub> (10 mg/kg diet) then decreased gradually in the other treatments.

The lowest value ( $0.55 \pm 0.02\%$ ) of SGR was detected in T<sub>1</sub> (0.00, control) and the same trend was obtained for the weight gain.

Also, data in table 2 revealed that HSI and GSI increased significantly in fish groups T<sub>2</sub> and T<sub>3</sub> (fed 10 and 20 mg folic acid/kg diet) and decreased gradually with the increase of folic acid levels over 20 mg/kg diet but still higher than control group.

Table 1: Effects of dietary folic acid on specific growth rate (SGR) and weight gain of Nile tilapia; *Oreochromis niloticus* after 8 weeks.

Parameters Treatments	Initial weight (g)	Final weight (g)	SGR%	WG (g)
T <sub>1</sub> (Control)	23.36±0.54	35.09±0.39 <sup>D</sup>	0.55 ± 0.02 <sup>D</sup>	11.73 ±0.09 <sup>E</sup>
T <sub>2</sub> (10 mg/kg)	22.98±0.31	44.77±0.77 <sup>B</sup>	1.03 ± 0.15 <sup>A</sup>	21.79 ±1.23 <sup>B</sup>
T <sub>3</sub> (20 mg/kg)	22.88±0.63	49.35±0.99 <sup>A</sup>	1.12 ± 0.10 <sup>A</sup>	26.52 ±1.53 <sup>A</sup>
T <sub>4</sub> (40 mg/kg)	22.20±0.10	38.68±0.15 <sup>C</sup>	0.95 ± 0.11 <sup>B</sup>	16.47 ±1.12 <sup>C</sup>
T <sub>5</sub> (80 mg/kg)	22.72±0.81	35.047±0.39 <sup>D</sup>	0.73 ± 0.12 <sup>C</sup>	12.33 ±0.97 <sup>D</sup>
T <sub>6</sub> (160mg/kg)	23.49±0.69	35.82±0.73 <sup>D</sup>	0.67 ± 0.01 <sup>CD</sup>	12.33 ±0.64 <sup>D</sup>

Data are expressed as means ± SE

Values in the same column with different superscripts are significantly different ( $P < 0.05$ ).

Table 2: Effects of dietary folic acid on hepato-somatic index (HSI) and gonado-somatic index (GSI) of Nile tilapia; *Oreochromis niloticus* after 8 weeks.

Parameters Treatments	HSI%	GSI%
T <sub>1</sub> (Control)	1.80 ± 0.17 <sup>D</sup>	0.50 ± 0.10 <sup>D</sup>
T <sub>2</sub> (10 mg/kg)	2.40 ± 0.23 <sup>A</sup>	0.99 ± 0.14 <sup>B</sup>
T <sub>3</sub> (20 mg/kg)	2.54 ± 0.19 <sup>A</sup>	1.80 ± 0.17 <sup>A</sup>
T <sub>4</sub> (40 mg/kg)	2.04 ± 0.28 <sup>B</sup>	0.87 ± 0.06 <sup>C</sup>
T <sub>5</sub> (80 mg/kg)	1.92 ± 0.22 <sup>C</sup>	0.54 ± 0.06 <sup>D</sup>
T <sub>6</sub> (160 mg/kg)	1.85 ± 0.12 <sup>CD</sup>	0.51 ± 0.12 <sup>D</sup>

Data are expressed as means ± SE

Values in the same column with different superscripts are significantly different ( $P < 0.05$ ).

Further, the folic acid treatments T<sub>2</sub> (10 mg/kg diet) and T<sub>3</sub> (20 mg/kg diet) showed significant increase in RBCs (1.63±0.04 & 1.69±0.09), Hb (7.09±0.90 & 6.86±0.65) and Hct (29.33±2.90 & 25.00±0.58) in comparison with control group T<sub>1</sub> (RBCs; 1.42±0.04, Hb; 4.63±0.17 & Hct; 23.00±0.58). All the hematological parameters were decreased and

fluctuated in the other treatments but still above the control group (Table 3). Accordingly, blood indices (MCH, MCV & MCHC) were changed but not in parallel with RBCs, Hb and Hct (Table 4).

Table 3: Effects of dietary folic acid on red blood cell counts (RBCs), hemoglobin contents (Hb), hematocrit (Hct) of Nile tilapia; *Oreochromis niloticus* after 8 weeks.

