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Impacts of different levels of vitamin K on the growth performance, hematological parameters, and immunological response of juvenile Nile tilapia (*Oreochromis niloticus*)

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Abstract
The current investigation was carried out to determine the impacts of different levels of vitamin K (VK) on the growth performance, hematological parameters, and immunological response of all-male juveniles of Nile tilapia (*O. niloticus*). VK3 (menadione) was added in five concentrations (0.0, 2.0, 4.0, 8.0, and 12.0 mg kg^{-1} diet) in five isonitrogenous (30% cp), isocaloric (18.61 MJ kg^{-1}) diets. The fish were fed the diets at a daily rate of 3% of their live weight, divided into 3 meals for 60 days. The results implied that supplemental VK did not provide any growth rate improvements and the efficiency of feed utilization over the control diet. Increasing dietary VK above 2 mg kg^{-1} feed resulted in significant retardation in fish performance and survival rates. Furthermore, supplemental VK up to 2–4 mg kg^{-1} increased hematological parameters, physiological functions, immune response, antioxidant capacity, and bone mineralization. Further increase in dietary VK resulted in a significant decline or level off in these parameters. Meanwhile, liver function enzymes increased progressively with dietary VK increasing. In conclusion, these findings suggest that supplemental VK maybe not be necessary for Nile tilapia growth performance, whereas about 2–4 mg kg^{-1} diet is required for other physiological functions. Increasing VK beyond these levels may pose adverse effects on Nile tilapia. However, further long-term studies are required to confirm these results.

Keywords Vitamin K · Nile tilapia · Hematological parameters · Growth performance · Physiological performance

Introduction

Vitamin K (VK) is a group of fat-soluble, quinone-derived compounds, sharing a common 2-methyl-1,4-naphthoquinone ring but differing in the side chain at the C3-position (Lambert and De Leenher 1992; Krossøy et al. 2011). This group includes at least two naturally occurring forms of VK: VK_1 (phylloquinone; 2-methyl-3-phytyl-1,4-naphthoquinone)

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and VK_2 (menaquinones; MK; 2-methyl-3-(prenyl)n-1,4-naphthoquinone) (Krossøy et al. 2011). A third, chemically synthesized form of VK is VK_3 (menadione; 2-methyl-1,4-naphthoquinone). Vitamin K has several functions in mammals and fish, including regulating the process of blood coagulation, through the production of prothrombin, which is a protein responsible for regulating clotting time for blood (Booth 1997; Jiang and Doolittle 2003). Vitamin K is also engaged in bone metabolism, quality, and health (Zhou et al. 2009) and Ca_2^+ homeostasis regulation (Oldenburg et al. 2008). Also, it plays a physiological role as an enzymatic co-factor, in addition to its role in inflammation, renal disease, sepsis, neoplasia, and energy metabolism (Arai et al. 2008; Booth 2009).

The VK requirement for cultivated fish is controversial and not fully comprehended. Varying, and sometimes contradictory, outcomes have been published on the impact of VK on farmed fish and its deficiency symptoms. For example, dietary VK deficiency induced a reduction in mineralization of bone and bone mass, leading to bone abnormalities in haddock (*Melanogrammus aeglefinus*) (Roy and Lall 2007) and mummichog (*F. heteroclitus*) (Udagawa 2001; 2004). A vitamin K-deficient diet also reduced growth performance and increased mortality in amago salmon (*Oncorhynchus rhodurus*) (Taveekijakarn et al. 1996). Increased blood coagulation time, hemorrhages, anemia, loss of fin tissue, weak bones, and occurrence of spinal curvature and short tails have also been documented as symptoms of VK deficiency (Taveekijakarn et al. 1996; Udagawa 2004; Lall and Lewis-McCrea 2007). Supplemental VK improved the growth, food digestion, and absorption in common carp (*C. carpio*), and anti-oxidant capacity in rainbow trout and common carp (Yuan et al. 2016). According to these studies, these fishes lack the ability to de novo synthesize VK, and thus, it needs to be provided with their diets.

