Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(1): 571 – 582 (2025)
www.ejabf.journals.ekb.eg

The Role of Vitamin K on Growth Performance, Hematology and Immune Responses of the African Catfish *Clarias Gariepinus*

Mahmoud Y. Abdelaziz[1], Ahmed M. Al-zayat[1], Mahmoud A. O. Dawood[2], Ahmed F. Abdelhamid[1*]

[1]Department of Fish production, Faculty of Agriculture in Cairo, Al-Azhar University, Egypt
[2]Department of Animal Production, Faculty of Agriculture, Kafrelsheikh University, Kafr El Sheikh, Egypt
***Corresponding Author:** ahmed.abdelhamid@azhar.edu.eg

ARTICLE INFO

Article History:
Received: Oct. 16, 2024
Accepted: Dec. 11, 2024
Online: Jan. 18, 2025

Keywords:
Vitamin K,
Clarias gariepinus,
Growth performance,
Hematological parameters,
Immune response

ABSTRACT

The African catfish (*Clarias gariepinus*) is a highly valuable species for aquaculture; it is extensively practiced in many countries across the world, involving Egypt. Therefore, the study aimed to investigate the potential effects of vitamin K (VK) on growth performance, haemato-chemical parameters, immune indices and antioxidant capacity. Four diets, each containing 30% crude protein, were formulated with 0.0% (control), 3mg kg^{-1} diet, 6mg kg^{-1} diet, and 9mg kg^{-1} diet of VK. These diets were fed to the African catfish with an initial weight of 77.64 ± 4.89g ad libitum, three times daily, for 90 days. The fish were fed the experimental diet at 3% of their biomass daily. The results show that supplemental VK improves growth performance, feed utilization, immune responses, total protein, albumin, hemoglobin, and red blood cell count compared to the control. This study suggests that the optimal dietary VK level is 9mg VK kg^{-1} diet due to its role in supporting growth, physiological functions, immune responses, welfare, and sustainability in the catfish diet industry.

INTRODUCTION

Vitamin K, a fat-soluble vitamin belonging to the naphthoquinone family, is distinguished by a unique side chain structure. There are two primary forms: K1 (phylloquinone) and K2 (menaquinones). K2 consists of a series of compounds known as menaquinone-n (MK-n), each with a different number of isoprene units (n=2-15). Among these, MK-4, MK-7, and MK-9 have been extensively researched **(Yan *et al.*, 2023)**. The synthetic, water-soluble version of vitamin K3, menadione, is transformed inside the liver to vitamin VK2. The majority of uses for vitamin K3 are in industry and research **(Vo *et al.*, 2023)**. There are further forms of vitamin K, such as K4 and K5, however they are only accessible in synthetic form, according to **Khalil *et al.* (2021)**. Green leafy vegetables are rich in vitamin K, particularly vitamin K1 (phylloquinone), a major dietary source. Additionally, algae produce vitamin K1 through photosynthesis. Human gut microbiota produces vitamin K2, which is mostly obtained through microbial sources in

food. Fermented foods like curds, cheese, cream, butter, sour cream, and fermented soybean product natto, along with eggs, poultry, and gammon, are common sources of vitamin K2 **(Wang *et al*., 2023)**. Alot of the vitamin K intake in the western diet comes from vitamin K1. However, vitamin K2 may be at least as significant for its bioactivity as vitamin K1 due to its greater ability for protection **(Piscaer *et al*., 2023)**. Vitamin K plays a crucial role in both fish and mammals, particularly in regulating blood clotting. It does so by facilitating the synthesis of prothrombin, a protein essential for controlling blood coagulation time **(Jiang & Doolittle, 2003)**. Additionally, vitamin K has an effect on bone quality, metabolism and health and management of Ca_2^+ homeostasis **(Zhou *et al*., 2009)**. Moreover, it functions as an enzymatic co-factor in physiological processes and is involved in inflammation, energy metabolism, sepsis, neoplasia, and diseases of renal **(Arai *et al*., 2008)**.

