

Original Research Articles

Combined effects of vitamin E and temperature on the growth, biochemistry, and natural immune response of juveniles snubnose pompano (*Trachinotus blochii*)

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This study investigates the combined effects of Vitamin E and temperature on the growth, biochemical composition, and immune responses of juvenile snubnose pompano (*Trachinotus blochii*). A 70-day experiment was conducted with six treatments combining two levels of dietary Vitamin E (0 and 400 mg/kg) and three temperature levels (28°C, 31°C, and 34°C). The results revealed that both temperature and Vitamin E significantly influenced growth performance, feed utilization efficiency, protein and lipid content, and certain immune parameters ($P < 0.05$). Fish reared at 34°C with 400 mg/kg vitamin E demonstrated the highest growth indicators (SGR_L , SGR_W), protein efficiency, hepatosomatic index (HSI), and immune response, as indicated by elevated serum lysozyme levels, phagocytic activity, and white blood cells. Temperature notably affected lysozyme and respiratory burst activity, with values increasing at higher temperatures. Vitamin E supplementation improved these metrics regardless of temperature, increasing protein efficiency and reducing feed intake and feed conversion ratio (FCR). Moreover, Vitamin E enhanced immune responses, including increased white blood cell (WBC) counts and phagocytic activity. No interactive effects between Vitamin E and temperature were observed ($P > 0.05$). Overall, Vitamin E (400 mg/kg) positively influenced growth, biochemical composition, and immunity in snubnose pompano, particularly at elevated temperatures. The findings demonstrate that dietary vitamin E can enhance growth and immune performance, particularly under elevated temperatures, providing a basis for optimizing vitamin E use in snubnose pompano aquaculture.

1. INTRODUCTION

Vitamin E is an essential micronutrient in the daily diet of animals, playing a vital role in many physiological and biochemical processes in fish.¹ Among these, the antioxidant function of Vitamin E is considered paramount in farmed fish, helping fish boost their resistance to stressors such as transportation, high-density farming, toxin exposure, and exposure to high temperatures.² Numerous studies have shown that fish supplemented with Vitamin E exhibit optimal hematological indices enhanced immune response, and improved growth.³⁻⁵ Fish are ectothermic animals, meaning their body temperature and physiological processes are influenced directly by their surrounding environment. Exposure to elevated temperatures can lead to increased metabolic rates, causing stress that negatively impacts growth, immune function, and overall health. In the context of global climate change, rising water temperatures

pose a significant challenge to fish farming, as these conditions can reduce the growth rates of fish and make them more susceptible to diseases.⁶⁻⁸ Fish, being ectothermic animals, are significantly affected by increasing environmental temperatures, which influence many of their physiological activities. To mitigate these negative effects, antioxidants such as vitamin E are crucial due to their ability to reduce oxidative stress and support immune function. Vitamin E is known to enhance the resilience of fish under stressful conditions, yet its role in countering the effects of high temperature specifically remains underexplored in aquaculture. Although many studies have demonstrated the role of Vitamin E in terrestrial animals under high-temperature conditions. However, this issue has not been extensively studied in fish. For instance, Chen et al.⁹ showed that when snubnose shiner fish (*Notemigonus crysoleucas*) were raised under high-temperature conditions and fed a diet supplemented with Vitamin E, they exhibited higher

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survival rates, visceral Vitamin E content, and hematological indices compared to fish not supplemented with Vitamin E.

Snubnose pompano (*Trachinotus blochii*) is one of the tropical marine fish species with fast growth rates and delicious meat quality. Along with the ability to facilitate seed supply and the fish's effective digestion and absorption of commercial feed, this species has been selected for commercial farming on an industrial scale in Vietnam. As with many other species developed on a large scale, nutrition and feed optimization for industrial feed are of great concern, and several studies on the nutritional composition of snubnose pompano have been published. However, information on the role of trace elements like Vitamin E remains limited, with only studying the effects of Vitamin E on growth, antioxidant capacity, and lipid metabolism in juvenile snubnose pompano.⁴ Moreover, there is still a lack of information on the impact of temperature on juvenile snubnose pompano, as well as the interactive effects of Vitamin E and temperature on this species. Therefore, this study was conducted to investigate the combined effects of Vitamin E supplementation and elevated temperature on the growth, biochemical composition, and immune responses of juvenile snubnose pompano (*Trachinotus blochii*). This research aims to provide insights into how nutritional interventions, like vitamin E, can improve fish health in the face of rising environmental temperatures, contributing valuable information to the field of tropical fish aquaculture.

2. MATERIALS AND METHODS

2.1. FISH FOR EXPERIMENT

Snubnose pompano fish were selected from a marine fish hatchery in Nha Trang city, Khanh Hoa province. The 70-day-old juvenile fish, uniform in size and healthy (with an average length of approximately 6.27 cm and an average weight of approximately 5.22 g), were transported in sealed oxygenated plastic bags to the laboratory. An acclimation period of one week was conducted to allow the fish to adjust to the experimental conditions. The experiment was carried out from July 2021 to July 2022 at the Center for Aquatic Breeding and Disease Research – Nha Trang University, Nha Trang city, Vietnam.

2.2. FEED PREPARATION

The feed used for the fish during the acclimation and experimental periods was a synthetic feed, formulated and produced by Van Xuan Co., Ltd. – Khanh Hoa. The ingredients and nutritional composition of the experimental fish feed are presented in [Table 1](#). The vitamin E used in the experiment was DL-alpha-tocopherol acetate (China). The feed mixture was processed into a paste and pelletized using a horizontal extrusion machine (Binh Minh, Vietnam) with pellet sizes ranging from 1 to 3 mm. After pelletization, the feed was dried at 30°C for 24 hours and stored at -20°C for gradual use. The vitamin E content in the feed was analyzed

by high-performance liquid chromatography (HPLC), corresponding to the experimental treatments of 2.31 and 382.1 mg/kg of vitamin E.

