MINISTRY OF EDUCATION AND TRAINING
NHA TRANG UNIVERSITY

PHAM THI HANH

ASSESSMENT OF THE IMPACT OF VITAMIN E AND C SUPPLEMENTATION IN THE DIET ON THE NATURAL IMMUNE RESPONSE OF SNUBNOSE POMPANO *Trachinotus blochii* (Lacepède, 1801) AT THE JUVENILE STAGE

SUMMARY OF PhD THESIS ON AQUACULTURE

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The study was completed at Nha Trang University

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INTRODUCTION

In aquaculture, farmed fish frequently face stress caused by various factors, with extreme temperatures being a significant influence. Prolonged exposure to such stressors and their strong intensity can reduce fish growth rates, survival rates, and suppress immune functions, making fish more susceptible to pathogens (Le MH, 2021). To enhance immune system functions and mitigate the harmful effects of oxidative stress, supplementing organisms with antioxidants is essential. Among the antioxidant groups provided to fish, vitamin E and vitamin C are considered the most potent. Fish cannot synthesize vitamin C and vitamin E on their own, so their vitamin requirements must be met through external sources, either from natural feed or through supplementation in formulated diets.

Snubnose pompano (*Trachinotus blochii*) is one of the key marine aquaculture species in many Asia-Pacific countries, including Vietnam. In recent years, numerous studies have focused on the nutritional requirements of this species to optimize production and commercial farming processes. However, research on the vitamin C and vitamin E requirements for this fish is very limited, particularly studies on the effects of these vitamins on the innate immune responses of juvenile snubnose pompano. Therefore, investigating the effects of vitamin E and vitamin C on the innate immune responses of snubnose pompano, especially under high-temperature conditions, is necessary to contribute to the sustainable development of intensive snubnose pompano farming.

General Objective: Aiming to improve the quality of industrial feed, support seed production, and develop sustainable commercial farming of snubnose pompano.

Specific Objectives: (i) Determine the optimal levels of vitamin E and vitamin C to enhance growth and improve the immune response of snubnose pompano under high-temperature conditions; (ii) Evaluate the effects of vitamin E and vitamin C on feed utilization efficiency, adaptability, and overall health of fish under high-temperature conditions; (iii) Provide a scientific basis for optimizing industrial feed formulations to improve the farming efficiency of snubnose pompano under high-temperature conditions.

Thesis Contents:

- The effects of dietary vitamin E levels on the immune response of snubnose pompano.
- The impact of dietary vitamin E on the immune response of snubnose pompano under high-temperature conditions.
- The effects of dietary vitamin C levels on the immune response of snubnose pompano.
- The impact of dietary vitamin C on the immune response of snubnose pompano under high-temperature conditions.

Scientific and Practical Significance of the Thesis:

The results of this study identify the required levels of vitamin C and E for growth, biochemical composition, and immune response in snubnose pompano. Additionally, the study highlights the positive effects of vitamin E and vitamin C on snubnose pompano under high-temperature conditions. These findings provide a scientific basis for improving the quality of formulated feeds for fish, contributing to the sustainable development of snubnose pompano farming in the context of climate change.

CHAPTER 1. LITERATURE REVIEW

Vitamins play an important role in the physiological functions of fish. Among the vitamins supplemented in feed, vitamin C and vitamin E are considered essential vitamins because of their strong antioxidant properties and disease resistance of animals.

Most teleosts are unable to synthesize vitamins. Therefore, the vitamins supplied to the body must be obtained from an exogenous source through natural foods. However, in intensive aquaculture, vitamin sources from natural foods are very limited, so adding vitamins to industrial feed is very necessary.

Vitamin requirements in aquatic animals vary depending on the species, stage of development, interaction between feed ingredients and experimental conditions. The quantitative requirements on vitamin C have been determined for several species and the recommended values varied from 10 to 10,000 mg AA/kg of feed. Vitamin C has been found to promote growth, increase survival rate and reduce deformities in fish. When supplemented in high concentrations, vitamin C has the effect of modulating the immune response. *P. crocea* juvenile needs 28,2 mg AA/kg for the best survival rate, but needs to supplement 489 mg/kg diet to increase lysozyme activity, complement system activity, phagocytic activity and respiratory burst of fish (Ai, 2006). Lysozyme activity of cobia also increased when vitamin C was supplemented at a concentration of 400 mg/kg diet, more than 20 times higher than the level required for growth and survival rate (Zhou, 2012).