On the contrary, several other studies revealed that supplemental VK is possibly not required for farmed fish. For instance, vitamin K-deficient feed did not cause detectable deficiency signs in rainbow trout (Kitamura et al. 1967), channel catfish (Murai and Andrews 1977), and Atlantic salmon (Graff et al. 2002; Krossøy et al. 2009). These researches concluded that the VK requirement of fish could be met by the amount found in basal diets (Graff et al. 2002; Krossøy et al. 2009), suggesting that supplemental VK may be not necessary for farmed fish.

Tilapia is currently the essential cultivated fish in the world, second only to carps (FAO 2020). They are currently farmed in over 120 countries worldwide, with Nile tilapia (*Oreochromis niloticus*) being the most great farmed tilapia species. This species represents 70–>80% of total production of tilapia during 2000–2019 (El-Sayed 2020; FAO 2020). Nile tilapia is also ranked second (after silver carp) in terms of global farmed fish production in 2019 (FAO 2020).

However, the Nile tilapia's requirement from VK has not been considered. It is unknown whether this species requires VK for healthy growth and physiological functions. As far as the authors know, only a single study (Lee 2003) evaluated the VK requirement of hybrid tilapia (*O. aureus*×*O. niloticus*). Therefore, the current study was conducted to assess the impacts of supplemental VK on growth performance, feed efficiency, hematological parameters, immune response, and antioxidant capacity in Nile tilapia juveniles.

Materials and methods

Fish and culture facility

All-mall Nile tilapia (*O. niloticus*) fingerlings used in the current study were obtained from the nursing unit of Fish Farming and Technology Institute (FFTI), Suez Canal University.

Healthy tilapia fingerlings with an initial weight of 30.28 ± 3.75 g were randomly distributed, in triplicates, into 15 indoor fiberglass, circular tanks with a maximum capacity of 3 m^3 (1.7 m diameter and 1.4 m high) filled with 1000 L dechlorinated tap water, at a density of 30 fish /m^3, under controlled photoperiods (12 L:12 D cycle) and temperature (25 ± 1 °C). The criteria for healthy fingerlings selection are eating when presented with food, not seeming disinterested, having bright coloration without abnormally dark spots, swimming actively with a symmetrical gait, and missing scales or not having tattered fins. They were adapted to the culture conditions for 2 weeks, during which they were fed with a commercial, extruded feed (30% CP; Aller aqua, 6th of October City, Egypt). The tank water used was filtered with sand filters and sterilized using ultraviolet units (Fujan Newland Entech Co., Ltd, China). Aeration was provided by an air blower (Rotary Blower, SWR, China) through diffuser stones. After the acclimation period, each tank's fish were netted, counted, weighed, and the average initial weights were recorded.

Water quality parameters, including temperature, dissolved oxygen (DO), ammonia (NH_4-N), nitrates (NO_3-N), nitrites (NO_2-N), and pH, were monitored weekly (ExStik II D-0600, FLIR Systems, Inc., USA) (Milwaukee MW-100). Twenty percent of the water capacity was changed per day. The scales of these parameters' values throughout the study were as follows: DO = 6.0–7 mg L^{-1}, NH4–N = 0.050–0.060 mg L^{-1}, NO_3–N = 7.0–8.3 mg L^{-1}, NO_2–N = 0.02–0.05 mg L^{-1}, and pH = 8.0–8.4.

Experimental diets and feeding regime

VK3 (menadione) was added in five concentrations (0.0, 2.0, 4.0, 8.0, and 12.0 mg kg^{-1} diet) in five experimental isonitrogenous (30% cp), isocaloric (18.61 MJ kg^{-1}), designated as D_0, D_2, D_4, D_8, and D_{12}, respectively (Table 1). The diets were prepared as described by El-Sayed et al. (2000) and El-Sayed et al. (2013). The fish were fed the test diets at a daily rate of 3% of the fish's live weight divided into 3 meals (9:00, 12:00, and 15:00 h) for 60 days.