2.3. EXPERIMENTAL DESIGN

The experiment was randomly designed with 6 treatments, combining 2 levels of vitamin E (0 and 400 mg/kg) with 3 temperature levels (28°C, 31°C, and 34°C). Each treatment was repeated 5 times simultaneously. Fish were placed into 30 composite tanks of 200 liters, with 30 individuals per tank. The composite tank system was covered to ensure sufficient natural light. The temperature in the experimental tanks was regulated using HZ-Q5 800W heaters (China).

The experiment lasted 70 days. The fish were fed twice a day, at 8:00 AM and 5:00 PM, at a rate of 5% body weight per day.

Environmental factors were regularly monitored and maintained at appropriate levels for the fish, including temperature maintained according to the experimental design, salinity (28 - 30‰), pH (7.5 - 8), and dissolved oxygen content (5.7 - 6.5 mg/l). Water quality was maintained by cleaning the tanks and replacing 50% of the water daily. To prevent fluctuations in rearing temperature during water replacement, we used seawater that was pre-adjusted to match the temperature of the rearing tanks. This approach ensured stable thermal conditions and reduced stress on the fish, providing a consistent environment that supports the well-being and growth of snubnose pompano. By carefully managing both temperature and water quality, we minimized potential stressors and maintained favorable conditions throughout the trial. Aeration was provided continuously.

2.4. SAMPLING AND CALCULATION

2.4.1. DETERMINATION OF GROWTH PARAMETERS

At the end of the experiment, all fish from the treatments were weighed and measured to determine growth indicators. Total length was measured using a technical measuring tape, accurate to 1 mm. Fish weight was measured using a BPA electronic scale (Ohaus, USA), accurate to 0.1 g. The survival rate of the fish was determined by the number of fish remaining at the end of the experiment.

Relative length growth rate (SGRL): $SGRL (\%/day) = \frac{(\ln L_2 - \ln L_1)}{t} \times 100$;

Relative weight growth rate (SGRW): $SGRW (\%/day) = \frac{(\ln W_2 - \ln W_1)}{t} \times 100$;

Survival rate (SR): $SR (\%) = \frac{(\text{Number of fish at the end of the experiment})}{(\text{Initial number of fish})} \times 100$; Where W1, W2 are the weights of the fish at the beginning and end of the experiment (g), and L1, L2 are the lengths of the fish at the beginning and end of the experiment (cm). t is the experimental duration (70 days).

Feed intake, protein efficiency (FI, PE), and feed conversion ratio (FCR) were determined based on the monitored feed intake during the entire experiment.

Daily feed intake (FI): $FI (\%) = 100 \times \frac{\text{Feed intake}}{(W_1 + W_2)/2} \times \text{days}$;

Table 1. Formulation and composition of experimental feed for juvenile snubnose pompano

Ingredient ^a (g/kg)	Dietary vitamin E levels (mg/kg)	
	0	400
Fish meal (defatted) ^b	430	430
Soybean meal	190	190
Wheat meal	100	100
Wheat flour	30	30
Fish oil-vitamin E free	57	57
Vitamin/Mineral ^c	36	36
Wheat gluten	140	140
Soy lecithin (70%)	12	12
Monocalcium phosphate	3	3
Choline	2	2
α-tocopherol acetate	0	400
Proximate composition (%)		
Crude protein	46.94	47.53
Crude lipid	10.6	10.73
Vitamin E (mg/kg)	2.31	382.1

^aSupplied by Long Sinh Feed Company, Khanh Hoa, Viet Nam.

^bprovided by T.C. Union, Tien Giang, Viet Nam

^cVitamin premix: Vitamin A, 1000000 UI; Vitamin D3, 300.000 IU, Vitamin C monophosphate 10.000 mg; Vitamin B6, 500 mg; Vitamin B2, 320 mg; Vitamin B12, 5 mg (Provimi Viet Nam Co. Ltd, Bien Hoa City, Dong Nai province, Viet Nam). Mineral premix: Zn (ZnO), 4.750 mg; Mn (MnSO₄.H₂O), 1.900 mg; Mg (MgO), 1.050 mg; Ca (CaHPO₄.2H₂O), 0.8%; Co (CoCO₃), 47.5 mg; Se (Na₂SeO₂), 47.5 mg (Provimi Viet Nam Co. Ltd, Bien Hoa City, Dong Nai province, Viet Nam).

Protein efficiency (PE): $PE = \text{Fish weight gain} / \text{Protein intake}$;

Protein intake was then calculated by multiplying the total feed intake by the percentage of protein in the feed. The formula used was: $\text{Protein Intake (g)} = \text{Feed Intake (g)} \times (\text{Protein Content} / 100)$;

Feed conversion ratio (FCR): $FCR = \text{Total feed intake} / \text{Fish weight gain}$;

Three fish were randomly sampled from each tank (9 fish per treatment) to dissect and collect liver and internal organs for weighing and analyzing internal organ index and liver index.

Viscera-somatic index (VSI): $VSI (\%) = (\text{Internal organ weight} / \text{Fish weight}) \times 100$;

Hepato-somatic index (HSI): $HSI (\%) = (\text{Liver weight} / \text{Fish weight}) \times 100$.

2.4.2. BIOCHEMICAL COMPOSITION ANALYSIS

Nine fish were randomly sampled per treatment and immediately stored in a deep freezer (-85°C). The biochemical composition of the feed and whole body of the fish was analyzed at the Practice and Experiment Center, Nha Trang University, using the following methods:

Protein content in the feed and fish body was analyzed using the Kjeldahl method (Kjeltec Auto 1030, Foss Tecator, Höganäs, Sweden).

Crude lipid was analyzed by the Soxhlet extraction method.

Moisture content was determined after drying the samples in an oven (UNE-600, Memmert, Germany) at 105°C until a constant weight was achieved.

Ash content was determined after the samples were burned in an electric furnace (SH-FU-5MG, SH Scientific, Korea) at 550°C for 8 hours until a constant weight was achieved.

2.4.3. HEMATOLOGICAL AND IMMUNE INDICATORS ANALYSIS

At the end of the experiment, 9 fish per treatment were randomly sampled and anesthetized using monophenyl ether glycol at a concentration of 150-200 ppm before blood collection. Blood samples were drawn from the caudal vein of the fish. A portion of the blood was analyzed for blood formula and biochemical composition, while the remaining portion was placed in Eppendorf tubes and kept overnight at 4°C before centrifugation to collect serum. The fish serum was stored at -80°C for lysozyme analysis.