It is debatable and unclear how much vitamin K is essential for farmed fish. There have been a variety of contradictory studies reported about the vitamin K effects on farmed fish and the symptoms of its shortage. For example, bone anomalies in haddock were caused by a decrease in bone mass and mineralization brought on by dietary vitamin K insufficiency (*Melanogrammus aeglefinus*) and mummichog (*F. heteroclitus*) **(Miho, 2004; Roy & Lall, 2007)**. Vitamin K deficiency in the diet of the amago salmon *(Oncorhynchus rhodurus)* leads to several negative health effects, including reduced growth rate and enhanced mortality rates **(Taveekijakarn *et al*., 1996)**. Other indications of vitamin K insufficiency that have been reported are: increasing blood coagulation time, anaemia, fins lost, the onset of spine curvature and bones weakness **(Lall & Lewis-McCrea, 2007)**.

Vitamin K supplementation has been shown to enhance growth, food absorption, and digestion in common carp (*C. carpio*), as well as increase antioxidant capacity in both the common carp and the rainbow trout **(Yuan *et al*., 2016)**. Notably, administering a supra-nutritional dose of MK4 preserved the survival period of alb creERT2; Gpx4fl/fl mice in the face of vitamin E deficiency, offering strong protection against associated pathological alterations and liver lipid peroxidation. Since fish cannot produce vitamin K on their own, it must be supplied through their diet **(Abdelhamid *et al*., 2024)**. The purpose of this investigation was to assess the effects of different vitamin K supplementation levels (0, 3, 6, and 9mg kg^{-1} diet) on the African catfish (*Clarias gariepinus*). The primary objectives were to evaluate the impact on growth performance, immune responses, and nutritional utilization.

MATERIALS AND METHODS

Experimental catfish

One hundred eighty adult African catfish (*Clarias gariepinus*) were sourced from a private hatchery in Kafr El-Sheikh City, Egypt, and carefully transported to the Fish Nutrition Laboratory at the National Institute of Oceanography and Fisheries (NIOF), Kafr El-Sheikh, Egypt. The fish underwent a two-week acclimation period in rectangular concrete tanks (5m × 5m × 1m) under very stringent guidelines, including a water temperature of 27 ± 3°C, 24-hour aeration (dissolved oxygen 5mg/ L), and a feeding regimen of a commercial basal diet (30% protein) three times daily (9:00, 12:00 and 15:00h) until satiation (Table 1). Healthy catfish with an initial weight of 77.64 ± 4.89g were dispersed at random, in triplicates, into 12 haps net (1x1x0.7m), at a density of 15 fish per hapa net, all haps of the experiment were fixed into (4 × 2 × 1m) concrete tanks under green house.

Table 1. Composition and proximate analyses of the basal diet (as fed)

Ingredient	g /kg	Chemical composition	(%)
Anchovy meal (60% CP)	20.00	Crud protein (CP)	30.19
Poultry by-product (60% CP)	90.00	Ether extract (EE)	12.46
Soybean meal (46% CP)	390.00	Ash	13.44
Yellow corn	98.50	NFE[c]	43.89
Wheat bran	185.00	GE[d]	4600 kcal/kg
Rice bran	185.00		
SB Oil	10.00		
Mono calcium phosphate	10.00		
Vitamin premix[a]	5.00		
Mineral premix[b]	5.00		
Methionine	0.50		
Lysine	0.50		
Vitamin C	0.50		
Total	1000		

[a]Vitamin premix (per kg of premix): Vitamin B1, 700 mg; Vitamin B2, 3500 mg; Vitamin B6, 1000 mg; Vitamin B12, 7 mg; Vitamin A, 8,000,000 IU; Vitamin D3, 2,000,000 IU; Vitamin E, 7000 mg; biotin, 50 mg; folic acid, 700 mg; nicotinic, 20,000 mg; pantothenic acid, 7000 mg.
[b]Mineral premix (per kg of premix): Calcium carbonate as carrier up to 1 kg for zinc, 40 g; iron, 20 g; copper, 2.7 g; iodine, 0.34 g; manganese, 53 g; selenium, 70 mg and cobalt, 70 mg.
[c]Nitrogen free extract (calculated by differences) (NFE) = 100- (CP%+ EE%+CF%+ Ash%)].
[d]Gross energy, was calculated using the 5.65, 9.45 and 4 for CP, EE and NFE, respectively.