Each tank's fish were sampled and weighed at 10-day intervals, their average weights were recorded and the daily amount of feed for each tank was readjusted accordingly. After the feeding trial ended, each tank's all fish were collected, counted, and weighed, and the average final weights were recorded.

Calculation of fish growth parameters

Growth performance and feed utilization efficiency were calculated as follows:

Percent weight gain = 100 (final weight (g) – initial weight (g) /initial weight (g).
Specific growth rate (SGR, % /day) = 100 (Ln W_f − Ln W_i) / t), where W_i and W_f are the initial and final weights (g), and t is the time of the experiment (days).
Feed conversion ratio (FCR) = dry feed intake (g)/ fish live weight gain (g).
Protein efficiency ratio (PER) = fish live weight gain (g)/dry protein fed (g).
Protein productive value (PPV) = 100}protein gain (g) /protein fed (g){.
Survival rate (SV) = (final stocking density / initial stocking density) × 100

Proximate composition analyses

After the trial ended, from each tank, five fish were randomly collected and frozen at − 20 °C for final body composition analyses. Initial body analysis was completed on a

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

pooled sample of 10 fish per treatment which was weighed and frozen before the study. Proximate analyses of whole-body protein, lipid, moisture, and ash were performed according to standard AOAC (2005) methods. Briefly, for protein was measured as nitrogen by a semi-automatic Kjeldahl (N × 6.25; VELP Scientific, UDK 126, Italy) following acid digestion. Lipid content was measured gravimetrically after Soxhlet extraction with petroleum ether (40–60 °C) as a solvent. For moisture content determination, fish samples were dried at 105 °C to constant weight. Ash content was determined after ignition in a muffle furnace at 550 °C for 6 h. All analyses were performed in triplicate samples for each parameter.

Hematological and immunological analyses

After the feeding trial ended, blood samples were collected from the caudal vein of five fish from each tank, using heparinized syringe needles (15 units/mL; 5000 IU, Amoun Pharmaceutical Co., Cairo, Egypt). From each tank, blood samples were split into two parts; one part was stored in heparinized Eppendorf tubes and used for hematology (red blood cell counting, total leucocytic count, hemoglobin, and hematocrit). The second part was kept in unheparinized tubes, left to clot at 4 °C and centrifuged at 5000 rpm (5 min) at room temperature, for separating plasma which was stored at − 18 °C and used for plasma analysis.

Red blood cell count (RBCs) was counted under light microscope using a Neubauer hemocytometer after blood dilution with phosphate-buffered saline (pH, 7.2) (Shalaby

et al. 2019; Abdel-Tawwab et al. 2020). Total leucocytic count (WBCs) (Rey V´azquez and Guerrero 2007) and hematocrit (PCV) were immediately determined according to Rehulka (2000) after sampling by placing fresh blood in glass capillary tubes, centrifuging it for 5 min in a microhematocrit centrifuge, and measuring the packed cell volume. Concentration of hemoglobin (Hb) was determined colorimetrically according to Jain (1993). Mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) were calculated according to Haney et al. (1992) as follows:

$$\text{MCV (fl)} = \text{PVC \%} \times \text{1000 RBCs } \left(\text{mill/mm}^3\right)$$