White blood cells, red blood cells, hematocrit, and hemoglobin were analyzed using a Sysmex XT-1800i blood analyzer (Sysmex Corporation, Hyogo, Japan). Plasma triglyceride and protein levels were measured using a Dx C600 chemistry analyzer.

Lysozyme content in the serum of snubnose pompano was determined following the modified method of Shugar (1952). In this method, 25 µL of the serum sample was placed into one well of a 96-well flat-bottom plate. 175 µL of a 0.075% *Micrococcus lysodeikticus* (ATCC 4698-M0508 Sigma) bacterial suspension was added to the wells, quickly

mixed, and the plate was placed in a spectrophotometer (*Titertek multiskan*). Optical density was measured at a wavelength of 450 nm every 30 seconds, continuously for 5 minutes. Lysozyme activity in the serum of snubnose pompano was determined based on a standard curve representing the lytic activity of lysozyme extracted from hen egg white (White hen egg lysozyme, Sigma, USA) at concentrations of 20, 10, 5 upto 0.625 µg/mL.

2.4.4. WHITE BLOOD CELL ISOLATION FOR DETERMINING PHAGOCYTIC ACTIVITY AND RESPIRATORY BURST

This was performed following the adjusted method of Samai et al. (2017). Kidney tissue was crushed in L-15 cell culture medium (Leibovitz's Lonza) supplemented with 2% snubnose pompano serum and filtered through a 100 µm membrane. 2 mL of the cell solution was added to a 10 mL centrifuge tube containing 7 mL of Percoll solution (Sigma) with a density of 1.05/1.075 g/cm³, centrifuged at 550 g for 35 minutes (1236R, Scanspeed, Denmark). White blood cells located between the two Percoll layers were collected, and cell density was determined using a trypan blue staining solution.

White blood cells isolated from the anterior kidney of snubnose pompano were washed with PBS (200 g, 10 minutes) and a cell suspension was prepared using L-15 medium. 100 µL of the cell suspension was added to wells of a 96-well flat-bottom plate and incubated for 2 hours at 20°C. White blood cells were washed with L-15 to remove non-adherent cells. The cells in the wells were incubated overnight at 20°C in L-15 medium supplemented with 4% Fetal Bovine Serum (FBS) and 100 µg.mL⁻¹ penicillin.

Phagocytic activity and the Phagocytic Index of kidney white blood cells in snubnose pompano were determined following the method of Siwicki et al. (1994). The steps included: 100 µL of macrophage solution (107 cells.mL⁻¹) were incubated with 100 µL of zymosan solution (108 cells.mL⁻¹, Sigma) for 2 hours at 22°C. The cells were stained with Congo red for 5 minutes, washed 3 times with PBS, and slide preparations were made, left to dry for 2 hours at room temperature. The slides were observed under a microscope at 400x and 1000x magnification to determine the phagocytic activity of 100 macrophages. Phagocytic activity (PA%) of white blood cells was determined as the ratio of total phagocytic white blood cells to 100 observed white blood cells. The phagocytic index was determined as the total number of yeast cells engulfed by the white blood cells.

Respiratory burst activity of the anterior kidney cells in snubnose pompano was determined using the method of Cheng et al. (2007). 100 µL of white blood cell solution (1 x 10⁶ cells/mL) was added to wells (96-well plate, Nunc MaxiSorp™) and incubated for 2 hours at 30°C. The cells were then sequentially incubated with 100 µL of zymosan solution (Sigma) and 100 µL of 0.3% Nitroblue tetrazolium (NBT, Sigma) for 30 minutes at room temperature. The reaction was stopped by adding 100 µL of methanol. 120 µL of 2M KOH solution and 140 µL of DMSO (dimethyl sulphoxide, Sigma) were added to each well to create a color reac-

tion. Respiratory burst activity (RB) of white blood cells was expressed through the color intensity formed from the reduction reaction of Nitroblue tetrazolium, determined by a spectrophotometer (iMark™, Bio-rad) at a wavelength of 630 nm. Each sample was repeated three times under the same conditions.

2.5. DATA ANALYSIS

The data collected from the experiment were calculated and presented as mean values (Mean) ± Standard Error (SE). The data were statistically analyzed using a two-way analysis of variance (Two-way ANOVA) with SPSS 22.0 software. Differences between mean values were determined using Duncan's test with a 95% confidence level.

3. RESULTS

3.1. COMBINED EFFECTS OF VITAMIN E AND TEMPERATURE ON THE GROWTH AND FEED UTILIZATION EFFICIENCY

The effects of vitamin E and temperature on the growth and feed utilization efficiency of juvenile snubnose pompano are presented in [Table 2](#). The results showed that both temperature and vitamin E significantly affected the growth and feed utilization efficiency of the fish ($P < 0.05$). Growth indicators, including final body length (FBL), specific growth rate in length (SGRL), final body weight (FBW), and specific growth rate in weight (SGRW), as well as protein efficiency (PE) were enhanced in fish reared at 31°C and 34°C. Feed intake (FI) and feed conversion ratio (FCR) were optimal at these two temperature levels. [Table 2](#) also showed that the addition of vitamin E to the feed significantly increased body weight (FBW, SGRW) and protein utilization efficiency, while reducing the FI and the FCR of the fish ($P < 0.05$). However, the analysis revealed no interactive effect between vitamin E and temperature on the growth and feed utilization efficiency of the fish ($P > 0.05$).

Vitamin E supplementation did not significantly impact the viscera somatic index (VSI) and hepatosomatic index (HSI) of the fish ($P > 0.05$), but temperature did affect these indices ($P < 0.05$). The lowest VSI and HSI values were observed at 28°C, with values of 6.27 and 1.00, respectively, while the highest values were recorded at 34°C, at 7.03 and 1.12, respectively. The results in [Table 2](#) indicated that there was no interactive effect between vitamin E and temperature on the VSI and HSI indices of the fish ($P > 0.05$).