Parameters Treatments	RBCs $\times 10^6$ cell/mm <sup>3</sup>	Hb (g/dl)	Hct %
T <sub>1</sub> (Control)	1.42 $\pm$ 0.04 <sup>B</sup>	4.63 $\pm$ 0.17 <sup>C</sup>	23.00 $\pm$ 0.58 <sup>B</sup>
T <sub>2</sub> (10 mg/kg)	1.63 $\pm$ 0.04 <sup>A</sup>	7.09 $\pm$ 0.90 <sup>A</sup>	29.33 $\pm$ 2.9 <sup>A</sup>
T <sub>3</sub> (20 mg/kg)	1.69 $\pm$ 0.09 <sup>A</sup>	6.86 $\pm$ 0.65 <sup>AB</sup>	25.00 $\pm$ 0.58 <sup>AB</sup>
T <sub>4</sub> (40 mg/kg)	1.45 $\pm$ 0.24 <sup>B</sup>	5.91 $\pm$ 0.21 <sup>B</sup>	22.67 $\pm$ 1.45 <sup>B</sup>
T <sub>5</sub> (80 mg/kg)	1.50 $\pm$ 0.07 <sup>B</sup>	4.29 $\pm$ 0.12 <sup>C</sup>	21.00 $\pm$ 0.58 <sup>B</sup>
T <sub>6</sub> (160 mg/kg)	1.61 $\pm$ 0.13 <sup>A</sup>	5.27 $\pm$ 0.65 <sup>BC</sup>	24.00 $\pm$ 2.08 <sup>B</sup>

Data are expressed as means  $\pm$  SE

Values in the same column with different superscripts are significantly different ( $P < 0.05$ ).

Table 4: Effects of dietary folic acid on blood indices (MCH, MCV & MCHC) of Nile tilapia; *Oreochromis niloticus* after 8 weeks.

Parameters Treatments	MCH (pg)	MCV (fl)	MCHC (g/dl)
T <sub>1</sub> (Control)	34.80 $\pm$ 1.76 <sup>BC</sup>	161.77 $\pm$ 3.75 <sup>AB</sup>	21.50 $\pm$ 0.84 <sup>C</sup>
T <sub>2</sub> (10 mg/kg)	43.47 $\pm$ 4.80 <sup>A</sup>	179.83 $\pm$ 14.26 <sup>A</sup>	24.07 $\pm$ 1.00 <sup>BC</sup>
T <sub>3</sub> (20 mg/kg)	40.57 $\pm$ 2.33 <sup>B</sup>	148.87 $\pm$ 5.94 <sup>B</sup>	28.27 $\pm$ 2.72 <sup>A</sup>
T <sub>4</sub> (40 mg/kg)	40.90 $\pm$ 1.99 <sup>B</sup>	156.87 $\pm$ 11.06 <sup>AB</sup>	26.70 $\pm$ 2.35 <sup>B</sup>
T <sub>5</sub> (80 mg/kg)	28.80 $\pm$ 1.95 <sup>C</sup>	140.63 $\pm$ 3.84 <sup>B</sup>	20.37 $\pm$ 0.90 <sup>C</sup>
T <sub>6</sub> (160 mg/kg)	32.63 $\pm$ 2.64 <sup>BC</sup>	149.40 $\pm$ 7.68 <sup>B</sup>	22.07 $\pm$ 0.94 <sup>BC</sup>

Data are expressed as means  $\pm$  SE

Values in the same column with different superscripts are significantly different ( $P < 0.05$ ).

Data in Table 5 showed the effect of dietary folic acid on blood sugar (glucose), total proteins and total lipids. Blood glucose in control group T<sub>1</sub> was 79.01±1.51 mg/dl increased insignificantly in T<sub>2</sub> and T<sub>3</sub> (80.83 ±1.49 & 80.33±1.83, respectively) and decreased gradually with the increase of folic acid levels recorded the lowest level (35.03±0.13 mg/dl) in T<sub>6</sub> fed on 160 mg folic acid/kg diet. Furthermore, plasma total proteins increased significantly in T<sub>2</sub> (4.35±0.55 mg/dl) and T<sub>3</sub> (4.62±0.15 mg/dl) and decreased significantly and gradually in the other groups. On the other hand, plasma total lipids in T<sub>2</sub> and T<sub>3</sub> increased insignificantly when compared to that of T<sub>1</sub> (control), while it increased more highly significant in T<sub>4</sub>, T<sub>5</sub> and T<sub>6</sub> (9.31±1.51, 10.44±0.89 & 12.88±1.75 g/dl, respectively).

Table 5: Effects of dietary folic acid on glucose, total proteins and total lipids in plasma of Nile tilapia; *Oreochromis niloticus* after 8 weeks.