Experimental diets

Four experimental isonitrogenous (30% CP), isocaloric (18.61 MJ/kg) diets were formulated, comprising four concentrations of vitamin K3 (menadione) (0.0, 3.0, 6.0, and

9.0mg kg^{-1} diet), designated as D_0, D_3, D_6 and D_9, respectively, which were purchased from Dakahlia Poultry Company. Table (1) shows the experimental diet composition as well as its proximate chemical analysis. The fish were given test diets for ninety days three separate times a day: 9:00, 12:00, and 15:00hr. Each day, the fish received 3% of their body weight in food. Moreover, every ten days, fish in each hapa were sampled and weighed. The daily amount of feed given to every hapa was recalculated based on the average weight of the fish within that hapa. Upon completion of the feeding experiment, all fish in every hapa were harvested, weighed individually, and the average final weight was recorded.

Growth parameters calculation

Growth performance and feed utilization efficiency were estimated as follows:

Weight gain (WG) = final weight (g) – initial weight (g)

The specific growth rate (SGR, %/d) = {Ln final mean body weight – Ln initial mean body weight / time intervals (days)} x 100.

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

Protein efficiency ratio (PER) = weight gain (g)/CP intake (g)

Proximate composition analyses

At the conclusion of the trial, 5 fish were randomly chosen from every hapa and were preserved at −20°C for the ultimate body composition study. A pooled sample of ten fish per treatment was stored frozen and weighed for the first body analysis prior to the research. Approximate assessments of whole-body hydration, protein, fat, and ash were performed using the standard **AOAC (2005)** procedures. In conclusion, the moisture content of fish samples was ascertained by drying them at 105°C until they reached a consistent weight.

Following acid digestion, protein was quantified as nitrogen using a semi-automatic Kjeldahl (N × 6.25; VELP Scientific, UDK 126, Italy). Petroleum ether (40–60°C) was used as the solvent in a gravimetric method to assess the lipid content after Soxhlet extraction. Ash concentration was measured six hours after ignition at 550°C in a muffle furnace. For every parameter, three samples were used for each analysis.

Hematology and immunological examination

Heparinized syringe needles (15 unit/mL; 5000 IU, Amoun Pharmaceutical Co., Cairo, Egypt) were used to draw blood from the caudal vein of five fish in each hapa at the conclusion of the feeding trial. The blood sample was divided into 2 parts. The first part that was utilized for hematology (including total leucocytic count, hemoglobin, hematocrit, and red blood cell count) was kept in heparinized Eppendorf tubes. After allowing the remaining portion to coagulate at 4°C in tubes devoid of heparin, the plasma was separated and stored at -18°C for use in plasma analysis. The plasma was then centrifuged for 5 minutes at an ambient temperature at 5000rpm.

Red blood cells (RBCs) were diluted with phosphate-buffered saline (pH, 7.2) and counted under a light microscope using a Neubauer hemocytometer **(Shalaby *et al.*, 2019)**. Hematocrit (Hct) and total leucocytic count (TLC) were measured according to **Rehulka (2000)**

A sample was obtained by centrifuging fresh blood for five minutes in a microhematocrit centrifuge, after which the packed cell volume was measured in glass capillary tubes. Hemoglobin (Hb) concentration was determined according to **Jain (1993)**.

Albumin content and total protein were assessed as described by **Cho and Giovannoni (2004)**. The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were defined colorimetrically according to **Reitman and Frankel (1957)**.

Biochemical and hepatic functions analyses

Total protein (TP) was measured with the Vitros TP instrument utilizing dry and wet biochemical slides and a clinical chemistry analyzer (Microlab 300, ELI Tech Group) **(Mariana *et al.*, 2011)**. Moreover, creatinine (CR) was measured using the clinical chemistry analyzer (Microlab 300, ELI Tech Group).

Immunological indices

Lysozyme activity assay

The Lysozyme (LZM) ELISA Kit (Cat No.:SL0050FI, SunLong Biotech Co., LTD, China) was used to evaluate the serum lysozyme activity. The *Micrococcus lysodeikticus* cells and the lysozyme sample were incubated in compliance with the manufacturer's instructions. The absorbance drop at 450nm was used to measure the response.