Both FBL and FBW increased with temperature, peaking at 34°C, with the highest values in the 34:400 group (14.87 cm and 66.31 g/fish). Vitamin E supplementation at 400 mg/kg significantly improved FBW ($P < 0.05$), with vitamin E-fed fish generally having higher FBW than non-supplemented groups. Furthermore, SGR in both length and weight increased with temperature, highest at 34°C with vitamin E (SGRw=3.91 %/day). SGRw also showed significant improvements with vitamin E supplementation ($P < 0.05$). FI values were slightly higher at 28°C (4.21-4.28) and lowest at 34°C with vitamin E (3.76). FCR improved (lower) with

Table 2. Combined effects of Vitamin E and Temperature on the Growth and Feed Utilization Efficiency of Snubnose pompano

Treatment	VSI	HSI	FBL (cm)	FBW (g/fish)	SGR _L (%/day)	SGR _W (%/day)	FI	FCR	PE
28:0	6.24±0.16	0.99±0.05	14.32±0.07	57.75±0.53	1.27±0.01	3.70±0.01	4.28±0.04	1.67±0.02	1.28±0.01
28:400	6.31±0.05	1.01±0.03	14.17±0.12	58.83±1.30	1.26±0.01	3.73±0.03	4.21±0.08	1.64±0.04	1.30±0.03
31:0	6.85±0.09	1.07±0.04	14.64±0.10	61.65±0.48	1.30±0.01	3.80±0.01	4.03±0.03	1.55±0.01	1.37±0.01
31:400	7.09±0.09	1.09±0.01	14.69±0.10	63.39±0.89	1.31±0.01	3.84±0.02	3.93±0.05	1.51±0.02	1.42±0.02
34:0	6.99±0.12	1.11±0.03	14.62±0.10	64.89±0.43	1.30±0.01	3.88±0.01	3.84±0.02	1.47±0.01	1.45±0.01
34:400	7.06±0.04	1.13±0.03	14.87±0.12	66.31±0.20	1.33±0.01	3.91±0.01	3.76±0.01	1.43±0.01	1.49±0.01
<i>Means of main effect in temperature</i>									
28	6.27 ^A	1.00 ^A	14.25 ^A	58.29 ^A	1.26 ^A	3.71 ^A	4.24 ^C	1.65 ^C	1.29 ^A
31	6.97 ^B	1.08 ^B	14.66 ^B	62.52 ^B	1.31 ^B	3.82 ^B	3.98 ^B	1.53 ^B	1.39 ^B
34	7.03 ^B	1.12 ^B	14.75 ^B	65.6 ^C	1.32 ^B	3.89 ^C	3.8 ^A	1.45 ^A	1.47 ^C
<i>Means of main effect in Vitamin E</i>									
0	6.69	1.06	14.53	61.43 ^X	1.29	3.79 ^X	4.05 ^X	1.56 ^X	1.37 ^X
400	6.82	1.08	14.58	62.85 ^Y	1.3	3.83 ^Y	3.97 ^Y	1.53 ^Y	1.4 ^Y
<i>P-values (Two-way ANOVA)</i>									
Temperature	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Vitamin E	NS	NS	NS	<0.05	NS	<0.05	<0.05	<0.05	<0.05
Temperature × Vitamin E	NS	NS	NS	NS	NS	NS	NS	NS	NS

The data are presented as the mean of five replicates (n=5). Different lowercase letters (a, b, c) within a column indicate significant differences between the experimental treatments. Different uppercase letters (A, B, C) within a column indicate significant differences ($P < 0.05$) between the means of the temperature levels. Different uppercase letters (X, Y, Z) within a column indicate significant differences ($P < 0.05$) between the means of the vitamin E levels.

increased temperature, reaching the best efficiency at 34°C (FCR=1.43-1.47). PE was highest at 34°C with vitamin E supplementation (1.49) and lowest at 28°C without vitamin E (1.28). Both temperature and vitamin E had significant effects on PE ($P < 0.05$), showing enhanced protein utilization at higher temperatures and with vitamin E supplementation.

3.2. COMBINED EFFECTS OF VITAMIN E AND TEMPERATURE ON THE BIOCHEMICAL COMPOSITION

The effects of temperature and vitamin E on the biochemical composition of juvenile snubnose pompano are shown in [Table 3](#). The results indicated that both temperature and vitamin E significantly influenced the biochemical composition of the fish. Overall, these results indicate that Vitamin E positively influenced protein, lipid, ash, and moisture content, while temperature primarily affected lipid content, with a significant interaction effect between temperature and Vitamin E on lipid levels. Temperature had a significant effect on lipid content ($P < 0.05$), with lower lipid levels observed at higher temperatures. The highest lipid content was found in the 34:400 treatment (10.66%) and the lowest in the 28:0 treatment (5.51%). Lipid levels generally increased with Vitamin E supplementation but decreased as temperature rose, although there was no significant difference in lipid content between the 31°C and 34°C groups. A significant interaction effect between temperature and Vitamin E was observed for lipid content ($P < 0.05$), with the highest levels in the 34:400 treatment group. In addition, Protein content was significantly affected by Vitamin E supplementation ($P < 0.05$), with Vitamin E-supplemented fish having higher protein levels (mean 19.47%) than non-supplemented fish (mean 18.75%). The highest protein content was observed in the 34:400 group (19.92%) and the lowest in the 28:0 group (18.45%). Temperature had no significant main effect on protein content, nor was there a significant interaction effect between temperature and Vitamin E on this parameter. Ash content was highest in the 28:0 treatment (5.76%) and lowest in the 34:400 treatment (4.18%). Vitamin E had a significant effect on ash content ($P < 0.05$), with lower ash values in Vitamin E-supplemented groups (mean 4.22%) compared to non-supplemented groups (mean 5.49%). No significant effect of temperature or interaction between temperature and Vitamin E was observed for ash content. Moisture content ranged from 67.19% in the 28:0 treatment to 69.88% in the 34:400 treatment. Vitamin E supplementation significantly increased moisture content ($P < 0.05$), with higher moisture levels in Vitamin E-supplemented fish (mean 68.78%) compared to non-supplemented fish (mean 67.4%). Temperature and the interaction between temperature and Vitamin E had no significant effect on moisture content.