It’s believed that the need for vitamin E in marine fish is higher than that of freshwater ones due to the higher amount of lipids and n-3 HUFA in marine fish food. According to Lewis (2018), vitamin E supplemented at high levels, far exceeding normal needs, will improve the organism's immune system, reduce the risk of infection, especially in the early stage of organismal development. Research on *A. regius* showed that fish needed 800 mg/kg diet to prevent granulomatous disease, while supplementation levels of 40 -100 mg/kg diet gave optimal growth performance (Ruiz, 2019). The respiratory burst activity of red snapper increased when the fish were supplemented with vitamin E at a level of 60 - 80 mg/kg diet, compared to lower levels of 10 - 40 mg/kg diet (Peng, 2009). Similar results were also recorded in parrotfish, the fish had a lower dietary requirement for optimal growth, which was 38 mg/kg diet. However, more than 500 mg/kg diet was needed to enhance the fish’s immune response (Galaz, 2010).

The chemical composition is considered reliable information that reflects the quality of the meat, nutritional value, physiological state and living environment of fish. Many factors have been identified that affect the composition of fish, of which ingredients in food is considered one of the factors that significantly affects the biochemical composition. Some studies show that vitamin E has a significant impact on fish body composition including moisture, lipid, ash and total protein content. According to Chen et al. (2004), fish not supplemented with vitamin E have low levels of protein, crude lipid and dry matter (Chen, 2004). Vitamin E also clearly affects the protein content of rohu fish *L. rohita* (Sau, 2004), hybrid tilapia *Oreochromis niloticus* x *O. aureus* (Huang, 2004). However, research on beluga sturgeon *H. huso* shows that vitamin E does not affect the protein, fat, ash and moisture content of fish (Amlashi, 2011).

Similar to vitamin E, vitamin C is also known to affect the body composition of fish. Research on the fish species *Heteropneustes fossilis* shows that supplementing the diet 1,200 mg of vitamin C/kg of food has the effect of increasing the protein content and reducing the body water ratio (Alam, 2009) or supplementing the diet of *T. ovatus* species at a dosage of 59.1 mg/kg diet increased protein and lipid content (Zhang, 2019). Adding vitamin C alone did not affect the protein, moisture, and ash content of Asian seabass *Lates calcarifer*, but vitamin C has a positive effect on these parameters when supplemented together with ginger extract (Abdelwahab, 2020). However, vitamin C did not affect the composition of cobia *R. canadum* juvenile when supplemented with vitamin E (Pham Anh, 2012). Temperature has been shown to have an impact on the body composition of fish, especially the content of essential fatty acids in the body (Khalili, 2018). Jobling (2003) reported that increased aquatic temperature leads to the accumulation of low amounts of saturated fatty acids and high amounts of unsaturated fatty acids (Jobling, 2003). Research on waigieu seaperch *P. waigiensis* shows that the protein and lipid levels of fish raised at a temperature of 28ºC are higher than 32ºC (Hoang, 2021). Salmon raised at 10°C exhibited higher levels of n-6 fatty acids in fillets than when raised at 20°C (Norambuena, 2016). Similar results were reported for rainbow trout raised at 15°C compared to 19°C (Mellery, 2016).

The fingerling period are sensitive. When they are transferred from a stable environment in hatcheries to an unstable condition in nature, they are often exposed to physical and chemical factors in the water that cause stress and anxiety weaken immunity. Therefore, supplementing vitamin C as well as vitamin E for fish is very necessary.

In our country, adding vitamin C and vitamin E to shrimp and fish diets is done very early, regularly. This significantly improves animal health. However, this supplement is only based on the manufacturer's recommendations and the dosage is generally applied to a group of organisms. Until now, there is limited research on the optimal supplementation levels or the effects of vitamin C and vitamin E on biological functions for each specific species. To date, research on the effects of vitamins E and C on growth and immune response in snubnose pompano *T. blochii* in Vietnam is still very limited.