Antioxidant activity assay

Colorimetric measurement of serum superoxide dismutase (SOD) was performed at 560nm (Cat NO.: SD2521, Biodiagnostic Co., Egypt) **(Nishikimi *et al.*, 1972)**. On the other hand, catalaze was colorimetrically measured at 510nm (Cat NO.: CA2517, Biodiagnostic Co., Egypt) **(Aebi, 1984)**. Lipid peroxide (Malondialdehyde, MDA) was colorimetrically assessed at a wavelength of 534nm (Cat NO.: MD2529, Biodiagnostic Co., Egypt) **(Ohkawa *et al.*, 1979)**. The Beers and Sizer method, which uses spectrophotometric detection of H_2O_2 breakdown, measured at 240nm, was used in the catalaze (CAT; EC 1.11.1.6) activity assay **(Beers & Sizer, 1952)**. Following the rate of NADPH oxidation at 340nm, glutathione reductaze and glutathione peroxidaze (GPx; EC 1.11.1.9) were coupled, and the activities of each enzyme were assessed. The specific activity was computed using the extinction coefficient of 6.22 mM/cm **(Carlberg & Mannervik, 1975)**. The WAKO LabAssay Series of kits (Shanghai Zhenzhun Biotechnology Co., LTD) was utilized to analyze cholesterol, triglycerides, urea, and natural immunoglobulin IgM using an automated biochemical analyzer (HITACHI7180).

Statistical analysis

The standard error of the mean (SEM) was calculated and expressed as mean ± SEM. One-way ANOVA was proceeded to determine if there were significant differences ($P< 0.05$) among the treatments. The statistical software SPSS Statistics, version 22.0 (IBM Corp., Armonk, NY), was used for the analyses. The means were compared using Duncan's multiple range test when the ANOVA F-values were significant *(*$P< 0.05$).

RESULTS

Growth performance and nutrient indices

Table (2) summarizes the growth performance of the African catfish (*Clarias gariepinus*) fed experimental diets enhanced with various levels of vitamin K (menadione VK3, VK). Fish fed vitamin K diets (D3, D6, D9) exhibited significantly superior growth performance than the control group (D0). This was reflected in higher final weight, weight gain, average daily gain, specific growth rate, feed efficiency ratio, and protein efficiency ratio. Conversely, the feed conversion ratio (FCR) was significantly lower in fish fed the highest vitamin K dose (D9) than in those fed the other treatments (D0, D3, D6), which did not show any significant differences among themselves.

Table 2. Growth performance and feed efficiency (mean± SE; *n* = 3) of the African catfish (*Clarias gariepinus*) fed on different concentrations of vitamin K for 90 days

Item	Treatments			
	D_0	D_3	D_6	D_9
IW	77.77±4.39	77.63±5.38	77.53±5.39	77.63±4.38
FW	$161.07±6.89^{c}$	$165.00±6.99^{bc}$	$171.07±5.27^{ab}$	$177.13±5.05^{a}$
WG	$83.30±2.50^{c}$	$87.37±2.37^{bc}$	$93.43±1.59^{ab}$	$99.60±2.15^{a}$
ADG	$0.92±0.03^{c}$	$0.97±0.03^{bc}$	$1.04±0.02^{ab}$	$1.10±0.02^{a}$
FCR	$1.14±0.03^{a}$	$1.07±0.03^{a}$	$1.06±0.02^{a}$	$0.82±0.02^{b}$
SGR	$1.04±0.02^{c}$	$1.08±0.03^{bc}$	$1.13±0.02^{ab}$	$1.18±0.02^{a}$
FER	$0.87± 0.08^{c}$	$0.99± 0.01^{bc}$	$1.09± 0.34^{ab}$	$1.14± 0.87^{a}$
PER	$1.92± 0.84^{c}$	$2.54± 0.06^{bc}$	$2.77± 0.04^{ab}$	$2.82± 0.09^{a}$

Means in the same row with various characters are significantly different ($P< 0.05$).

Immunological and antioxidant responses

As presented in Table (3), the inclusion of vitamin K in the diet (D3, D6, D9) did not result in significant differences in antioxidant enzyme activities (superoxide dismutase, catalase, glutathione peroxidase) or lipid peroxidation (malondialdehyde) compared to the control group (D0). However, fish fed vitamin K diets exhibited significantly higher levels of IgM and lysozyme, particularly in the D6 and D9 groups, suggesting a positive impact on the immune system.