3.3. COMBINED EFFECTS OF VITAMIN E AND TEMPERATURE ON THE IMMUNE RESPONSE

The hematological indices of juvenile snubnose pompano under the influence of temperature and vitamin E are presented in [Table 4](#). The analysis revealed no interactive ef-

fect between vitamin E and temperature on the hematological parameters as a whole ($P > 0.05$); however, both temperature and vitamin E independently affected these parameters ($P < 0.05$). White blood cells (WBC) counts varied from 5.75 (28:0) to 7.54 (34:400), with the highest values observed at 34°C in the Vitamin E-supplemented group. Both temperature and Vitamin E significantly influenced WBC counts ($P < 0.05$), with WBC levels increasing at higher temperatures and with Vitamin E supplementation. Additionally, a significant interaction effect between temperature and Vitamin E was noted for WBC counts ($P < 0.05$). Red blood cells (RBC) counts were lowest in the 28:0 group (1.79) and highest in the 34:400 group (3.11). Both temperature and Vitamin E had a significant effect on RBC counts ($P < 0.05$), with RBC levels increasing as temperature and Vitamin E supplementation increased. There was also a significant interaction effect between temperature and Vitamin E on RBC counts ($P < 0.05$). Hemoglobin (Hb) levels ranged from 6.7 g/dL (28:0) to 8.98 g/dL (34:400). Temperature and Vitamin E independently had significant effects on Hb levels ($P < 0.05$), with higher Hb concentrations observed at 34°C and in the Vitamin E-supplemented groups. No significant interaction effect between temperature and Vitamin E was detected for Hb. Hematocrit (Hct) levels were lowest in the 28:0 group (19.8%) and highest in the 34:400 group (25.68%). Temperature and Vitamin E both significantly influenced Hct levels ($P < 0.05$), with Hct levels generally increasing at higher temperatures and with Vitamin E supplementation. A significant interaction effect was observed between temperature and Vitamin E for Hct levels ($P < 0.05$). Platelets (PLT) counts ranged from 22.2 (28:0) to 23.8 (28:400). Vitamin E supplementation significantly affected PLT levels ($P < 0.05$), though temperature and the interaction effect were not significant for this parameter. Triglyceride levels were lowest at 28°C (3.99 mmol/L) and highest at 34°C with Vitamin E supplementation (5.15 mmol/L). Temperature had a significant effect on triglyceride levels ($P < 0.05$), with higher levels recorded at elevated temperatures. Neither Vitamin E nor the interaction between temperature and Vitamin E showed a significant effect on triglycerides. Protein levels ranged from 36.06 g/L (28:0) to 40.36 g/L (34:400). Both temperature and Vitamin E had significant effects on protein levels ($P < 0.05$), with higher protein levels observed at increased temperatures and in the Vitamin E-supplemented groups. There was also a significant interaction effect between temperature and Vitamin E on total protein levels ($P < 0.05$). In summary, temperature significantly influenced WBC, RBC, Hb, Hct, triglyceride, and protein levels, while Vitamin E had significant effects on WBC, RBC, Hb, Hct, PLT, and protein levels. Interaction effects between temperature and Vitamin E were significant for WBC, RBC, Hct, and protein levels, indicating that both factors contribute interactively to these hematological parameters.

The effects of vitamin E and temperature on immune parameters, including serum lysozyme levels, respiratory burst activity, phagocytic activity, and the phagocytic index of snubnose pompano are presented in [Table 5](#). Lysozyme levels ranged from 6.70 µg/mL in the 28:0 group to 9.40 µg/

Table 3. Combined effects of temperature and vitamin E on the biochemical composition of the fish body of snubnose pompano

Treatment	Ash (%)	Moisture (%)	Protein (%)	Lipid (%)
28:0	5.76±0.39	67.19±0.69	18.45±0.09	5.51±0.46 ^a
28:400	4.20±0.46	67.72±0.68	19.17±0.17	9.37±0.49 ^c
31:0	5.30±0.30	67.48±0.31	18.89±0.29	7.86±0.22 ^b
31:400	4.27±0.08	68.74±0.48	19.30±0.23	9.42±0.30 ^c
34:0	5.43±0.21	67.54±0.30	18.91±0.25	8.33±0.39 ^{bc}
34:400	4.18±0.17	69.88±0.38	19.92±0.35	10.66±0.20 ^d
<i>Means of main effect in temperature</i>				
28	4.98	67.56	18.81	7.44 ^A
31	4.79	68.11	19.1	8.64 ^B
34	4.8	68.71	19.42	9.50 ^C
<i>Means of main effect in Vitamin E</i>				
0	5.49 ^X	67.4 ^X	18.75 ^X	7.24 ^X
400	4.22 ^Y	68.78 ^Y	19.47 ^Y	9.82 ^Y
<i>P-values (Two-way ANOVA)</i>				
Temperature	NS	NS	NS	<0.05
Vitamin E	<0.05	<0.05	<0.05	<0.05
Temperature × Vitamin E	NS	NS	NS	<0.05

The data are presented as the mean of five replicates (n=5). Different lowercase letters within a column indicate significant differences between the experimental treatments. Different uppercase letters (A, B, C) within a column indicate significant differences ($P < 0.05$) between the means of the temperature levels. Different uppercase letters (X, Y, Z) within a column indicate significant differences ($P < 0.05$) between the means of the vitamin E levels.