Parameters Treatments	Glucose (mg/dl)	T. proteins (g/dl)	T. lipids (g/dl)
T <sub>1</sub> (Control)	79.01±1.51 <sup>A</sup>	2.96±0.08 <sup>C</sup>	4.22±0.36 <sup>C</sup>
T <sub>2</sub> (10 mg/kg)	80.83 ±1.49 <sup>A</sup>	4.35±0.55 <sup>A</sup>	4.90±1.48 <sup>C</sup>
T <sub>3</sub> (20 mg/kg)	80.33±1.83 <sup>A</sup>	4.62±0.15 <sup>A</sup>	4.72±1.68 <sup>C</sup>
T <sub>4</sub> (40 mg/kg)	58.72±1.29 <sup>B</sup>	3.56±0.19 <sup>B</sup>	9.31±1.51 <sup>B</sup>
T <sub>5</sub> (80 mg/kg)	56.15±1.08 <sup>B</sup>	2.45±0.24 <sup>D</sup>	10.44±0.89 <sup>AB</sup>
T <sub>6</sub> (160 mg/kg)	35.03±0.13 <sup>C</sup>	2.30±0.23 <sup>D</sup>	12.88±1.75 <sup>A</sup>

Data are expressed in means ± SE

Values in the same column with different superscripts are significantly different ( $P < 0.05$ ).

Data in table 6 showed that ALT and AST increased insignificantly in T<sub>2</sub> (13.37±3.26 & 14.58±1.47) and T<sub>3</sub> (13.15±2.28 & 14.02±1.93) compared to control T<sub>1</sub> (11.60±0.34 & 13.80±1.21) but it elevated significantly in T<sub>4</sub>, T<sub>5</sub> and T<sub>6</sub> compared to the T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>.

Table 6: Effects of dietary folic acid on transaminases (ALT & AST) in plasma of Nile tilapia; *Oreochromis niloticus* after 8 weeks.

Parameters Treatments	ALT (iμ/l)	AST (iμ/l)
T <sub>1</sub> (Control)	11.60±0.34 <sup>C</sup>	13.80±1.21 <sup>C</sup>
T <sub>2</sub> (10 mg/kg)	13.37±3.26 <sup>C</sup>	14.58±1.47 <sup>C</sup>
T <sub>3</sub> (20 mg/kg)	13.15±2.28 <sup>C</sup>	14.02±1.93 <sup>C</sup>
T <sub>4</sub> (40 mg/kg)	33.06±1.79 <sup>B</sup>	18.13±1.95 <sup>B</sup>
T <sub>5</sub> (80 mg/kg)	46.80±2.19 <sup>A</sup>	18.47±1.00 <sup>B</sup>
T <sub>6</sub> (160 mg/kg)	45.50±1.32 <sup>A</sup>	20.68±1.55 <sup>A</sup>

Data are expressed as means ± SE

Values in the same column with different superscripts are significantly different ( $P < 0.05$ ).

## DISCUSSION

Fish farmers usually aim to cause the fish to grow rapidly and keeping feed costs as low as possible. In the present study, higher growth rates and weight gain were observed in Nile tilapia; *Oreochromis niloticus* fed diet containing 20 followed by that containing 10 mg folic acid/kg diet. These results agreed with those of **Duncan *et al* (1993)** and **Cowey and Woodward (1993)** who has proven and stressed the important role of folic acid in growth of Channel catfish and rainbow trout. Also, agreed with **Shiau and Huang (2001 a, b)** who recorded the same results in case of tilapia and Grass shrimp. In addition, **Smith (1968)** and **Smith and Halver (1969)** stated that deficiency of folic acid is consistently resulted in megaloblastic anemia together with anorexia and associated low weight gain (WG) in Coho salmon (*O. kisutch*). **Lin *et al.* (2011)** mentioned that folic acid improve growth and health of most aquatic animals. **Firouz *et al.* (2013)** showed that treatments of fingerling rainbow trout (*Oncorhynchus mykiss*) with 10 mg folic acid /Kg dried food for two months could improve the specific growth rate (SGR). Also, **Wei *et al.* (2016)** recorded significant increase of specific growth rate in juvenile crabs fed the diets containing  $\geq 2.0$  mg folic acid/kg for 8 weeks. Typical symptoms of folic acid deficiency include anorexia, anemia, slow growth and poor feed conversion in some aquatic animals (**NRC 2011**). Therefore,



it is necessary to determine the requirements of folic acid for Nile tilapia; *Oreochromis niloticus*.

The gonado-somatic index (GSI) is bioindicator that supply structural information, more than functional to respect of health status. Liver is the metabolic organ. It is a target for the metabolism in the fish body, the liver index (HSI) is a useful biomarker to detect the hazardous effects of the environmental stressors (**Pait and Nelson, 2003**). In this study, diets with 10-20 mg folic acid/kg could improve the GSI and HSI better than other treatments. **Halver (1989)** and **Halver (2002)** recorded that folic acid is an essential nutrient for improvement of hematopoietic activity, regulation of blood glucose, cell membrane activity and eggs' hatchability in rainbow trout (6-10 mg/kg).