Table 3. Biochemical immunological and antioxidant parameters (mean ± SE; n = 3) of the African catfish (*Clarias gariepinus*) fed on different concentrations of vitamin K for 90 days

Item	Treatments			
	D_0	**D_3**	**D_6**	**D_9**
IgM (mg/mL)	384.00±28.31^b	373.00±17.73^b	488.00±24.04^a	484.67±25.2^a
Lysozyme (µg/mL)	5.88±0.29^b	6.13±0.18^b	5.98±0.13^a	6.10±0.17^a
SOD (U/g protein)	55.00±5.15	55.20±6.15	55.90±6.73	56.00±4.15
CAT (U/g protein)	5.90±0.52	5.30±0.17	5.90±0.23	5.22±0.17
GPX (U/g protein)	3.60±0.23	3.23±0.04	3.78±0.23	3.90±0.46
MDA (nmol/g protein)	5.90±0.35	5.77±0.40	5.60±0.35	5.05±0.40

Means in the same row with various characters are significantly different (P< 0.05).

Haemato-biochemical parameters

As presented in Table (4), the inclusion of vitamin K in the diet resulted in a progressive increase in albumin and total protein levels, indicating an improved liver function. However, vitamin K supplementation did not significantly alter the levels of alanine transaminase, aspartate transaminase, cholesterol, triglycerides, creatinine, or urea. Table (5) further demonstrates that vitamin K supplementation significantly enhanced hematological parameters, including hemoglobin concentration, red blood cell count, hematocrit, and monocyte count, particularly at higher dietary levels (D6, D9). In contrast, white blood cells, eosinophils, basophils, and neutrophils remain unaffected by vitamin K supplementation.

Table 4. Blood biochemical parameters of the African catfish (*Clarias gariepinus*) fed on different concentrations of vitamin K for 90 days

Item	Treatments			
	D_0	**D_3**	**D_6**	**D_9**
ALT (U/L)	26.11±3.58	25.09±1.58	24.01±2.87	26.21±1.58
AST (U/L)	279±12.31	286±14.04	282±12.89	291±11.73
Cho (Mg/dl)	229±12.31	201±10.15	211±12.15	219±11.45
Tri (Mg/dl)	287±10.30	266±9.40	274±16.70	290±8.50
Total protein (g/dl)	2.81±0.20^d	3.89±0.19^c	4.33±0.30^b	4.98±0.15^a
Albumin (g/dl)	0.79±0.01^d	1.02±0.02^c	1.44±0.04^b	1.96±0.05^a
Cr (Mg/dl)	0.24±0.02	0.22±0.01	0.23±0.06	0.21±0.02
Urea (Mg/dl)	0.56±0.03	0.62±0.02	0.64±0.10	0.59±0.01

Means in the same row with various characters are significantly different (P< 0.05).

Table 5. Impact of vitamin K levels on haematological responses of the African catfish (*Clarias gariepinus)*

Measurement	Treatments			
	D_0	**D_3**	**D_6**	**D_9**
Hb (g/dl)	5.90 ± 0.52^{c}	10.40 ± 0.23^{b}	15.13 ± 0.35^{a}	15.19 ± 0.17^{a}
RBCs ($(10^{6}\mu L^{-1})$)	1.10 ± 0.06^{c}	1.93 ± 0.04^{b}	2.86 ± 0.23^{a}	2.96 ± 0.17^{a}
WBCs($(10^{4}\mu L^{-1})$)	95.59±0.18	95.12±0.18	96.40±0.18	97.56±0.18
Hct %	25.29 ± 1.17^{c}	33.89 ± 2.51^{b}	40.61 ± 3.35^{a}	42.00 ± 2.29^{a}
Lymphocytes %	74.33 ± 5.17^{c}	79.47 ± 6.96^{b}	82.20 ± 6.24^{a}	83.34 ± 5.62^{a}
Monocytes %	6.56 ± 0.35^{c}	8.05 ± 0.29^{b}	10.00 ± 0.40^{a}	10.50 ± 0.46^{a}
Eosinophils %	2.00±0.12	2.21±0.18	2.07±0.18	2.10±0.26
Basophils%	1.21±0.02	1.41±0.03	1.13±0.14	1.19±0.21
Neutrophil %	6.32±0.29	6.90±0.17	6.66±0.29	6.30±0.17

Means in the same row with various characters are significantly different ($P< 0.05$).