mL in the 34:400 group. Temperature had a significant effect on lysozyme levels ($P < 0.05$), with lysozyme concentrations increasing at higher temperatures, reaching a mean of 8.30 µg/mL at 34°C. Vitamin E supplementation also significantly increased lysozyme levels ($P < 0.05$), with Vitamin E-supplemented groups averaging 8.21 µg/mL compared to 7.03 µg/mL in non-supplemented groups. No significant interaction effect between temperature and Vitamin E was found for lysozyme. Phagocytic activity values ranged from 69.80% in the 28:0 group to 78.00% in the 34:400 group. Vitamin E had a significant effect on phagocytic activity ($P < 0.05$), with higher phagocytic activity observed in Vitamin E-supplemented fish (mean 75.53%) compared to non-supplemented fish (mean 71.87%). However, temperature and the interaction between temperature and Vitamin E had no significant effect on phagocytic activity. The phagocytic index ranged from 1.78 in the 28:0 group to 2.02 in the 34:400 group. Temperature did not significantly influence the phagocytic index ($P > 0.05$), with the highest values observed at 34°C (mean 1.99). There was no significant effect of Vitamin E supplementation or any interaction effect between temperature and Vitamin E on the phagocytic index. Respiratory burst activity varied from 1.22 in the 28:0 group to 1.48 in the 34:400 group. Both temperature and Vitamin E significantly influenced respiratory burst activity ($P < 0.05$), with higher values observed at increased temperatures and with Vitamin E supplementation. The highest mean respiratory burst activity (1.41) was recorded at 34°C. No significant interaction effect between temperature and Vitamin E was found for respiratory burst activity. In summary, temperature significantly affected lysozyme levels,

and respiratory burst activity, with the highest values generally observed at 34°C. Vitamin E supplementation significantly increased lysozyme levels, phagocytic activity, and respiratory burst activity. However, there were no significant interaction effects between temperature and Vitamin E for any of the immune parameters measured.

4. DISCUSSION

The present study investigated the combined effects of temperature and dietary vitamin E supplementation on the growth performance, biochemical composition, and immune response of juvenile snubnose pompano. The results indicate that both temperature and Vitamin E significantly impact growth and immune parameters, although no interaction between temperature and Vitamin E was observed for these factors.

Due to climate change, global temperature patterns have changed significantly and are predicted to become more extreme. This will affect the fitness and performance of fish species, especially those in tropical regions. Tropical fish have different temperature limits compared to cold-water and warm-water fish. They will die at temperatures between 10°C and 20°C and most stop growing at temperatures below 25°C.¹⁰ Some studies on tropical fish show that Waigieu seaperch *Psammoperca waigiensis*⁷ grow and have a good immune response at 28°C, but in contrast, their growth and immune response decrease sharply when reared at 34°C. At 32°C, the secretion of collagen type II in golden pompano *Trachinotus ovatus* larvae is inhibited compared to 24°C and 28°C,⁶ while the hematological parameters of

Table 4. Combined effects of temperature and vitamin E on hematological parameters of snubnose pompano

Treatment	WBC ($\times 10^3/\text{mm}^3$)	RBC ($\times 10^6/\text{mm}^3$)	Hb (g/dL)	Hct (%)	PLT ($\times 10^9/\text{mm}^3$)	Triglyceride (mmol/L)	Protein (g/L)
28:0	5.75 $\pm$ 0.07 ^a	1.79 $\pm$ 0.01 ^a	6.70 $\pm$ 0.08	19.8 $\pm$ 0.45 ^a	22.2 $\pm$ 0.37	3.99 $\pm$ 0.15	36.06 $\pm$ 0.63 ^a
28:400	6.51 $\pm$ 0.15 ^b	1.87 $\pm$ 0.03 ^a	7.12 $\pm$ 0.09	22.12 $\pm$ 0.51 ^b	23.8 $\pm$ 0.58	4.19 $\pm$ 0.20	36.20 $\pm$ 0.46 ^a
31:0	6.65 $\pm$ 0.13 ^b	2.21 $\pm$ 0.03 ^b	6.92 $\pm$ 0.27	21.92 $\pm$ 0.31 ^b	22.4 $\pm$ 0.24	4.75 $\pm$ 0.34	36.76 $\pm$ 0.39 ^a
31:400	7.15 $\pm$ 0.04 ^c	2.21 $\pm$ 0.03 ^b	7.90 $\pm$ 0.34	22.78 $\pm$ 0.40 ^b	23.4 $\pm$ 0.40	4.92 $\pm$ 0.32	39.52 $\pm$ 0.46 ^b
34:0	6.35 $\pm$ 0.05 ^b	2.21 $\pm$ 0.04 ^b	7.42 $\pm$ 0.29	22.08 $\pm$ 0.25 ^b	22.4 $\pm$ 0.51	4.41 $\pm$ 0.04	39.24 $\pm$ 0.38 ^b
34:400	7.54 $\pm$ 0.13 ^d	3.11 $\pm$ 0.05 ^c	8.98 $\pm$ 0.27	25.68 $\pm$ 0.66 ^c	23.2 $\pm$ 0.58	5.15 $\pm$ 0.38	40.36 $\pm$ 0.56 ^b
<i>Means of main effect in temperature</i>							
28	6.13 ^A	1.83 ^A	6.91 ^A	20.96 ^A	23	4.09 ^A	36.13 ^A
31	6.9 ^B	2.21 ^B	7.41 ^A	22.35 ^B	22.9	4.83 ^B	38.14 ^B
34	6.95 ^B	2.66 ^C	8.2 ^B	23.88 ^C	22.8	4.78 ^B	39.8 ^C
<i>Means of main effect in Vitamin E</i>							
0	6.25 ^X	2.07 ^X	7.01 ^X	21.27 ^X	22.33 ^X	4.38	37.35 ^X
400	7.07 ^Y	2.4 ^Y	8.0 ^Y	23.53 ^Y	23.47 ^Y	4.75	38.69 ^Y
<i>P-values (Two-way ANOVA)</i>							
Temperature	<0.05	<0.05	<0.05	<0.05	NS	<0.05	<0.05
Vitamin E	<0.05	<0.05	<0.05	<0.05	<0.05	NS	<0.05
Temperature $\times$ Vitamin E	<0.05	<0.05	NS	<0.05	NS	NS	<0.05

The data are presented as the mean of five replicates (n=5). Different lowercase letters within a column indicate significant differences between the experimental treatments. Different uppercase letters (A, B, C) within a column indicate significant differences ($P < 0.05$) between the means of the temperature levels. Different uppercase letters (X, Y, Z) within a column indicate significant differences ($P < 0.05$) between the means of the vitamin E levels.