$$\text{MCH (pg)} = \text{Hb (g/dl)} \times 10/\text{RBCs mill/mm}^3\big)$$

$$\text{MCHC (g/dl)} = \text{Hb (g/dl)/PVC\%}$$

Biochemical and hepatic functions analyses

Plasma Ca^{2+} (mmol/l) was measured using a Stat Profile pHOx plus analyser (Nova Bio. Co., Waltham, USA), whereas total calcium in plasma (mmol/l) was measured using ICP-AES and ALP was measured using DGKC (Deutsche Gesellschaft für Klinische Chemie) method. Plasma PTH (Para thyroid hormone) level (nmol/l) was measured with a homologous radioimmunoassay (RIA) according to Rotllant et al. (2003). The TAC (total antioxidant capacity) was determined spectrophotometrically (Roberta et al. 1999). TP (total protein) was measured by Vitros TP device using dry and wet biochemical slides with Clinical Chemistry Analyzer (Microlab 300, ELI Tech Group) (Mariana et al. 2011). Globulin and creatinine were determined using Clinical Chemistry Analyzer (Microlab 300, ELI Tech Group). Activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined colorimetrically according to Reitman and Frankel (1957).

Immunological assays

Serum lysozyme activity (LYZ) was measured by turbidometric assay according to Ellis (1990) using *M. luteus* suspension (Sigma, USA). Lysozyme activity for all samples was calculated from a standard curve prepared from the white of a chicken egg lysozyme (Sigma, USA).

Statistical analysis

The current results were stated as (mean ± standard error of mean (SE)). One-way analysis of variance (ANOVA) was carried out to test the significant difference ($P < 0.05$) between treatments. Statistical analyses were conducted using (SPSS Inc.) program v22.0 (Chicago, IL). Duncan's Multiple Range Test was applied to compare means when *F*-values from the ANOVA were significant ($P < 0.05$).

Table 2 Growth rates and feed efficiency (mean ± SE; $n = 3$) of Nile tilapia (*Oreochromis niloticus*) fed on different concentrations of vitamin K for 60 days

Parameter	D_0	D_2	D_4	D_8	D_{12}
IW	31.47 ± 0.15^{ab}	31.67 ± 0.26^{a}	28.47 ± 0.17^{d}	30.43 ± 0.72^{bc}	29.40 ± 0.11^{cd}
FW	61.80 ± 0.80^{a}	63.70 ± 0.35^{a}	52.90 ± 1.01^{b}	45.77 ± 0.42^{c}	41.77 ± 0.61^{d}
WG	30.33 ± 0.78^{a}	32.033 ± 0.18^{a}	24.40 ± 1.04^{b}	15.33 ± 0.33^{c}	12.37 ± 0.73^{c}
ADG	0.51 ± 0.03^{a}	0.53 ± 0.04^{a}	0.42 ± 0.03^{ab}	0.26 ± 0.00^{bc}	0.21 ± 0.03^{c}
SGR	0.93 ± 0.03^{a}	0.94 ± 0.03^{a}	0.69 ± 0.06^{b}	0.64 ± 0.1^{bc}	0.47 ± 0.1^{c}
FCR	1.77 ± 0.06^{c}	1.63 ± 0.06^{c}	1.80 ± 0.10^{bc}	2.06 ± 0.12^{b}	2.36 ± 0.08^{a}
PER	1.93 ± 0.06^{a}	2.03 ± 0.08^{a}	1.90 ± 0.1^{a}	1.60 ± 0.11^{b}	1.40 ± 0.06^{b}
PPV	$30.300.57^{a}$	31.27 ± 1.51^{a}	25.00 ± 0.56^{b}	17.53 ± 1.04^{c}	13.50 ± 0.51^{d}
SV	93.07 ± 1.36^{ab}	97.23 ± 2.76^{a}	86.10 ± 2.80^{b}	75.03 ± 4.16^{c}	72.20 ± 1.40^{c}

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

Table 3 Proximate analysis (% dry weight basis) of Nile tilapia (*Oreochromis niloticus*) fed on different concentrations of vitamin K for 60 days

