Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(2): 449 – 465 (2025)
www.ejabf.journals.ekb.eg

Effect of Folic Acid (Vitamin B9) on the Reproductive Performance of the Nile tilapia *Oreochromis niloticus*

Haytham A. Abd El-Ghaffar[1, 2]*, Sheren A. Al-Zahaby[3], Wafaa G. El-Nagar[3], Mohamed A. Al-Zahaby[1]

[1]Agricultural Research Center, Central Lab. for Aquaculture Res., Abbassa, Sharkia, Egypt
[2]WorldFish, Abbassa, Abu Hammad, Sharkia, Egypt
[3]Zoology department, Faculty of Science, Zagazig University, Sharkia, Egypt
***Corresponding Author: dr_haytham1983@hotmail.com**

ARTICLE INFO

Article History:
Received: Feb. 4, 2025
Accepted: March 7, 2025
Online: March 10, 2025

Keywords:
Nile tilapia,
Reproductive,
Biochemical,
Folic acid,
Histology and gonads

ABSTRACT

The study investigated the effects of folic acid on the reproductive efficiency and health of the Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758). The adult broodstock fish were fed commercial diets (36% crude protein) supplemented with different graded levels of folic acid (2.5, 5.0, and 7.5mg/ kg diet) in addition to the fourth control group of un-supplemented feed. Fish were stocked in separate hapas widened in four concrete ponds over three months. It was found that folic acid increased the total blood protein, albumin, globulins, and estradiol levels but suppressed blood ALT and AST activities and urea and creatinine levels in the adult Nile tilapia. Dietary folic acid (Vitamin B9) also plays a crucial role in enhancing reproductive performance since higher nutritional levels of folic acid significantly improve the fertility of fish males and females. It positively affected fish gonads histology with notable improvements. Additionally, the present study demonstrated non-significant adverse effects on fish blood biochemical and aminotransferases at moderate levels of supplemented folic acid. The current study suggested folic acid safety and efficacy as a dietary additive for fish well-being and good reproductive performance. The results advocate further exploring dietary folic acid in fish farming practices to optimize growth and reproductive health.

INTRODUCTION

Increased public demand for seafood against decreasing natural resources has encouraged scientists to study how to improve aquatic food production, particularly fish. This makes aquaculture a growing field of research that aims to overcome the obstructions of good fish with high productivity. Natural and artificial supplementary fish feeding is crucial for fish production **(Akter *et al*., 2024)**. Supplementary fish feeding represents about 40-50% of main fish farming expenses. However, research on the qualification and quantification of many food components, such as vitamins, is limited for many farmed fish species. Earlier studies by **Halver (1989)** and **Craig and Helfrich (2002)** certified the importance of vitamin B, particularly B9 (Folic acid), as a nutrient element for all aquatic animals, including fish. Recent investigations have focused on the effects of vitamins on the well-being and health of fish by studying some biological, physiological, and histological parameters.

Dietary folic acid is an indispensable micro-nutrient, despite its deficiency typical symptoms including anorexia, anemia, slow growth, and poor feed conversion **(Kocabas & Gatlin, 1999)**. Folic acid (mono pteroylglutamic acid) is a synthetic molecule used as vitamin supplements in foods; its oxidized form is stated as a vitamin, so folate is the common name of all derivatives of the B-vitamins **(Miao *et al*., 2013)**. It is a water-soluble, odorless vitamin having the molecular formula $C19H19N7O6$ and a molecular weight of 441.4g/ mol and appears as orange-yellow needles or platelets **(Guo, 2009)**. It functions as a one-carbon donor or acceptor in various reactions involved in amino acid and nucleotide metabolism **(Miao *et al*., 2013)**. Folic acid is not stored in body tissues to meet the permanent requirement for a longer period, so a continuous source of these vitamins is necessary **(Yeganeh Kari *et al*., 2022)**.

Folic acid is essential for the growth, health, and metabolism of aquatic organisms in the aquaculture industry **(Miao *et al.*, 2013; Asaikkutti *et al*., 2016; Xu *et al*., 2024)**. It is important to metabolize amino acids and nucleotides in most aquatic animals, including fish **(Halver & Hardy, 2002; Miao *et al*., 2013)**. The optimum level of dietary folic acid has been studied and recognized only for fingerlings and juveniles of many fish species, including the rainbow trout fries, *Oncorhynchus mykiss* **(Cowey & Woodward, 1993; Firouz *et al.*, 2013)**; the channel catfish, *Ictalurus punctatus* **(Duncan *et al*., 1993)**; the hydride of *Oreochromis niloticus* and *O. aureus* **(Shiau & Huang, 2001)**; grass carp, *Ctenopharyngodon idella* **(Zhao *et al*., 2008; Shi *et al*., 2015)**; the blunt Nile tilapia, *Oreochromis niloticus* **(Barros *et al.*, 2009)**; the green catfish, *Mystus nemurus* **(Hien & Doolgindachbaporn, 2011)**; the grouper, *Epinephelus malabaricus* **(Lin *et al*., 2011)**; the snout bream, *Megalobrama amblycephala* **(Sesay *et al*., 2016, 2017)**, the Nile tilapia, *Oreochromis niloticus* **(Shalaby *et al*., 2019)** and Catla, *Catla catla* **(Khan & Khan 2020)**. However, it does not significantly influence animal survival **(Miao *et al*., 2013)**.

In addition, folic acid plays an important role in erythrogenesis **(Lee *et al.*, 2017; Jamalzad Falah *et al.*, 2020)** as well as in various metabolic, biochemical, and physiological processes that improve fish growth performance **(Badran & Ali, 2021)**, respiratory rate **(Kari *et al*., 2022)**, as well protecting fish from hypoxic stress **(Kari *et al*., 2022)**. Otherwise, **Jamalzad *et al*. (2020**) showed that folic acid has a competitive binding mechanism to remove free radicals from living tissue and to maintain the fish's health status **(Shi *et al*., 2022)**.

In the present study, the importance of dietary folic acid (vitamin B9) on the reproductive performance of the adult Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) was investigated through a demonstration of the effect of different small folic acid levels on some physiological and biochemical blood parameters as well histological aspects of gonads (tastes and ovaries) closely related to the fish reproductive performance. In addition, since the liver plays an essential role in the metabolism of vitamins, liver status is used to indicate vitamin level in fish **(Xiang *et al*., 2016)**.