Fish are ectothermic animals, they cannot control their own body temperature. Their body temperature is determined by the temperature of the surrounding water environment. Therefore, temperature is an important factor affecting the growth and survival rate of fish. To date, there have been many studies on the effects of temperature on fish’s development. However, information about the impact of this factor on snubnose pompano is still very limited. Most previous studies have just been evaluated the impact of temperature on some indicators of growth, survival rate, gene expression, deformability of pompano in the larvae stage (Han, 2021) and on intestinal histology, antioxidant enzyme activity, metabolism, and blood biochemistry of fish in the juvenile stage (Prabu, 2023). Research on the impact of temperature on snubnose pompano in Viet Nam, particularly the interaction effects of vitamins and temperature on growth, biochemical composition and immune response of snubnose pompano juvenile has not been conducted yet.

CHAPTER 2. GENERAL MATERIALS AND METHODS

2.1 Subjects, time and location of research

- Research object: Snubnose pompano *Trachinotus blochii* (Lacepède, 1801) juvenile.
- Research period: from 2020 to 2023
- Research location: Nha Trang University

2.2 Research content:

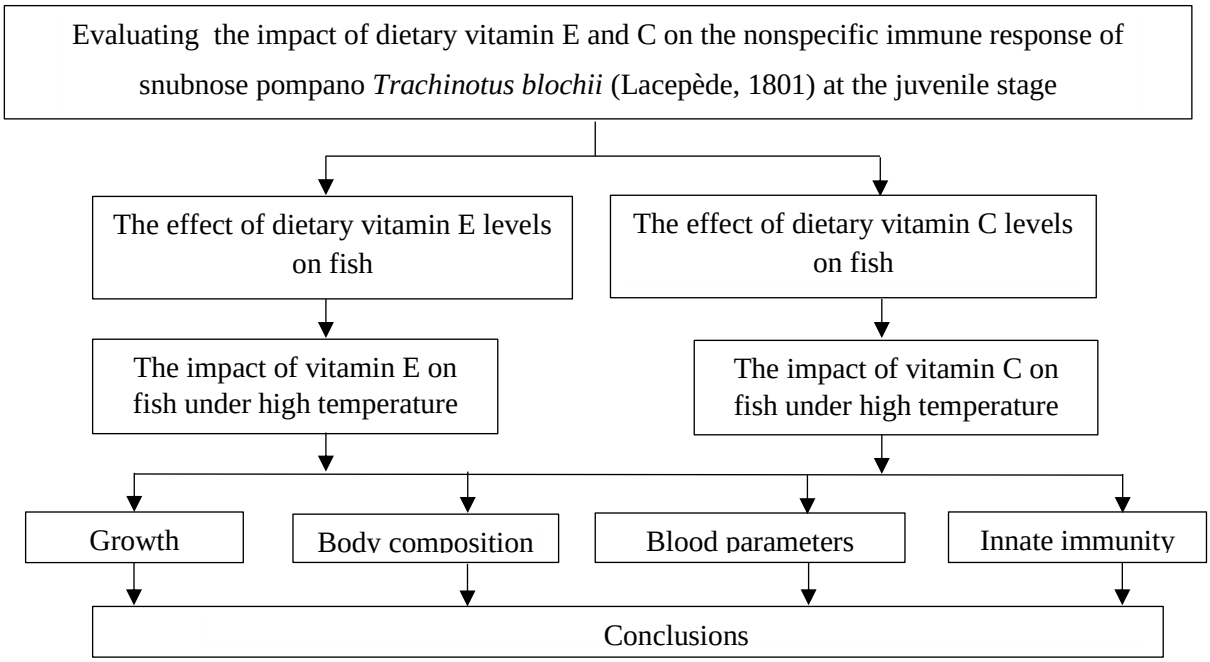


Figure 2.1 Block diagram of research content

2.3 Research Methods

2.3.1 Experimental diets

The base feed formula (47.26% protein, 10.7% lipid) was pelleted, dried at 30^{o}C for 24 hours and stored at 4^{o}C until analysed and used in the trials.

2.3.2 Experimental animals

Snubnose pompano were selected for the experiment to ensure uniform size (about 5 cm), healthy, flexible swimming, bright body color, and no deformities.