Experimental diets						
Parameters	Initial	D_0	D_2	D_4	D_8	D_{12}
Moisture	74.10 ± 0.31	74.50 ± 0.54^{a}	73.67 ± 0.67^{a}	73.00 ± 0.00^{a}	72.06 ± 0.11^{b}	70.67 ± 0.33^{c}
Crude protein	57.09 ± 0.44^{bc}	60.07 ± 0.57^{a}	60.02 ± 0.50^{a}	59.00 ± 0.54^{ab}	58.10 ± 0.47^{bc}	57.06 ± 0.32^{bc}
Crude lipid	19.04 ± 0.60^{c}	22.33 ± 0.22^{a}	21.45 ± 0.11^{ab}	19.98 ± 0.65^{bc}	19.05 ± 0.59^{c}	19.07 ± 0.58^{c}
Total ash	22.33 ± 0.57^{a}	16.89 ± 0.57^{c}	18.67 ± 0.75^{b}	20.19 ± 0.67^{b}	22.25 ± 0.57^{a}	23.01 ± 0.57^{a}

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

Results

Fish performance

Nile tilapia growth performance was significantly affected ($P < 0.05$) by dietary vitamin K (VK) (Table 2). The control diet (D_0) and D_2 produced significantly better performance than other diets ($P < 0.05$). However, D_0 and D_2 did not change significantly ($P < 0.05$). Further increase in supplemental VK resulted in significant retardation in growth rates, feed efficiency, and survival rates ($P < 0.05$). The best vitality and fastest readily consumption of the diets were observed in D_2 followed by D_0 while the worst vigor and longest readily consumption of the diets were observed in D_{12}.

Body composition

The Nile tilapia whole-body chemical composition in the current study was impacted significantly by VK doses (Table 3). Body moisture, protein, and lipids tended to decrease, whereas body ash increased ($P < 0.05$), with increasing VK levels. However, these parameter values did not change significantly at 0 and 2 mg VK/kg.

Hematological, immunological, and hepatic and kidney functions analyses

Dietary VK supplementation had a significant impact on hematological parameters ($P<0.05$) (Table 4). The concentrations of red blood cells (RBCs) count, hemoglobin concentration (Hb), hematocrit (PCV), mean cell volume (MCV), and white blood cells (WBC), mean cell hemoglobin (MCH) and total antioxidant capacity (TAC) significantly increased ($P<0.05$) with increasing VK levels up to 2 mg/kg, and decreased or leveled off with a further increase in VK levels.

Immunological parameters and hepatic and kidney function analyses were also significantly affected ($P<0.05$) by dietary VK levels (Table 5). Total protein and albumin resort to raise with increasing VK levels up to 4 mg/kg (D_4) and decreased afterward. Globulin concentration was also significantly decreased at D_4 ($P<0.05$), whereas at other VK levels, it did not significantly differ ($P<0.05$). Supplemental VK significantly enhanced plasma total calcium ($P<0.05$), but increasing VK from 2 to 12 mg kg^{-1} did not alter Ca concentrations ($P<0.05$). The ionized calcium was significantly increased at 2 mg/kg (D_2) ($P<0.05$) and decreased afterward. Lysozyme activity also increased up to 12 mg VK ($P<0.05$). PTH showed a significant rise with increasing VK levels up to 4 mg/kg (D_4). Otherwise, AST, ALT, and creatinine levels significantly enhanced with increasing VK levels ($P<0.05$).

Discussion

Controversial, varying, and sometimes conflicting outcomes have been reported on VK requirements of farmed fish. The current investigation revealed that supplemental VK did not result in any increase in fish performance, and feed utilization, even at the lowest concentration (2 mg kg^{-1}). Moreover, fish performance was significantly retarded with a further increase in dietary VK levels. These findings suggest that the Nile tilapia requirement of VK is low and could be met by the amount of VK found in the basic diet. Comparable results were reported in channel catfish (*Ictalurus punctatus*) (Murai and Andrews 1977), rainbow trout (Kitamura et al. 1967), and Atlantic salmon (Graff et al. 2002; Krossøy et al. 2009), where VK-deficient diets did not cause deficiency signs, suggesting that supplemental VK might not be necessary for these fishes.