MATERIALS AND METHODS

The experiment was performed in the central laboratory for aquaculture research, Abbassa in Abu-Hammad, Sharkia, Egypt). Two groups (males and females) of the Nile tilapia (*Oreochromis niloticus*) broodstock of individual weight 230±20g/ fish were obtained from the Center Laboratory of Aquaculture Research (CLAR). They were acclimated for two weeks prior to the experimentation in two concrete ponds ($8m^3$ each) for males and females separately. Then after, the acclimated fish were stocked in hapas of (1×2×1m) with one male and one female each, widened in four concrete ponds with three hapas in each for each one of the three experimental treatments as the following:

T1: fed on an un-supplemented commercial diet of 36% crude protein (control group).

T2: fed on 2.5 mg folic acid/kg 36% crude protein feed.

T3: fed on 5.0 mg folic acid/kg 36% crude protein feed.

T4: fed on 7.5 mg folic acid/kg 36% crude protein feed.

The experimental period lasted for three months, from March to June; fish in each group were fed twice daily with about 2% of fish weight, which decreased to about 0.3% of fish weight during spawning. In spite of this, one-third of the water in each pond was renewed daily with fresh water, and the other remains were continuously aerated with dissolved oxygen of about 5.5–6.5mg/ l while keeping the slandered water environmental variables at about 25.0–28.0°C, pH 7.2–8.2, and ammonia and nitrite ≤ 0.01mg/ l.

After the three months of the experimental period, blood samples were collected from a caudal vein of each male and female with a hypodermic syringe. The extracted blood was stored in Eppendorf tubes containing EDTA, which was used as an anticoagulant. However, the fresh fish bodies were dissected to extract gonads for the histological investigations of the liver and gonads (testes and ovaries).

The following parameters were estimated calorimetrically in blood serum: aminotransferases (AST and ALT) **(Reitman & Frankel, 1957)**, glucose and urea **(Trinder, 1969)**, protein, creatinine **(Henry, 1974)**, and albumin and globulins **(Busher, 1990)**.

However, the histology of the liver and gonads was described using histological techniques of hematoxylin and eosin staining. The livers and gonads (testes and ovaries) of the experimented fish immediately after fish dissection were removed, and a suitable portion of each was fixed in a neutral 4% formaldehyde solution overnight and subsequently transferred to 70% ethanol. Afterward, they were dehydrated in graded ethanol solutions, and then embedded in paraffin wax. Cross-sections of 4–6µm thick were made in a rotary microtome, stained with hematoxylin and eosin, and mounted on glass slides for light microscopy scrutiny **(Suvarna *et al.*, 2012; Bancroft & Layton, 2019)**. The sections were analyzed and photo-documented by using an Olympus BX50 microscope.

Statistical analysis

All the results were subjected to the one-way method for analysis of variance (ANOVA). Duncan's multiple range test **(Duncan, 1955)** evaluated the mean differences between groups at 0.05 significant levels.

RESULTS

The experimented *Oreochromis niloticus* showed the following effect of adding folic acid to its diet on the blood biochemical constituents.

Biochemical blood constituents

The total plasma proteins fluctuated in values among the four groups with the addition of folic acid. T2 and T4 showed insignificant decreases. The same fluctuation occurred with albumin, which was insignificant. Globulins showed the same trend, the slightly insignificant one, and also the albumin/globulins ratio, as shown in Table (1)

Table 1. Total protein, albumin, globulin, and albumin/globulin ratio in blood serum of the Nile tilapia after the experimental period

Treatment (mg folic/kg feed)	**Protein g/dl**	**Albumin g/dl**	**Globulins g/dl**	**Albumin /Globulins ratio**
(T1) control	3.4489±0.745	1.0071±0.13514	2.1084±0.44683	0.5947±0.14893
(T2) 2.5mg	3.3139±0.4221	0.9701±0.05818	2.201±0.36543	0.5218±0.08584
(T3) 5.0mg	3.6300±0.57309	1.2924±0.15578	2.3376±0.5319	0.6594±0.12743
(T4) 7.5mg	3.2444±0.82382	0.9317±0.01528	2.3127±0.81037	0.5834±0.26681
***P*-value**	0.976	0.168	0.986	0.898

Means having different letters in the same column for the same parameter are significant ($P< 0.05$).

Furthermore, data in Table (2) show insignificant changes in all treatment including blood glucose, and urea. However, creatinine showed high significant differences ($P< 0.05$) marked with different superscripts, as it increased significantly in T2 (0.6565 ±

0.07326 b) in comparison with T1 (0.3953 ± 0.03502 mg/dl). It decreased significantly in T3 (0.0605 ± 0.00774 mg/dl) and increased significantly in T4 (0.9945 ± 0.06302 mg/dl).

Table 2. Glucose, urea, and creatinine in blood serum of the Nile tilapia after experimentation

Treatment (mg folic/kg feed)	Glucose mg/dl	Urea mg/dl	Creatinine mg/dl
(T1) Control	35.0193±2.17093	195.1307±65.73455	0.3953±0.03502 c
(T2) 2.5mg	34.5300±3.31300	170.2241±39.97288	0.6565±0.07326 b
(T3) 5.0mg	33.3163±0.91138	110.1438±21.73999	0.0605±0.00774 d
(T4) 7.5mg	36.6667±1.12918	110.1961±23.86552	0.9945±0.06302 a
***P*-value**	0.878	0.508	0 (P < 0.05)

Means having different letters in the same column for the same parameter are significant ($P< 0.05$).

Data in Table (3) show the effects of dietary folic acid on aminotransferases aspartate transaminase (AST) and alanine aminotransferase (ALT) in the plasma of the Nile tilapia, whereas AST increased insignificantly, ALT value showed a significant decrease in T2, T3, and T4 (27.8262±3.36923, 26.8299±2.6553, and 20.2861±3.34133iµ/ l, respectively) compared to the control T1 fish group of un-supplemented diet (40.7893 ± 6.14908iµ/ l). The significance was lost when the ratio AST/ALT was measured as the value increased insignificantly.

Table 3. Blood aminotransferases (AST & ALT) of Nile tilapia after the experimental period

Treatment (mg folic/kg feed)	AST iµ/l	ALT iµ/l	AST/ALT
(T1) control	39.2123±20.22313	40.7893±6.14908 a	6.0672±2.97958
(T2) 2.5mg	48.2509±18.05581	27.8262±3.36923 ab	13.5549±11.06223
(T3) 5.0mg	23.2102±4.14913	26.8299±2.6553 ab	1.4421±0.34681
(T4) 7.5mg	44.7343±13.0271	20.2861±3.34133 b	0.4845±0.05987
***P*-value**	0.702	0.04	0.577

Means different letters in the same column for the same parameter are significant ($P< 0.05$).