The hematological parameters render valuable information for biologists to determine the health status of fishes (**Asadi *et al.*, 2011**). In this study, treatments with 10-20 mg folic acid/kg ration led to significant increase in RBCs, Hb and Hct which were not in parallel with increase of the MCV, MCH and MCHC indices. **Smith and Halver (1969)** stated that megaloblastic anemia is reported as a prominent feature of folate deficiency in coho salmon and **Duncan *et al.* (1993)** confirm the same results in channel catfish. **Shiau and Huang (2001b)** and **Miao *et al.* (2013)** stated that folic acid is important in amino acid and nucleotide metabolism. Also, **Wei *et al.* (2016)** found that folic acid is necessary to relieve oxidative stress. These results disagreed with those of **Firouz *et al.* (2013)** who found that dietary 10 mg folic acid to rainbow trout decreased RBCs, Hb and Hct. Totally, the results indicate that increase in folic acid levels (10-20 mg/kg diet) could improve blood parameters. In general, it appears that increase the level of folic acid above 20 mg/kg has adverse effect. The hematopoietic activity of animals, including fishes, is a result of synergy between a different essential factors, including vitamin B12, B6, K, E, D and folic acid as well as some minerals such as iron, zinc, copper and so on (**Choi and Kim, 2005**).

Levels of folic acid from 10 to 20 mg/kg don't affect the blood and liver functions, since, no significant changes were recorded in concentrations of blood glucose, total proteins, total lipids or transaminases (AST, ALT) compared with the control treatment. On the other hand, high levels (40-160 mg/kg) exhibit decrease in concentrations of blood glucose and total proteins with increase in total lipids and transaminases (AST, ALT) which indicate to the probable impairment in liver functions and other organs.

As a conclusion, it must be noticed that the taken supplementary elements and vitamins are variable based on size and age of fish.

Furthermore, since folic acid is not the only taken supplementary factor (macro/micro elements and vitamins) for fish farming, the effects of each factor needs to be interpreted with the respect to the synergic or antagonistic effects of other effective elements on fish growth. Such these synergisms have been seen, for instance, between niacin and folic acid (Shafaeipour *et al.*, 2011) and between vitamin C and Folic acid (Hien and Doolgindachbaporn, 2011).

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## تأثير مستويات مختلفة من حمض الفوليك على النمو وبعض النواحي الفيسيولوجية في سمكة البلطي النيلي

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### الملخص العربي

حمض الفوليك هو فيتامين أساسي في تغذية الأسماك ، وقد يسبب نقصه أو الإفراط في تناوله اضطرابات فيسيولوجية تقلل من معدل النمو والإنتاج.

لذلك أجريت هذه الدراسة لتقييم آثار مستويات مختلفة من حمض الفوليك (١٠ ، ٢٠ ، ٤٠ ، ٨٠ و ١٦٠ مجم/كجم من العليقة المجففة) ومقارنتها مع المجموعة الضابطة (٠,٠٠ حمض الفوليك) على معدل النمو النسبي (SGR) والوزن المكتسب، وبعض القياسات الفيسيولوجية في إصبعيات البلطي النيلي.

صممت التجربة في ستة مجموعات بأحواض زجاجية وكل مجموعة في ثلاثة مكررات بكل مكرر ١٠ اصبعيات (٢٠-٣٠ جم) وذلك بعد أقلمتها في الظروف المعملية ، حيث وزنت اسماك كل مكرر على حدة وتم إطعام كل مجموعة بالعليقة المجهزة لها إلى حد الإشباع مرتين يوميًا لمدة ٨ أسابيع.

أخذت أوزان الاسماك في كل مكرر على حدة في نهاية التجربة لحساب معدل النمو النوعي وكذلك الوزن المكتسب. ثم أخذت عينات الدم للفحص الفيسيولوجي.

وقد أظهرت النتائج تحسنا واضحا في معدل النمو النسبي والقياسات الفيسيولوجية في الاسماك التي تغذت على العليقة المضاف لها ٢٠ مجم من حمض الفوليك ثم التي بها ١٠ مجم مقارنة بالمجموعة الضابطة في حين سجلت المجموعات الاخرى ذات التركيزات العالية من حمض الفوليك (٤٠ - ١٦٠ مجم/كجم ) انخفاضا واضحا في معدل النمو النسبي وجميع القياسات الفيسيولوجية الاخرى.

ولذا يمكن أن نخلص إلى أن تغذية أسماك البلطي النيلي بعلائق تحتوي على مستويات ما بين ١٠- ٢٠ مجم من حمض الفوليك/كجم عليقة يحسن النمو والحالة الصحية لأسماك البلطي النيلي وبالتالي يحسن من انتاجها.