Table 4 Hematological responses (mean ± SE; $n=3$) of Nile tilapia (*Oreochromis niloticus*) fed on different concentrations of vitamin K for 60 days

Parameters	Experimental diet				
	D_0	D_2	D_4	D_8	D_{12}
HB (g/dl)	3.93 ± 0.09^{c}	5.63 ± 0.29^{a}	5.30 ± 0.58^{ab}	4.37 ± 0.07^{bc}	3.67 ± 0.09^{c}
RBCs ($(10^6\ \mu L^{-1})$)	3.35 ± 0.06^{b}	3.83 ± 0.09^{a}	3.50 ± 0.15^{b}	2.45 ± 0.03^{c}	2.21 ± 0.03^{c}
PCV (%)	18.50 ± 0.29^{bc}	22.67 ± 1.46^{a}	20.50 ± 0.87^{ab}	15.67 ± 0.33^{d}	17.07 ± 0.53^{cd}
MCV (fl)	54.27 ± 2.23^{c}	68.27 ± 3.49^{ab}	65.60 ± 7.28^{bc}	65.43 ± 0.52^{bc}	78.90 ± 2.31^{a}
MCH (pg)	11.57 ± 0.03^{b}	16.60 ± 0.69^{a}	16.47 ± 0.72^{a}	17.47 ± 0.29^{a}	16.43 ± 0.18^{a}
MCHC (%)	21.40 ± 0.81^{bc}	24.40 ± 0.23^{ab}	25.60 ± 1.73^{a}	26.53 ± 0.37^{a}	20.90 ± 0.87^{c}
Platelets ($(10^4\ \mu L^{-1})$)	6.77 ± 1.01^{ab}	8.25 ± 1.01^{a}	5.21 ± 0.69^{bc}	4.58 ± 0.58^{bc}	3.07 ± 0.09^{c}
WBCs ($(10^4\ \mu L^{-1})$)	1.55 ± 0.029^{c}	2.40 ± 0.35^{a}	2.24 ± 0.92^{a}	1.83 ± 0.08^{ab}	2.07 ± 0.12^{ab}
TAC (mM/L)	1.03 ± 0.03^{b}	1.43 ± 0.07^{a}	1.43 ± 0.03^{a}	1.33 ± 0.07^{a}	1.13 ± 0.07^{b}

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

Table 5 Biochemical immunological and hepatic and kidney functions analysis (mean ± SE; $n = 3$) of Nile tilapia (*Oreochromis niloticus*) fed on different concentrations of vitamin K for 60 days

Parameters	Experimental diets				
	D_0	D_2	D_4	D_8	D_{12}
Total protein (g/dl)	3.05 ± 0.14^{bc}	3.34 ± 0.04^{ab}	3.53 ± 0.15^{a}	2.88 ± 0.07^{c}	2.99 ± 0.06^{c}
Albumin (g/dl)	0.65 ± 0.20^{b}	1.00 ± 0.12^{b}	1.8 ± 0.17^{a}	0.80 ± 0.06^{b}	0.88 ± 0.03^{b}
Globulin (g/dl)	2.40 ± 0.06^{a}	2.35 ± 0.14^{a}	1.73 ± 0.03^{b}	2.15 ± 0.14^{a}	2.19 ± 0.16^{a}
Lysozyme activity (µg/ml)	2.38 ± 0.15^{c}	4.14 ± 0.063^{b}	5.42 ± 0.24^{a}	5.37 ± 0.31^{a}	5.36 ± 0.27^{a}
Ca (mg/dl)	15.98 ± 0.14^{b}	18.80 ± 0.29^{a}	18.83 ± 0.56^{a}	18.23 ± 0.61^{a}	19.55 ± 0.32^{a}
Ca ionized (mg/dl)	13.22 ± 0.26^{c}	15.07 ± 0.17^{a}	13.93 ± 0.09^{b}	13.97 ± 0.15^{b}	14.60 ± 0.31^{ab}
PTH	1.98 ± 0.09^{ab}	2.25 ± 0.03^{a}	2.30 ± 0.06^{a}	1.60 ± 0.17^{c}	1.83 ± 0.12^{bc}
AST (U/L)	241.00 ± 24.25^{b}	279.33 ± 45.61^{b}	616.00 ± 50.81^{a}	498.67 ± 44.49^{a}	512.00 ± 31.26^{a}
ALT (U/L)	24.67 ± 0.88^{c}	150.00 ± 13.28^{bc}	146.67 ± 3.76^{bc}	302.33 ± 41.28^{b}	572.67 ± 159.80^{a}
Creatinine (mg/dl)	0.30 ± 0.01^{c}	0.33 ± 0.01^{c}	0.35 ± 0.01^{bc}	0.39 ± 0.01^{b}	0.50 ± 0.03^{a}