The level of plasma sex hormones (estradiol and testosterone) in the Nile tilapia is shown in Table (4). Estradiol had insignificant variations with the different levels of dietary folic acid, which is marked with different given superscripts. However, in the case of testosterone, the differences were also significantly varied, as marked by the given superscripts; the value increased from 4.6567 ± 0.18774ng/ml in T1 (control group) to 5.2733 ± 0.17638ng/ml in fish of T2 but then continued to decrease in T3 and T4 (1.95 ± 0.10693 and 1.87 ± 0.18193ng/ ml, respectively (Table 4).

Table 4. Estradiol and testosterone levels in blood serum of the Nile tilapia after the experimental period

Treatment (mg folic/kg feed)	Blood serumestradiol (ng/ml) in females	Blood serum testosterone (ng/ml) in males
(T1) control	2182.667±175.1955 ab	4.6567±0.18774 b
(T2) 2.5mg	2099.333±51.3333 b	5.2733±0.17638 a
(T3) 5.0mg	2706.667±54.8736 ab	1.95± 0.10693 c
(T4) 7.5mg	2888.667±410.3805 a	1.87±0.18193 c
***P*-value**	0.099	0 ($P < 0.05$)

Means that having different letters in the same column for the same parameter are significant ($P< 0.05$).

Histological observations

Examining the histological sections of Nile tilapia's liver with a light microscope (Fig. 1) made it look healthy; folic acid had no notable effect on liver construction. Hepatic parenchyma exhibiting regular polyhedral hepatocytes (Hc) with central normal spherical nuclei. They form cord-like structures with many hepatic sinusoids (HS) in-between, which are dilated out in a larger blood hepatic vein (HV), in addition to spreading the hepatopancreatic acini (HA). There are no differences in liver tissue structure between the control normal fish group (T1) of the un-supplemented diet and those fed folic acid added to their feed ration, which means that the livers of these later treated fish are healthy and not disordered.

However, histological sections of Nile tilapia's ovaries fed on commercial feed with micro addition of folic acid showed some changes compared with the control un-supplemented ones. The number of intact vitellogenic oocytes increased with the increase of folic acid concentration, to the degree of their crowding, particularly in females of the group (T3) fed on 5.0mg folic acid/Kg feed. The number of atrophied ova appeared more in the control un-supplemented group than in the other groups, indicating an increase in the ovulation rate in females fed supplementary dietary folic acid (Fig. 2).

Likewise, histological sections of the male Nile tilapia's testis fed supplementary dietary folic acid prior to spawning also showed some histological changes. The normal intact structure of seminiferous tubules, spermatocytes, spermatids, and sperms appeared in all experimented fish groups. The dilation of seminiferous lobules (SL**)** embracing intact spermatozoa (Sz) at the expense of the in-between interstitial tissue (ICT) is apparent in all males fed with dietary folic acid, particularly in fish of (T3) fed with 5.0mg folic acid /kg feed (Fig. 3).

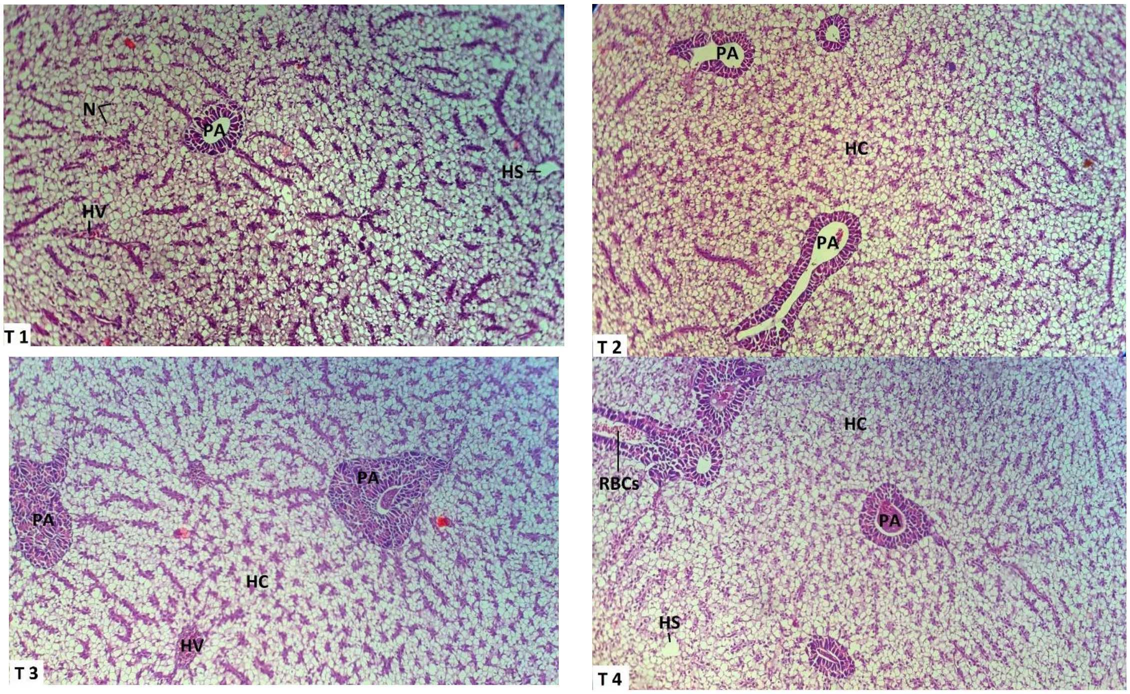

Fig. 1. Histological section in the liver of the Nile tilapia ; (T1) - Control group, (T2) – folic acid 2.5 mg/kg feed, (T3) - folic acid 5.0mg/ kg feed, (T4) - folic acid 7.5mg/ kg feed; hepatic sinusoid (**HS),** hepatocyte (**Hc)**, hepatic vein (**HV)**, hepatopancreatic acini **(PA)**, red blood cell (**RBCs)**, Nuclei **(N)**