Table 5. Combined effects of temperature and vitamin E on serum lysozyme levels, respiratory burst activity, phagocytic activity, and the phagocytic index of snubnose pompano

Treatment	Lysozyme ($\mu\text{g/mL}$)	Phagocytic activity	Phagocytic index	Respiratory burst activity
28:0	6.70 $\pm$ 0.33	69.80 $\pm$ 1.85	1.78 $\pm$ 0.06	1.22 $\pm$ 0.03
28:400	7.52 $\pm$ 0.59	73.80 $\pm$ 2.75	1.90 $\pm$ 0.06	1.25 $\pm$ 0.02
31:0	7.19 $\pm$ 0.42	71.40 $\pm$ 2.42	1.86 $\pm$ 0.07	1.29 $\pm$ 0.03
31:400	7.72 $\pm$ 0.24	74.80 $\pm$ 1.46	1.85 $\pm$ 0.07	1.37 $\pm$ 0.03
34:0	7.20 $\pm$ 0.27	74.40 $\pm$ 1.89	1.96 $\pm$ 0.06	1.34 $\pm$ 0.03
34:400	9.40 $\pm$ 0.63	78.00 $\pm$ 1.73	2.02 $\pm$ 0.06	1.48 $\pm$ 0.03
<i>Means of main effect in temperature</i>				
28	7.11 ^A	71.8	1.84	1.23 ^A
31	7.46 ^B	73.1	1.86	1.33 ^B
34	8.30 ^C	76.2	1.99	1.41 ^C
<i>Means of main effect in Vitamin E</i>				
0	7.03 ^X	71.87 ^X	1.87	1.29 ^X
400	8.21 ^Y	75.53 ^Y	1.92	1.36 ^Y
<i>P-values (Two-way ANOVA)</i>				
Temperature	< 0.05	NS	NS	< 0.05
Vitamin E	< 0.05	< 0.05	NS	< 0.05
Temperature $\times$ Vitamin E	NS	NS	NS	NS

The data are presented as the mean of five replicates (n=5). Different lowercase letters within a column indicate significant differences between the experimental treatments. Different uppercase letters (A, B, C) within a column indicate significant differences ($P < 0.05$) between the means of the temperature levels. Different uppercase letters (X, Y, Z) within a column indicate significant differences ($P < 0.05$) between the means of the vitamin E levels.

striped catfish *Pangasianodon hypophthalmus* increase at 35°C compared to 27°C and 31°C.¹¹

Snubnose pompano is a tropical marine fish species with a historically narrow temperature tolerance range, fluctuating between 24°C and 28°C, with optimal growth observed at 27°C. This species exhibits limited heat tolerance, becoming inactive and at risk of mortality below 12°C. At temperatures exceeding 33°C, survival rates significantly decrease, leading to death within a few days.¹² However, the current research shows that the fish grew well and had a good immune response at temperatures between 28°C and 34°C, with the best results at 34°C, and no individual fish died at this temperature. In addition, another study reported that snubnose pompano exhibited good growth, high survival rates, and effective antioxidant responses at 32°C.⁸ This indicates that the temperature range of snubnose pompano has expanded, and the fish have adapted well to higher temperatures. Therefore, the temperature range in the current study is considered the new adaptive limit for the experimental fish. In this study, we chose 34°C as one of the experimental temperatures to investigate the upper thermal tolerance limit of juvenile snubnose pompano. Although the FAO reports that water temperatures above 33°C can be fatal for certain species within a few days, recent studies on tropical fish have indicated that species like *Trachinotus blochii* may withstand higher temperatures under controlled conditions, especially when dietary antioxidants, such as Vitamin E, are included.⁸ The inclusion of 34°C allowed us to assess the combined effects of high temperature and Vitamin E supplementation in extreme conditions. To ensure the safety and well-being of

the fish, we gradually acclimated them to the higher temperatures (31°C and 34°C) over a period of seven days, increasing the temperature incrementally by 1°C per day. This approach minimized thermal shock and allowed the fish to adapt physiologically. Throughout the 70-day trial, no mortality was observed, indicating that the fish were able to tolerate these elevated temperatures when supplemented with Vitamin E. The results of this experiment suggest that snubnose pompano can adapt to temperatures as high as 34°C without adverse effects, potentially expanding their known thermal range under appropriate conditions. However, further research is needed to confirm the long-term effects of such high temperatures on survival and health in commercial farming settings. Within this temperature range, as temperature increases, the rate of metabolic reactions in the fish body accelerates, leading to faster growth. This is reflected in the increased length and weight of the fish, as well as the rise in hematological parameters to meet the body's metabolic demands.

The experimental temperature did not affect the biochemical composition of snubnose pompano except for total lipid content. Lipid levels decreased as the temperature increased from 28°C to 34°C, with the lowest levels observed at 34°C. This is reasonable since snubnose pompano's metabolic demands are higher at 34°C, requiring more energy, thus necessitating the breakdown of lipids to supply energy for the fish's activities. Additionally, temperature influenced immune indicators, such as serum lysozyme levels and respiratory burst activity, but did not affect phagocytic activity or the phagocytic index. Serum lysozyme levels and strong bacterial agents produced dur-

ing the respiratory burst of macrophages in snubnose pompano were highest at 34°C and gradually decreased at 28°C. According to Magnadottir et al.,¹³ phagocytosis is one of the most important processes in poikilothermic animals, as it is less affected by temperature. Similarly, Biller and Takahashi¹⁴ suggested that respiratory burst, which produces strong bactericidal agents, is significantly influenced by temperature. Thus, the phagocytic activity and respiratory burst of snubnose pompano's white blood cells differed from Biller's findings. This could be because the respiratory burst of white blood cells in this experiment were consistent with the findings of Magnadottir et al.¹³ and.¹⁴

The ability of the innate immune system to eliminate foreign invaders depends not only on the phagocytic activity of white blood cells and the destruction of foreign matter by oxidants produced during the respiratory burst of macrophages but also on humoral components like lysozyme. Many studies have shown that serum lysozyme levels in fish are influenced by their adaptation to different temperatures. Fletcher and White¹⁵ reported that serum lysozyme levels in European plaice *Pleuronectes platessa* L. decreased by 70% when living at low temperatures (5°C) for three months. A similar trend has been observed in common carp *Cyprinus carpio* L. (cited in Saurabh and Sahoo¹⁶) and gilthead sea bream *Sparus aurata*.¹⁷ Conversely, Kumari et al.¹⁸ reported the lowest lysozyme levels at the highest environmental temperature (32.5°C) in Asian catfish *Clarias batrachus*. Similarly, Japanese eel *Anguilla japonica* reared at 20–30°C had lower serum lysozyme levels compared to 15°C.¹⁶ In the current study, serum lysozyme levels in snubnose pompano gradually increased within its adaptive temperature range of 28°C to 34°C, with the highest levels observed at 34°C.