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

Fish may require extremely low VK concentrations for optimum performance. For example, Krossøy et al. (2009) demonstrated that only 0.1 mg phylloquinone (VK1) kg^{-1} feed was sufficient to meet the requirement for regular growth and bone veracity in juvenile Atlantic salmon. Less than 0.2 mg VK kg^{-1} has also been suggested as a minimum requirement for Atlantic cod (*G. morhua*) (Grahl-Madsen and Lie 1997). Along the same line, no considerable impact of dietary (VK3) on the growth of yellow croaker juvenile (*Pseudosciaena crocea*) was observed (Cheng et al. 2015). Also, no external signs of insufficiency of VK or toxicity were detected (Cheng et al. 2015). However, VK was desired for the period of blood coagulation (< 3.45 mg kg^{-1}), and for the maximum accumulation of menaquinone-4 in fish muscle (10.42 mg kg^{-1}) and liver (10.55 mg kg^{-1}). Similar results were also found in white shrimp (*L. vannamei*), where growth performance was not considerably affected by supplemental VK3 from 9.97 to 156.02 mg kg^{-1} diet (Dai et al. 2022a, b).

On the other hand, excessive dietary VK can be toxic, causing various toxicity symptoms in fish, including a reduction in growth rates (Grisdale-Helland et al. 1991; Grahl-Madsen and Lie 1997), oxidation-mediated liver toxicity, and bone abnormality (Udagawa 2001). These findings may explain the reduced growth in Nile tilapia feed excessive VK in the current study. The reduction (or leveling off) of the biochemical parameters (protein, albumin, globulin, lysozyme), together with the significant increase in liver function enzyme activities, at high VK doses (beyond 4 mg kg^{-1} feed), may also support this assumption, thereby suggesting that excessive dietary VK may be toxic to Nile tilapia. However, further, long-term investigations are in demand to verify these results.

It should be mentioned, however, that toxicity symptoms of VK may depend on the source of supplemental VK used. For example, Udagawa (2001) found that high doses of dietary VK3 (menadione sodium bisulfite—MSB) caused a high incidence of bone abnormality in mummichog (*F. heteroclitus*), compared with phylloquinone (VK1) which showed no significant variation in bone deformities. Similar toxicity symptoms of Menadiones (VK3) and its peers have also been reported in mammals (Rebhun et al. 1984; Suttie, 1991), whereas no symptoms of toxicity were detected for VK1 or VK2. These findings imply that phylloquinone is more suitable as a vitamin K source than MSB in fish feed.

In contrast with the outcomes above, several other studies revealed that supplemental VK is necessary for farmed fish. Dietary VK enhanced the growth rates and feed consumption in common carp (Yuan et al. 2016) and anti-oxidant capacity in rainbow trout (Stephensen et al. 2002) and common carp (Yuan et al. 2016). VK deficiency resulted in reduced growth performance and increased mortality in amago salmon (*O. rhodurus*) *(*Taveekijakarn et al. 1996*)*, and also caused bone abnormality in haddock (*M. aeglefinus*) (Roy and Lall 2007) and mummichog (*F. heteroclitus*) (Udagawa 2001, 2004). Hemorrhages, anemia, increased blood coagulation time, loss of fin tissue, and occurrence of spinal curvature have also been documented as symptoms of VK deficiency (Taveekijakarn et al. 1996; Udagawa 2004; Lall and Lewis-McCrea 2007). These studies suggest that these fishes may lack the ability to de novo synthesize VK, and thus, supplemental VK becomes necessary.