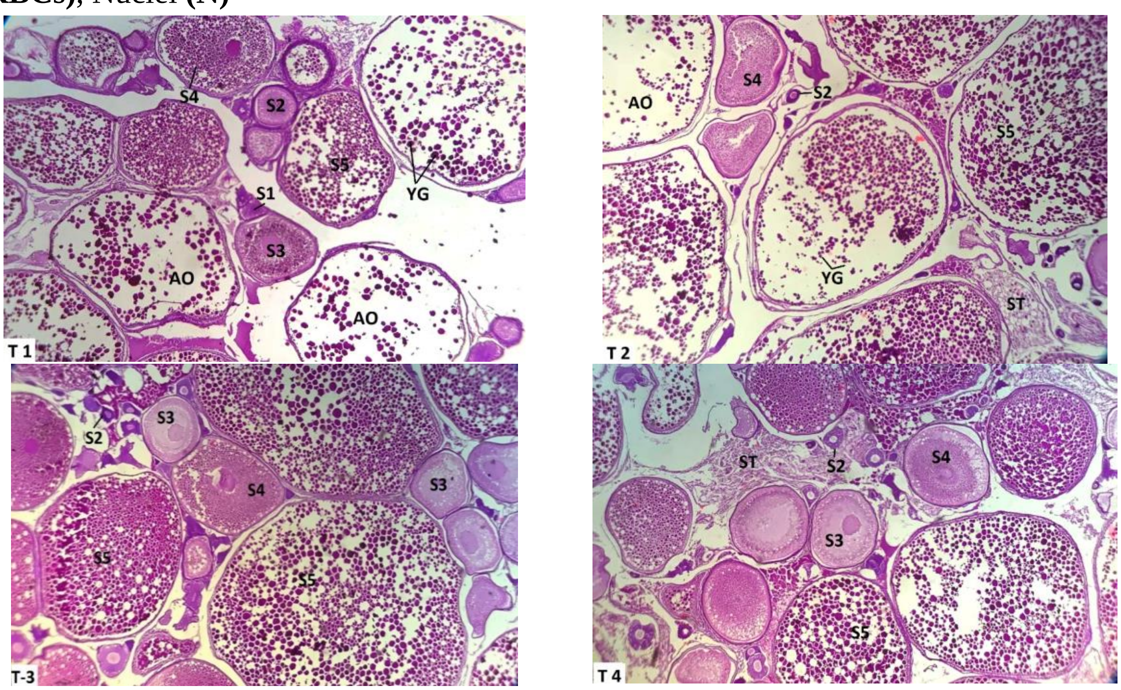

Fig. 2. Histological section in the ovary of the Nile tilapia ; (T1) - Control group, (T2) – folic acid 2.5 mg/kg feed, (T3) - folic acid 5.0 mg/kg feed, (T4) - folic acid 7.5 mg/kg feed; stromal supporting tissue (**St)** yolk granules **(YG)**, vitellogenic oocyte **(VO)**, pre-vitellogenic oocyte **(PvO),** atrophied oocyte (**AO)**

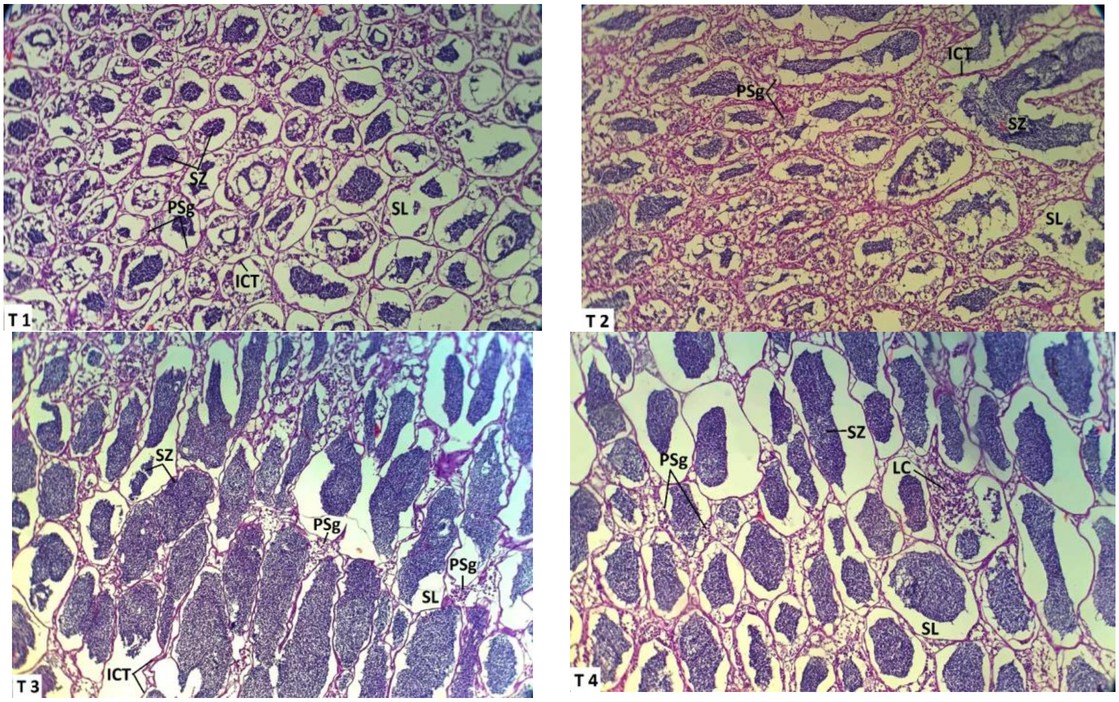

Fig. 3. Histological section in the testis of the Nile tilapia ; (T1) - Control group, (T2) – folic acid 2.5 mg/kg feed, (T3) - folic acid 5.0 mg/kg feed, (T4) - folic acid 7.5 mg/kg feed; primary spermatogonia (**PSg)**, seminiferous lobules (**SL)**, spermatozoa (**Sz)**, interstitial connective tissue (**ICT)**, Leydig cells **(LC)**

DISCUSSION

Folic acid (mono pteroylglutamic acid) is a synthetic molecule used as vitamin supplements in foods; its oxidized form is stated as a vitamin, so folate is the common name of all derivatives of the B vitamins. It functions as a one-carbon donor or acceptor in various reactions involved in amino acid and nucleotide metabolism **(Miao *et al*., 2013)**.

Folic acid is an essential micronutrient for aquatic and terrestrial animals. **Duncan *et al.* (1993)** summarized the symptoms of folic acid deficiency as a reduction in growth and megaloblastic anemia characterized by pale livers, spleens, and kidneys and inappetence as well as the attenuated immune system and reduced resistance to diseases **(Tasbozan & Erbas, 2023)**. Consequently, retardation of growth and abnormal hematopoiesis in rainbow trout and the Nile tilapia **(Cowey & Woodward, 1993; Lim & Klesius, 2001)**, respectively. Some fish species need dietary folic acid for normal growth and hematopoiesis **(Lin *et al.*, 2011)**. **Choi and Kim (2005)** proved that the hematopoietic activity of fishes results from the synergic effect of many essential vitamins such as K, E, and D, as well as B12, B6, and B9 (folic acid).