DISCUSSION

The results of studies on vitamin K in fish vary, likely due to differences in vitamin K is a requirement among fish species. Research on the vitamin K needs of the catfish species is limited and requires further investigation **(Abdelhamid *et al*., 2024)**. The existing investigation reported that supplemental vitamin K improves the growth performance and feed utilization. These results agree with those of **Taveekijakarn *et al*. (1996)**, who observed that the deficiency of vitamin K in fish diet decreased growth performance and improved mortality in the Amago salmon (*Oncorhynchus rhodurus*). This is contradictory to **Abdelhamid *et al*. (2024)**, who reported that increasing VK levels improved the growth performance of the Nile tilapia (*Oreochromis niloticus*) up to a certain point, after which higher levels led to poor growth. Thus, while vitamin K plays a key role in supporting growth, this effect is limited and not continuous. It is worth noting that the feed conversion ratio (FCR) improved significantly in the fourth treatment (D9), a result consistent with **Abdelhamid *et al.* (2024)**. The improvement in FCR has a positive effect on economic returns, which encourages the sustainability of African catfish production.

The primary defense mechanism against pathogens is the fish's inherent immune system **(Saurabh & Sahoo, 2008)**. In the present study, supplementation of vitamin K (VK) in the diet of the African catfish (*Clarias gariepinus*) significantly increased the levels of lysozyme (LYZ) and immunoglobulin M (IgM), particularly at higher VK doses (D6 and D9) compared to the control group (D0) and lower VK doses (D3). These findings suggest that dietary VK enhances the humoral immune response in the African catfish. The positive effects of vitamin K (VK) on fish and shrimp may be attributed to its ability to boost immune responses and maintain the antioxidant defense system.

Therefore, it is evident that VK enhances immunological responses and modulates the activities of antioxidant enzymes in farmed fish **(Abdelhamid *et al*., 2024)**.

In the current study, dietary supplementation of VK significantly decreased hepatic malondialdehyde (MDA) levels, a marker of lipid peroxidation. Additionally, VK-fed fish groups exhibited a non-significant increase in the activities of hepatic superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) compared to the control group. The levels of SOD, CAT, and GPX in the serum serve as biomarkers of the body's antioxidant defense system. These enzymes are essential for the removal of free radicals and the prevention of oxidative damage to fish tissues **(Hoseinifar *et al.*, 2020)**. The total protein test measures the combined concentration of albumin and globulin, two major protein classes found in the fluid component of blood. Proteins are essential constituents of all cells and organs

One important factor in preventing blood vessel fluid leakage is albumin. The correlation between elevated levels of total protein and albumin and a stronger innate immune response in fish suggests that these proteins are crucial for supporting the fish's defense mechanisms **(Ayoub *et al.*, 2019)**. Based on the current findings, fish fed vitamin K exhibited significantly higher levels of total protein and albumin compared to the control group. These results suggest that VK positively impacts immune response in the striped catfish.

Hemato-biochemical parameters are commonly used to assess the nutritional status, general health, and environmental adaptability of fish **(Abdelhamid *et al.*, 2021)**. In this study, fish fed vitamin K diets showed significant increases in hemoglobin (Hb), red blood cell (RBC) count, and hematocrit (Hct) levels. An elevated RBC count indicates a higher oxygen-carrying capacity of the blood. These results are consistent with several previous studies that incorporated vitamin K into fish diets.

CONCLUSION

Finally, the current research indicates that the African catfish (*Clarias gariepinus*) grow more successfully when supplemented with vitamin K. Innate immune indicators significantly improved when vitamin K was included in the diet. Overall, the results suggest that adding vitamin K to aquafeeds at levels up to 9mg kg^{-1} diet is a promising strategy for promoting growth.

ACKNOWLEDGMENTS

All researchers of the research study extend their thanks and gratitude to the Administration of the Fish Nutrition Laboratory, Baltim Research Station, National Institute of Oceanography and Fisheries (NIOF) for providing the opportunity to conduct the research experiment and providing the necessary tools for that.

REFERENCES

Abdelhamid, A.F.; Ayoub, H.F.; Abd El-Gawad, E.A.; Abdelghany, M.F. and Abdel-Tawwab, M. (2021). Potential effects of dietary seaweeds mixture on the growth performance, antioxidant status, immunity response, and resistance of striped catfish (*Pangasianodon hypophthalmus*) against Aeromonas hydrophila infection. *Fish & Shellfish Immunology*, *119*, 76-83.

Abdelhamid, A.F.; Gewida, A.G.; El-Sayed, A.F.M. and Badran, M.F. (2024). Impacts of different levels of vitamin K on the growth performance, hematological parameters, and immunological response of juvenile Nile tilapia (Oreochromis niloticus). *Aquaculture International*, *32*(1), 477-488.