Hematology can provide important and reliable information on metabolic disorders, stress, nutrient deficiencies, and adaptation mechanisms to environmental variations; thereby, they are a practical tool for monitoring fish health (Makled et al. 2017; El-Sayed et al. 2021). The present hematological parameters values, including Hb%, RBC, WBC, PCV, MCV, MCH, blood platelets, and TAC, were significantly increased in Nile tilapia fed VK-supplemented diets up to 2 mg kg^{-1} feed. Beyond this level, these limitations were significantly reduced or leveled off. Similar findings were discovered in lake trout, where 2 mg VK kg^{-1} feed was sufficient for normal coagulation and satisfactory PCV in lake trout (Poston 1976). It has also been suggested that VK deficiency causes time-prolonged coagulation and anemia (Taveekijakarn et al. 1996; Steinberg 2022) .

Although supplemental VK was not necessary for regular growth performance in the current study, about 2–4 mg kg^{-1} were required for optimum immunological responses and for maintaining the antioxidant defence mechanism. This could be due to that VK suppresses the formation of lipid peroxide (Tampo and Yonaha 1996). These peroxides are free radicals, strongly involved in lipid peroxidation and protein oxidation (Kohen and Nyska 2002). Supplemental VK is effective in preventing reactive oxygen species (ROS) through the production of enzymes of antioxidant (such as CAT, SOD, GPx, and GST), thereby blocking the accumulation of free radical and exerting a protective defense against oxidative stress (Fang et al. 2002; Yuan et al. 2016). Similar to this, dietary VK3 had no effect on the growth performance of *L. vannamei*, but significantly improved their immunological response and the capacity of antioxidant (Dai et al. 2022a, b). Thus, it is evident that VK plays a significant role in mediating the activities of antioxidant enzymes and improves immune responses of farmed fish and shrimp.

In addition to the previous functions, VK plays an essential function in other biological processes in fish, such as bone mineralization (Udagawa 2000; Roy and Lall 2007). However, VK requirement for regular growth and survival in fish is generally lower than the requirement for normal bone development (Udagawa 2000). The same author (Udagawa 2001) found that when a VK-free diet was fed to mummichog (*F. heteroclitus*) larvae, it caused severe incidences of deformities in the backbone and caudal skeleton. Moreover, the larvae from broodfish fed a VK-deficient diet had vertebral deformities compared to larvae from broodfish were fed a VK-rich diet (Udagawa 2004). Vitamin K-deficient diets also caused the formation of weak and thin bones, and induced abnormalities in bone structure, including vertebral fusion and row irregularity (Udagawa 2001, 2004). Also, in haddock (*M. aeglefinus*) feeds, VK insufficiency reduced the mineralization of bone and caused bone deformities (Roy and Lall 2007). In current consideration, the increase in body ash, total plasma Ca, and ionized Ca with supplemental VK agrees with these findings, thereby supporting this vitamin role in bone structure and mineralization.

Conclusion

In conclusion, the present findings detected that supplemental VK is possibly not necessary for growth and feed efficiency of Nile tilapia, assuming that the VK contents in the basal feed may fulfill the requirement of these fish. On the other hand, about 2–4 mg VK kg^{-1} diet is required for optimum physiological functions, immune response, bone mineralization, and the capacity of antioxidant.

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Data availability Data are available on request due to privacy.

Declarations

Ethical approval The Committee of Scientific Research Ethics, Faculty of Agriculture granted ethical approval in compliance with Suez Canal University norms for the ethics of scientific research (Ref. No.: 65/2022).

Competing interests The authors declare no competing interests.

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