Despite this, a plurality of previous studies have focused on the effect of folic acid on physiological and biochemical growth measurements, which are used as biomarkers for animal health status. Many of these studies manifest the effect of folic acid as a growth promotor of fish, particularly on fingerlings and juveniles **(Shiau & Huang, 2001; Barros *et al*., 2009; Sesay *et al*., 2016; Essay *et al*., 2017; Shalaby *et al*., 2019; Khan & Khan, 2020; Yeganeh Kari *et al*., 2022)**. Furthermore, folic acid may enhance nutrient bioavailability for aquatic animals and regulate glucose metabolism to improve their growth performance **(Xu *et al*., 2024)**. However, very few studies have been conducted on the effects of folic acid on fish reproductive performance.

Therefore, in the present study, some biochemical blood measurements such as blood total protein, albumin and globulins and aminotransferases (ALT, AST), urea, and creatinine, as well as sex hormones estradiol and testosterone of the adult Nile tilapia, *Oreochromis niloticus*, broodstock have been investigated in four experimental adult fish groups including control group of un-supplemented diet and the three treated fish groups with 2.5, 5.0 and 7.5mg folic acid/kg 30% crude protein commercial feed.

According to the results, some physiological and biochemical parameters were elevated in the fish fed with dietary folic acid compared to the control fish. Folic acid suppressed the urea, ALT, and AST liver enzymes as its amount increased in fish fodder. However, estradiol was lowered in T2 compared to control T1 after increasing in T3 and T4, and on the contrary, testosterone increased in T2 after decreasing in T3 and T4 simultaneously. The lowering level of both liver aminotransferases compared with those of the control fish group designates that folic acid did not hurt the liver of the experimented fish but, on the contrary, ameliorated liver status. Since ALT and AST are aggregated in the cytosol of hepatocytes, they are ordinarily measurable in the serum at low concentrations **(Knell, 1980)**. However, any disadvantage may lead to a loss of hepatocyte membrane integrity or necrosis, releasing these enzymes into the bloodstream, where their concentrations elevate **(Senior, 2012; Oh *et al*., 2017)**. Living animals, including fish with elevated blood aminotransferases, will have severe liver disorders **(Ioannou *et al*., 2006)**. ALT (alanine aminotransferase) and AST (aspartate aminotransferase) are liver enzymes. The first one helps in converting protein into energy **(Qian *et al*., 2015)**, but AST helps break down amino acids **(Ndrepepa, 2021)**.

Otherwise, the total blood protein, even its two main constituents, albumin and globulins, showed its highest levels in T3 of 5mg folic acid, demonstrating the lowest blood glucose. **Miao *et al*. (2013)** showed that dietary folic acid improves cell membrane function and mediates and regulates blood glucose.

Dietary folic acid also provoked obvious histological alterations in the gonads of the studied adult Nile tilapia. The liver appeared healthy in all treated fish. Therefore, folic acid does not have a notable effect on liver building. Hepatic parenchyma has regular polyhedral intact hepatocytes with many hepatic sinusoids in-between aggregating in small hepatic venules and large hepatic veins. The liver of fish fed with supplementary folic acid also showed hepatopancreatic acini, acquainted in all teleosts **(Nejedli and Gajger, 2013; Abdelrhman *et al*., 2022; Abusrer & Shtewi, 2023)**.

The liver is involved in the metabolism of all nutrients, even vitamins. Furthermore, it plays a principal role in removing toxins from the blood **(Hou *et al*., 2020)**. It has been well established by **Rinchard and Kestemont (2003)** that, dramatic changes occur in the liver of fish related to the gonadal maturation and vitellogenesis. Since vitellogenesis requires considerable metabolic energy **(Jalabert, 2005; Ladisa *et al*., 2021)**, helping in the production of many mature eggs filled with lipoprotein yolk. Metabolism accompanies seasonal variation in the reproductive cycle to sustain energy-demanding gonadal development **(Ladisa *et al*., 2022)**.

The manufactured vitellogenin in the liver is motivated by estradiol, which is significantly increased with the upgrading of supplementary folic acid in the present Nile tilapia. This vitellogenin is transported through the bloodstream to the ovaries to be processed into yolk proteins in the oocytes **(Reading & Sullivan, 2011; Mcbride *et al*., 2015)**.

Both gonads (ovary and testis) of the Nile tilapia fed dietary folic acid exposed some histological alterations that differed with the different acid doses. These alterations were evaluated by the degree of tissue change based on the lesion's severity **(Araujo *et al*., 2019)**. The Nile tilapia's ovaries fed supplementary folic acid revealed that the intact vitellogenic oocyte increased with increasing folic acid concentration, particularly in females of the T3 group fed on a 5.0mg folic acid/Kg diet. On the contrary, the atrophied ova appeared more in the control group than in the other groups, even those fed a 2.5mg folic acid/Kg diet. This indicates that the fish's ovulation rate intensified with supplementary folic acid.

Similarly, the tilapia's testis fed dietary folic acid also showed normal intact histological assembly. The seminiferous tubules and all developmental stages of sperms appeared in all experimented fish groups, particularly in fish fed with 5.0mg folic acid/kg. It also showed a significant positive increase with folic acid in double amounts.

Steroid hormone production decreased in response to vitamin E supplementation **(Mondul *et al*., 2011)**. Therefore, this vitamin has an irreplaceable role in promoting the ovary development of aquatic animals, including fishes, since it increases yolk granules in the oocytes **(Zhang *et al.*, 2007)**. Similarly, **Emata *et al.* (2000**) stated that in milkfish (*Chanos chanos*), supplementary Vitamin E improves the laying egg and its hatching since it ameliorates egg hatchability **(Miao *et al*., 2013)**.