Aebi H (1984) [13] Catalase in vitro. Methods in enzymology: Elsevier. pp. 121–126.

AOAC (2005) Association of official analytical chemists. Official Methods of Analysis (16th edn). Author Arlington, VA, USA.

Arai, H.; Nagai, K. and Doi, T. (2008). Role of growth arrest-specific gene 6 in diabetic nephropathy. *Vitamins & Hormones*, *78*, 375-392.

Ayoub, H.F.; Abdelghany, M.F. and El-Sayed, A.B. (2019). Effects of Diatoms Amphora coffeaeformis on growth parameters, non-specific immunity and protection of the Nile tilapia (*Oreochromis niloticus*) to Aeromonas hydrophila infection. *Egyptian Journal of Aquatic Biology and Fisheries*, *23*(1), 413-426.

Beers, R.F. and Sizer, I.W. (1952). A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J Biol chem*, *195*(1), 133-140.

Carlberg, I.N.C.E.R. and Mannervik, B.E.N.G.T. (1975). Purification and characterization of the flavoenzyme glutathione reductase from rat liver. *Journal of biological chemistry*, *250*(14), 5475-5480.

Cho, J.C. and Giovannoni, S.J. (2004). Oceanicola granulosus gen. nov., sp. nov. and Oceanicola batsensis sp. nov., poly-β-hydroxybutyrate-producing marine bacteria in the order 'Rhodobacterales'. *International Journal of Systematic and Evolutionary Microbiology*, *54*(4), 1129-1136.

Hoseinifar, S.H.; Yousefi, S.; Van Doan, H.; Ashouri, G.; Gioacchini, G.; Maradonna, F. and Carnevali, O. (2020). Oxidative stress and antioxidant defense in fish: the implications of probiotic, prebiotic, and synbiotics. *Reviews in Fisheries Science & Aquaculture*, *29*(2), 198-217.

Jain NC (1993) Essentials of Veterinary Hematology. Lea and Febiger, Philadelphia, pp 76–250.

Jiang, Y. and Doolittle, R. F. (2003). The evolution of vertebrate blood coagulation as viewed from a comparison of puffer fish and sea squirt genomes. Proc. Natl. Acad. Sci. USA 100, 7527–7532.

Khalil, Z.; Alam, B.; Akbari, A.R. and Sharma, H. (2021). The medical benefits of vitamin K2 on calcium-related disorders. *Nutrients*, *13*(2), 691.

Lall, S.P. and Lewis-McCrea, L.M. (2007) Role of nutrients in skeletal metabolism and pathology in fish – an overview. Aquaculture, 267, 3–19.

Mariana L; Cristea V; Coprean D; Mirela M; Tanţi P. and Elena B (2011) Biochemical determinations and oxidative stress evaluation on Oncorhynchus mykiss grown in recirculating system. Lucrări Ştiinţifice - vol. 55, Seria Zootehnie

Nishikimi M; Rao N. and Yagi K (1972) The occurrence of superoxide anion in the reaction of reducedphenazine methosulfate and molecular oxygen. Biochemical and biophysical research communications 46: 849–854. https://doi.org/10.1016/s0006-291x(72)80218-3.

Ohkawa H; Ohishi N. and Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical biochemistry 95: 351–358. https://doi.org/10.1016/0003-2697(79)90738-3.

Piscaer, I.; Janssen, R.; Franssen, F. M.; Schurgers, L. J. and Wouters, E. F. (2023). The pleiotropic role of vitamin K in multimorbidity of chronic obstructive pulmonary disease. *Journal of Clinical Medicine*, *12*(4), 1261.

Rehulka J. (2000) Influence of astaxanthin on growth rate, condition, and some blood indices of rainbow trout. O Mykiss Aquaculture 190:27–47. https:// doi. org/ 10. 1016/ S0044- 8486(00) 00383-5

Reitman S. and Frankel S. (1957) A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 28:56–63. https:// doi. org/ 10. 1093/ ajcp/ 28.1. 56

Rey V´azquez G. and Guerrero GA. (2007) Characterization of blood cells and hematological parameters in C dimerus (Teleostei, Perciformes). Tissue Cell 39:151–160

Roy, P. and Lall, S. (2007). Vitamin K deficiency inhibits mineralization and enhances deformity in vertebrae of haddock (*M. aeglefinus*). Comp. Biochem. Physiol., 148, 174–183.