Snubnose pompano is a pelagic species that swims continuously, so it requires a highly nutritious diet to supply the energy needed for its vigorous metabolism. The protein content in commercial feed for snubnose pompano typically ranges from 40% to 50%, with lipid levels between 7% and 10%.¹² In addition to the main nutrients such as protein, lipids, and carbohydrates, vitamin E and other vitamins play an important role in the daily diet of fish. Numerous studies have shown that fish fed with adequate vitamin E exhibit good growth, favorable biochemical composition, and strong immune responses.^{3,4,19} The current study also demonstrates that vitamin E significantly affected the growth, biochemical composition, hematological indices, and serum lysozyme levels in snubnose pompano.

Phagocytosis is an essential mechanism of innate immunity and is considered an indicator of immune status in various fish species, influenced by several factors, including nutrition.¹⁴ To date, few studies have examined the effect of vitamin E on fish phagocytic function. The current study shows that vitamin E supplementation influenced the phagocytic function of juvenile snubnose pompano. Although fish in the treatments without vitamin E remained healthy throughout the experiment and showed no abnormal behavior, phagocytic activity and the phagocytic index were significantly lower compared to the group supplemented with vitamin E. These results are consistent

with studies on rainbow trout *Oncorhynchus mykiss*.²⁰ According to Blazer,²¹ some studies have shown that phagocytic activity increases when fish are supplemented with high levels of vitamin E, while others have demonstrated that phagocytic function depends on the amount of vitamin E provided. Thus, the differences in phagocytic ability in the current study compared to other research may be related to the amount of vitamin E supplemented in the fish feed.

Respiratory burst is the result of phagocytosis by macrophages and neutrophils. The products of the respiratory burst are strong bactericidal agents, free oxygen radicals, and reactive oxygen species.¹⁴ However, when reactive oxygen species exceed the body's needs, they can negatively affect the phagocytic ability of macrophages and neutrophils, as these free radicals attack and damage the membranes of immune cells.²² Vitamin E protects the membranes of macrophages from damage caused by free radicals and reactive oxygen species such as peroxide and superoxide, which are produced during the respiratory burst.²¹ Therefore, the absence of vitamin E in this experiment may have affected the respiratory burst process in white blood cells, with lower levels of reactive oxygen species in the group without vitamin E compared to the group with vitamin E.

According to El-Sayed,²³ temperature is one of the factors significantly affecting the vitamin E requirements of fish, though this has not yet been widely demonstrated in aquatic animals. Chen et al.⁹ showed the role of vitamin E in improving survival rates, vitamin E content in organs, and hematological indices of snubnose shiner *Notemigonus crysoleucas* under high-temperature conditions. In the current study, there was no interactive effect of vitamin E and temperature on growth, feed utilization efficiency, body biochemical composition, or natural immune responses in snubnose pompano. The experimental temperatures were within the fish's adaptive range, so their bodies did not experience significant damage from free radicals generated by high-temperature stress. Thus, the protective role of vitamin E against cellular damage and free radicals under high-temperature conditions was not evident in this study. Therefore, future research should determine the maximum temperature limit that snubnose pompano can tolerate to further explore the interaction between vitamin E and temperature on the fish's physiological processes.

In conclusion, the results of this study demonstrate that both temperature and Vitamin E supplementation play a significant role in enhancing growth, biochemical composition, and immune responses of juvenile snubnose pompano. Fish reared at 34°C displayed optimal growth and feed efficiency, with the highest performance metrics observed in groups supplemented with 400 mg/kg of vitamin E. This supplementation notably improved serum lysozyme, white blood cell counts, and phagocytic activity, indicating a strengthened immune response. The findings confirm that snubnose pompano can adapt to higher temperatures when supported by adequate dietary antioxidants, suggesting that 400 mg/kg of vitamin E is effective in promoting resilience in warmer aquaculture environments.

Future research should explore long-term impacts and potential upper temperature limits to further optimize vitamin E utilization for snubnose pompano health and productivity in changing climate conditions.

Implications and Perspectives: The findings highlight the importance of Vitamin E supplementation in aquaculture, particularly for snubnose pompano farmed in environments with elevated temperatures. Future research should focus on identifying the upper temperature limits that this species can tolerate to better understand the potential of Vitamin E in protecting fish against thermal stress. Additionally, studies on long-term effects of Vitamin E on the immune system and interactions with other dietary elements would provide further insights for optimizing nutrition strategies in commercial aquaculture systems. Exploring these factors will help improve fish health and productivity in the context of global climate change.

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AUTHORS' CONTRIBUTION

Formal Analysis: Thi-Hanh Pham (Equal), Minh-Hoang Le (Equal). **Investigation:** Thi-Hanh Pham (Equal). **Funding acquisition:** Thi-Hanh Pham (Lead). **Methodology:** Vi-Hich Tran (Equal), Minh-Hoang Le (Equal). **Writing – original**

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COMPETING OF INTEREST – COPE

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ETHICAL CONDUCT APPROVAL – IACUC

In accordance with Vietnam's National Regulations on the Use of Animals in Research (Decree 32/2006/ND-CP, 2006), snubnose pompano is not classified under either Group IB (endangered and critically endangered species) or Group IIB (threatened and rare species). As such, conducting this study did not necessitate obtaining a permit or ethical approval. Nonetheless, the researchers adhered to the highest standards of ethical practice in their treatment of animals throughout the study.

INFORMED CONSENT STATEMENT

All authors and institutions have confirmed this manuscript for publication.

DATA AVAILABILITY STATEMENT

All are available upon reasonable request

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