Vitamin E increases reproductive performance and promotes reproduction of the Nile tilapia **(Zhang *et al.*, 2021)**. Similarly, vitamin B9 (folic acid) of the present study enhanced egg maturation and yok granules in the ovum's cytoplasm leading to the rising reproductive performance of tilapia. Furthermore, **Izquierdo *et al.* (2001)** and **Naiel *et al.* (2023)** declared that improvement of broodstock nutrition with supplementary items has been shown to greatly improve not only egg and sperm quality but also seed production, i.e., the reproductive performance of many cultured fish species. Vitamin E can also protect the sperm of the giant tiger prawn, *Penaeus monodon,* from oxidation and increase its survival **(Liu *et al.*, 2018)**. Amplification of folic acid levels in fodder of East Asian river prawn, *Macrobrachium nipponense*, significantly increases female and male fecundity and improves offspring survival **(Jiang *et al.*, 2023)**. Folic acid deficiency may result in impairing fish gill health status **(Shi *et al.*, 2015**) as well as mitigating disorders (anorexia) and megaloblastic anemia **(Sesay *et al*., 2017; Mohamed & Badran, 2021)**.

CONCLUSION

Dietary folic acid is an indispensable micro-nutrient in aquatic animals. It has many positive effects on metabolism and growth parameters. Furthermore, it is beneficial for the adequacy of fish' reproductive performance of the Nile tilapia broodstock. Therefore, we recommend adding folic acid as a nutritional supplement to broodstock before hatching in fish hatcheries.

Ethical approval

Applying all required measures, reviewed and approved by the ZU-IACUC Committee following the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, the National Research Council's Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978) and in compliance with the ARRIVE guidelines. With Approval number : ZU-IACUC/1/F/9/2025

REFERENCES

Abdelrhman, M.A.; Ashour, M.; Al-Zahaby, M.A.; Sharawy, Z.Z.; Nazmi, H.; Zaki, M.A.A.; Ahmed, N.H.; Ahmed, S.R.; El-Haroun, E.; Van Doan, H. and Goda, A.M.A. (2022). Effect of polysaccharides derived from brown macroalgae Sargassum dentifolium on growth performance, serum biochemical, digestive histology and enzyme activity of hybrid red tilapia, Aquaculture Reports, (25): 101212, ISSN 2352-134. https://doi.org/10.1016/j.aqrep.2022.101212.

Abusrer, **S.A. and Shtewi**, **H.H.** (2023). Morphological and histological structure of hepatopancreas in rock goby *Gobius paganellus* on the western coast of Libya. Open Vet. J., 13(10):1251-1258. doi: 10.5455/OVJ.2023.v13.i10.3

Akter, M.; Schrama, J.W.; Adhikary, U.; Alam, M.d.S.; Mamun-Ur-Rashid, M. and Verdegem, M. (2024). Effect of pellet-size on fish growth, feeding behaviour and natural food web in pond polyculture. Aquaculture, 593, 741342. https://doi.org/10.1016/j.aquaculture.2024.741342

Araujo, F.G.; Gomes, I.D.; Nascimento, A.A.; Santos, M.A. and Sales, A. (2019). Histopathological analysis of liver of the catfish, *Pimelodus maculatus* in a tropical eutrophic reservoir from South-eastern Brazil. Acta Scientiarum Biol. Sci., vol. 41, https://doi.org/10.4025/actascibiolsci.v41i1.41039

Asaikkutti, A.; Bhavan, P.S. and Vimala, K. (2016). Effects of different levels of dietary folic acid on the growth performance, muscle composition, immune response, and antioxidant capacity of freshwater prawn *Macrobrachium rosenbergii*. Aquaculture, 464, 136–144.

Bancroft, J.D. and Layton, C. (2019). The hematoxylins and eosin. In: Bancroft's Theory and Practice of Histological Techniques (Eds. Suvarna S.K., Layton C. and. Bancroft J.D), 8th Edit. Elsevier, Chapter 10 pp.126–138. https://doi.org/10.1016/B978-0-7020-6864-5.00010-4

Barros, M.M.; Ranzani-Paiva, M.J.T.; Pezzato, L.E.; Falcon, D.R. and Guimara, I. (2009). Haematological response and growth performance of Nile tilapia (*Oreochromis niloticus* L.) fed diets containing folic acid. Aquacul. Res., 40, 895–903.

Busher, J.T. (1990). Serum Albumin and Globulin. In Clinical Methods: The History, Physical, and Laboratory Examinations. Walker, H.K.; Hall, W.D. and Hurst, J.W., editors. 3rd edition. Boston, Butterworths Publishers, Chapter 101

Choi, J.W. and Kim, S.K., (2005). Relationship of Led, Copper, Zinc, and Cadmium levels versus hematopoiesis and iron parameters on healthy Adolescents. Ann. Clin. Lab. Sci., 35: 428–432.

Cowey, C.B. and Woodward, B. (1993). The dietary requirement of young rainbow trout (*Oncorhynchus mykiss*) for folic acid. Journal of Nutrition, 123, 1594–1600. https://doi.org/10.1093/jn/123.9.1594

Craig, S. and Helfrich, L.A. (2002). Understanding fish Nutrition, Feeds, and Feeding. Virginia Cooperative Extension Publication. 420-256. Virginia State, Petersburg. VT/028/0402//420256

Duncan, D.B. (1955). Multiple range and multiple F tests. Biometrics, 11, 1–41. https://doi.org/10.2307/3001478

Duncan, P.L.; Lovell, R.T.; Butterworth, C.E.; Jr Freeberg, L.E. and Tamura, T. (1993). Dietary folate requirement determined for channel catfish, *Ictalurus punctatus*. J. Nutrition, 123, 1888–1897. https://doi.org/10.1093/jn/123.11.1888

Emata, A.C.; Borlongan, I.G. and Damaso, J.P. (2000). Dietary vitamin C and E supplementation and reproduction of milkfish, *Chanos* Forsskal. Aquacul. Res., 31, 557– 564.

Firouz, A.; Soheil, L.; Hossein, K.; Shabanali, N.; Mohammad, B. and Mehdi, M. (2013). The effects of folic acid treatment on biometric and blood parameters of fingerling rainbow trout fishes (*Oncorhynchus mykiss*). J. Aquacul. Res. Devel., 4, 175. https://doi.org/10.4172/2155-9546.1000175

Guo, M. (2009). Vitamins and minerals as functional ingredients. In: Functional Foods, Principles and Technology. A volume in Woodhead Publishing. Series in Food Scie., Technology and Nutrition, Chapter 6. pp.197-236

Halver, J.E. (1989). The vitamins. In: Fish nutrition, 2nd edition, edited by J.E. Halver. Academic Press, New York & London., pp.31–109.

Halver, J.E. and Hardy, R.W. (2002). Fish Nutrition (3rd ed.), **Academic Press,** ISBN: 0123196523, 9780123196521, pp. 824.