Saurabh, S. and Sahoo, P.K. (2008). Lysozyme: an important defence molecule of fish innate immune system. *Aquaculture research*, *39*(3), 223-239.

Shalaby A; Ghareeb A; Abd El-Rahman M. and Abd El-Hamid E. (2019) Effect of different levels of folic acid on the growth and some physiological aspects of Nile tilapia *"O. niloticus*." Egypt J Aquac 9:33–45. https:// doi. org/10. 21608/ eja. 2019. 19184. 1008

Taveekijakarn, P.; Miyazaki, T.; Matsumoto; M. and Arai, S. (1996) Studies on vitamin K deficiency in amago salmon (*O. rhodurus*) (Jordan & McGregor). J. Fish Dis., 19, 209–214.

Udagawa, M. (2004). The effect of parental vitamin K deficiency on bone structure in Mummichog Fundulus heteroclitus. J. World Aquac. Soc., 35, 366–371.

Vo, H.V.T.; Nguyen, Y.T.; Kim, N. and Lee, H.J. (2023). Vitamin A, D, E, and K as Matrix Metalloproteinase-2/9 Regulators That Affect Expression and Enzymatic Activity. Int. J. Mol. Sci. 2023, 24, 17038.

Wang, A.Y.M.; Afsar, R. E.; Sussman-Dabach, E.J.; White, J. A.; MacLaughlin, H. and Ikizler, T. A. (2023). Vitamin Supplement Use in Patients With CKD: Worth the Pill Burden? *American Journal of Kidney Diseases*.

Yan, Q.; Zhang, T.; O'Connor, C.; Barlow, J.W.; Walsh, J.; Scalabrino, G.; and Sheridan, H. (2023). The biological responses of vitamin K2: A comprehensive review. *Food Science & Nutrition*, *11*(4), 1634-1656.

Yuan, J.; Feng, L.; Jiang, W.D.; Liu, Y.; Jiang, J.; Li, S.H. and Zhou, X.Q. (2016). Effects of dietary vitamin K levels on growth performance, enzyme activities and antioxidant status in the hepatopancreas and intestine of juvenile J ian carp (*Cyprinus carpio*) var. Jian). *Aquaculture Nutrition*, *22*(2), 352-366.

Zhou, H.Y.; Hong, J.L.; Shu, P.; Ni, Y.J. and Qin, M.J. (2009). A new dicoumarin and anticoagulant activity from Viola yedoensis Makino. *Fitoterapia*, *80*(5), 283-285.

Arabic summary

أسماك القرموط الأفريقي هي من الأسماك ذات الأهمية الكبيرة في الإنتاج السمكي، والذي يتم تربيته على نطاق واسع في العديد من البلدان في جميع أنحاء العالم بما في ذلك مصر. لذلك، تم إجراء البحث الحالي لدراسة التأثيرات المحتملة لـفيتامين ك على أداء النمو، مقاييس الدم، الإستجابة المناعية ومضادات الأكسدة. تم تجهيز أربعة علائق ذات مستوى (30٪ بروتين خام) تحتوي على صفر٪ فيتامين (ك) (مجموعة الكنترول)، (3، 6 و 9 مليجرام/ كجم عليقة) من فيتامين (ك) وتم تغذية أسماك القرموط الأفريقي ذات الوزن الإبتدائى (4.89± 77.64 جرام) حتى الشبع الظاهري ثلاث مرات يوميا لمدة 90 يومًا. تم تغذية الأسماك على النظام الغذائي بمعدل يومي 3٪ من وزنها الحي. تشير النتائج إلى أن فيتامين (ك) قام بتحسين أداء النمو، الكفاءة الغذائية للأعلاف، الاستجابات المناعية، بروتين الدم، الألبومين، الهيموجلوبين و خلايا الدم الحمراء مقارنة بالمجموعة الضابطة. توصي الدراسة الحالية بإضافة فيتامين (ك) بتركيز 9 مليجرام/كجم إلى علائق أسماك القرموط الأفريقي حيث تأثيره الإيجابى على كل من أداء النمو، الوظائف الفسيولوجية والاستجابات المناعية.

الكلمات الدالة: فايتمين (ك)، القرموط الأفريقى، أداء النمو، مقاييس الدم والإستجابات المناعية.