Henry, R.J. (1974). Clinical chemistry: principles and techniques. 2**nd** edition, Harper and Row, New York, USA. 190.

Hien, D.V. and Doolgindachbaporn, S. (2011). Effect of niacin and folic acid in feed rations on growth and live weights of green catfish (*Mystus nemurus* Valenciennes 1840). Pakistan J. Biol. Sci., 14, 64–68.

Hou, Y.; Hu, S.; Li, X.; He, W. and Wu, G. (2020). Amino acid metabolism in the liver: Nutritional and physiological significance. Adv. Exp. Med. Biol., 1265: 21–37. doi: 10.1007/978-3-030-45328-2_2.

Ioannou, G.N.; Boyko, E.J. and Lee, S.P. (2006). The prevalence and predictors of elevated serum aminotransferase activity in the United States in 1999-2002. Am. J. Gastroenterol., 101(1):76-82. [PubMed]

Izquierdo, M.S.; Fernández-Palacios, H. and Tacon, A.G.J. (2001). Effect of broodstock nutrition on reproductive performance of fish. Aquaculture, 197, 25– 42. https://doi.org/10.1016/S0044-8486(01)00581-6

Jalabert, B. (2005). Particularities of reproduction and oogenesis in teleost fish compared to mammals. Reprod. Nutr. Dev. 45, 261–279. https://doi.org/10.1051/rnd:2005019

Jamalzad Falah, F.; Rajabi Islami, H. and Shamsaie, M.M. (2020). Dietary folic acid improved growth performance, immuno-physiological response, and antioxidant status of fingerling Siberian sturgeon, *Acipenser baerii* (Brandt, 1896). Aquaculture Reports, 17, 100391. https://doi.org/10.1016/j.aqrep.2020.100391.

Jiang, G.; Xue, Y. and Huang, X. (2023). Effects of dietary folic acid supplementation on sex differences in oriental river prawn, *Macrobrachium nipponense*. Animals, 13 (23), 3677. doi: 10.3390/ani13233677

Khan, Y.M. and Khan, M.A. (2020). Dietary folic acid requirement of fingerling Catla, *Catla catla* (Hamilton). Aquacul. Nutr., 26:1035–1045. https://doi.org/10.1111/anu.13061

Knell, A.J. (1980). Liver function and failure: the evolution of liver physiology. J. R. Coll. Physicians Lond., 14(3): 205–8.

Kocabas, A.M. and Gatlin, D.M., (1999). Dietary vitamin E requirement of hybrid striped bass (*Morone chrysops* female × *M. saxatilis* male). *Aquaculture Nutrition*, 5:3–7.

Ladisa, C.; Ma, Y. and Habibi, H.R. (2021). Seasonally related metabolic changes and energy allocation associated with growth and reproductive phases in the liver of male goldfish (*Carassius auratus*). J. Proteomics 241, 104237. https://doi.org/10.1016/j.jprot.2021.104237

Ladisa, C.; Ma, Y. and Habibi, H.R. (2022). Metabolic changes during growth and reproductive phases in the liver of female goldfish (*Carassius auratus*). Front. Cell Dev. Biol. 10:834688. doi: 10.3389/fcell.2022.834688

Lee, S.; Sonmez, O.; Hung, S.S. and Fadel, J.G. (2017). Development of growth rate, body lipid, moisture, and energy models for white sturgeon (*Acipenser transmontanus*) fed at various feeding rates. Animal Nutrition, 3(**1**), 46-60. https://doi.org/10.1016/j.aninu.2016.10.005.

Lim, C. and Klesius, P.H. (2001). Influence of dietary levels of folic acid on growth response and resistance of Nile tilapia, *Oreochromis niloticus* to *Streptococcus iniae*. Sixth Asian Fisheries Forum. Book of Abstracts, pp. 150.

Lin, Y.H.; Lin, H.Y. and Shiau, S.Y. (2011).Dietary folic acid requirement of grouper, *Epinephelus malabaricus*, and its effects on non-specific immune responses. Aquaculture, 317 (1–4): 133-137. https://doi.org/10.1016/j.aquaculture.2011.04.010

Liu, D.; Huang, J.; Zhou, F.; Yang, Q.; Jiang, S.; Yang, L.; Zhu, C. and Jiang, S. (2018). Synergistic effect of Vitamin E and *Schizochytrium* sp. on spermatophore regeneration in *Penaeus monodon*. South China Fisheries Science, 14(1), 20– 26. DOI: 10.3969/j.issn.2095-0780.2018.01.003

McBride, R.S.; Somarakis, S.; Fitzhugh, G.R.; Albert, A.; Yaragina, N.A.; Wuenschel, M.J.; Alonso-Fernández, A. and Basilone, G. (2013). Energy acquisition and allocation to egg production in relation to fish reproductive strategies. Fish and Fisheries. Wiley., 16, 23–57. http://doi.org/10.1111/faf.12043

Miao, S.; Zhang, M.S.W.; Xu, W. and Mai, K. (2013). Dietary folic acid requirement of juvenile abalone, *Haliotis discus hannai.* Ino. *Aquaculture*, 400–401, 73-76. https://doi.org/10.1016/j.aquaculture.2013.03.005

Mohamed, M.A. and Badran, M.A.F. (2021). Effects of folic acid on growth performance and blood parameters of flathead grey mullet, *Mugil cephalus*. Aquaculture, 536, 736459. https://doi.org/10.1016/j.aquaculture.2021.736459

Mondul, A.M.; Rohrmann, S.; Menke, A.; Feinleib, M.; Nelson, W.G.; Platz, E.A. and Albanes, D. (2011). Association of Serum α-Tocopherol with Sex Steroid Hormones and Interactions with Smoking: Implications for Prostate Cancer Risk. Cancer Causes Control, 22(6): 827–836. doi:10.1007/s10552-011-9753-4.

Naiel, M.A.E.; Eissa, E.H.; El-Aziz, Y.M.; Saadony, S.; Abd Elnabi, H.E. and Sakr, S.E. (2023). The assessment of different dietary selenium resources on reproductive performance, spawning indicators, and larval production of red tilapia (*Oreochromis mossambicus* × *O. niloticus*) broodfish. Aquacult. Nutr., Article ID 5596619, 11 pages. https://doi.org/10.1155/2023/5596619

Ndrepepa, G. (2021). Aspartate aminotransferase and cardiovascular disease-a narrative review. J. Lab. Precis. Med., Vol. 6: 6. doi: 10.21037/jlpm-20-93

Nejedli, S. and Gajger, I.T. (2013). Hepatopancreas in some sea fish from different species and the structure of the liver in teleost fish, common pandora, *Pagellus erythinus* (Linnaeus, 1758) and whiting, *Merlangius merlangus euxinus* (Nordmann, 1840). Vet. Arhiv. 83(4), 441-452.

Oh, R.C.; Hustead, T.R.; Ali, S.M.; Pantsari, M.W. (2017). Mildly Elevated Liver Transaminase Levels: Causes and Evaluation. Am. Fam. Physician., 96(11):709–715. [PubMed]

Qian, K.; Zhong, S.; Xie, K.; Yu, D.; Yang, R. and Gong, D.W. (2015). Hepatic ALT isoenzymes are elevated in gluconeogenic conditions including diabetes and suppressed by insulin at the protein level. Diabetes Metab. Res. Rev., 31(6):562–571. doi: 10.1002/dmrr.2655

Reading, B.J. and Sullivan, C.V. (2011). The Reproductive Organs and Processes: Vitellogenesis in Fishes. Maryland Heights, MO: Elsevier, 635–646. https://doi.org/10.1016/B978-0-12-374553-8.00257-4

Reitman, S. and Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American J. Clinical Pathol., 28(1): 56-63. DOI: 10.1093/ajcp/28.1.56

Rinchard, J. and Kestemont, P. (2003). Liver changes related to oocyte growth in roach, a single spawner fish, and in bleak and white bream, two multiple spawner fish. Internat. Rev. Hydrobiol. 88, 68–76. https://doi.org/10.1002/iroh.200390006

Senior, J.R. (2012). Alanine aminotransferase: a clinical and regulatory tool for detecting liver injury-past, present, and future. Clin. Pharmacol. Ther., 92(3): 332-9.

Sesay, D.F., Habte-Tsion, H-M.; Zhou, Q.; Ren, M.; Xie, J.; Liu, B.; Chen, R. and Pan, L. (2016). Effects of dietary folic acid on the growth, digestive enzyme activity, immune response and antioxidant enzyme activity of blunt snout bream

(*Megalobrama amblycephala*) fingerling. Aquaculture, 452, 142–150. https://doi.org/10.1016/j.aquaculture.2015.10.026

Sesay, D.F.; Tsion, H.M.; Zhou, Q.; Ren, M.; Xie, J.; Liu, B.; Ruli, C. and Pan, L. (2017). The effect of dietary folic acid on biochemical parameters and gene expression of three heat shock proteins (HSPs) of blunt snout bream (*Megalobrama amblycephala*) fingerling under acute high temperature stress. Fish Physiology and Biochemistry, 43, 923–940. https://doi.org/10.1007/s10695-016-0311-6

Shalaby, A.M.; Ghareeb, A.A.; Abd El-Rahman, M.M. and Abd El-Hamid, E.A.A. (2019). Effect of different levels of folic acid on the growth and some physiological aspects of Nile tilapia, *Oreochromis niloticus*. Egypt. J. for Aquacul., 9 (3):33-45 DOI: 10.21608/eja.2019.19184.1008

Shi, L.; Feng, L.; Jiang, W.D.; Liu, Y.; Jiang, J.; Wu, P.J.; Zhao Kuang, S.Y.; Tang, L.; Tang, W.N.; Zhang, Y.A. and Zhou, X.Q. (2015). Folic acid deficiency impairs the gill health status associated with the NF-KB, MLCK and Nrf2 signalling pathways in the gills of young grass carp (*Ctenopharyngodon idella*). Fish and Shellfish Immunology, 47(1), 289–301. doi: 10.1016/j.fsi.2015.09.023.

Shi, L.; Zhai, H.; Wei, L.; Zeng, F.; Ren, T. and Han, Y. (2022). Effects of dietary folic acid on growth performance, body wall folate content, immune and antioxidant enzyme activity of juvenile sea cucumber, *Apostichopus japonicus*. Aquaculture Research, 53, 6219–6226. https://doi.org/10.1111/are.16095

Shiau, S.Y. and Huang, S.Y. (2001). Dietary folic acid requirement for maximum growth of juvenile tilapia, *Oreochromis niloticus* × *O. aureus*. Fisheries Sci., 67, 655–659.

Suvarna, S.K.; Layton, C. and Bancroft, J.D. (2012). Bancroft's Theory and Practise of Histological Techniques. (Co-author), 7th edition, Elsevier, China: Churchill Livingstone;

Tasbozan, O. and Erbas, C. (2023). Antioxidant additives in fish feeds. Black Sea J. of Agricul. 6(3). DOI: 10.47115/bsagriculture.1246497

Trinder, P. (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor, Ann. Clin. Biochem., 6, 24-25.

Xiang, X.; Zhou, X.Q.; Chen, G.F.; Wu, P. and Zheng, Z.L. (2016). Effect of graded levels of **dietary thiamine** on the growth performance, body composition and hemato-biochemical parameters of juvenile *Sclizothorax prenanti*. Aquaculture Nutrition, **22**, 691–697.

Xu, X.; Ping, S.; Wang, F.; Chang, M.S. and Song, G. (2024). Folic acid improves the growth and immune response of crayfish (*Procambius clarkii*) by regulating glucose metabolism. Aquaculture Rep. 36, 1-10. 102077, https://doi.org/10.1016/j.aqrep.2024.102077

Yeganeh Kari, A.; Ershad Langeroudi, H.; Valipour, A. and Alinezhad, S. (2022). Fingerling beluga sturgeon, *Huso huso* (Linnaeus, 1758) growth, hematological, biochemical parameters and opercular respiratory rate under hypoxia challenge with levels of dietary folic acid. Iranian J. Fish. Scie., 21(6) 1558-1572. DOI: 10.22092/ijfs.2023.128564

Zhang, G.; He, R.; Zhang, S.; Cao, K. and Gao, H. (2007). Effect of vitamin E in broodstock diet on reproductive performance of *Monopterus albus*. Acta Hydrobiologica Sinica, 02, 196– 200.

Zhang, X.; Ma, Y. and Xiao, J. (2021). Effects of vitamin E on the reproductive performance of female and male Nile tilapia (*Oreochromis niloticus*) at the physiological and molecular levels. Aquacul. Res., (52): 3518–3531. https://doi.org/10.1111/are.15193

Zhao, Z.; Wen, H.; Wu, F. and Liu, A. (2008). Dietary folic acid requirement for grass carp fingerling, *Ctenopharyngodon idella*. Journal of Shanghai Fisheries University, 17, 187–192.