2.3.3 Experimental arrangement

2.3.3.1 Experiment 1: Effects of different dietary vitamin E levels on the immune response of snubnose pompano

Food: Vitamin E used for the experiment was DL-alpha-tocopherol acetate (China). The base feed was supplemented with vitamin E at levels of 100, 200, 400, 800 and 1,600 mg/kg diet.

Experimental fish: Fish were randomly arranged into 200 L composite tanks with a density of 30 fish/tank. The experiment duration is 10 weeks, and the growth is checked every 2 weeks.

Experimental design: The experiment was designed with 6 treatments, including 5 treatments supplemented with vitamin E (100, 200, 400, 800 and 1,600 mg/kg diet) and 1 treatment without vitamin E supplementation (control treatment). Each treatment was repeated 3 times at the same time.

Care and management regime: Fish feeding time is 2 times a day, at 8:00 a.m. and 5:00 p.m. Fish are fed a diet of about 5% of body weight. Environmental factors are measured every 2 days. The water is changed 50% every day and aerated continuously.

2.3.3.2 Experiment 2: The impact of dietary vitamin E on the immune response of pompano under high temperature conditions

Experimental design: Experiment 2 was randomly arranged with 6 treatments, including 2 vitamin E treatments (0, 400 mg/kg diet) combined with 3 temperature treatments (28ºC, 31ºC and 34ºC). Each treatment was repeated 5 times at the same time.

The temperature in the experimental tanks was adjusted using an HZ-Q5 800W heater (China). Food preparation, fish source, care and management of the experimental system were carried out as in experiment 1 (sections 2.3 and 2.3.3.1)

2.3.3.3 Experiment 3: Effects of different dietary vitamin C levels on the immune response of pompano

Experimental design: The experiment was designed with 6 treatments, including 5 treatments supplemented with vitamin C (50, 100, 200, 400 and 800 mg/kg diet) and 1 treatment without vitamin C supplementation (control treatment). Each treatment was repeated 3 times at the same time.

The form of vitamin C used for the experiment was L-ascorbate-2-phosphate 35%, CSPC (China). Methods of food production, fish preparation, care and management were carried out as experiment 1, described in sections 2.3 and 2.3.3.1.

2.3.3.4 Experiment 4: The impact of dietary vitamin C on the immune response of pompano under high temperature conditions

Experimental design: Experiment 4 was randomly arranged with 6 treatments, including 2 vitamin C treatments (0, 150 mg/kg diet) combined with 3 temperature treatments (28ºC, 31ºC, 34ºC). Each treatment was repeated 5 times concurrently. The fish source, care method, and experimental system management were similar to those described in experiment 2.

2.4 Methods of collecting, analyzing samples and processing data

2.4.1 Sample collection and analysis methods

Method for determining environmental parameters

Environmental parameters such as temperature, pH, and DO content were determined using a multi-parameter environmental meter WQC-22A (ToaDkk, Japan). Salinity was measured using an AR8012 salinity meter (SmartSensor, China).

Method for analyzing vitamin content in food

* Vitamin content was analyzed using high-pressure liquid chromatography, corresponding to 6 experimental treatments of vitamin E, which were 2.31 mg/kg diet; 117.25 mg/kg diet; 219.03 mg/kg diet; 392.1 mg/kg diet; 788.54 mg/kg diet; 1,537.32 mg/kg diet and 6 experimental treatments of vitamin C were 7.3 mg/kg diet; 50.5 mg/kg diet; 99.6 mg/kg diet; 186.5 mg/kg diet; 394.8 mg/kg diet; 786.3 mg/kg diet.

Method to determine growth, survival rate, feed efficiency and body index

* At the end of the experiment, all fish in each treatment were weighed and measured to determine the growth indicators.

- Fish were measured and weighed to determine their length and weight using a technical measuring ruler and a BPA electronic scale (Ohaus, USA). The survival rate (%) of fish was determined as the number of fish remaining at the end of the experiment.

- Feed utilization, protein utilization efficiency (FI, PE) and feed conversion ratio (FCR) were determined through food intake monitored daily throughout the experiment.

- Three fish (9 fish/treatment) were collected randomly from each tank, dissect the liver and internal organs to weigh and analyze the VSI and HSI.

Method for determining biochemical indicators

* Proximate composition of diets and fish whole body: Protein, Lipid, Ash and Moisture were analyzed according to the AOAC method (2000).

Method for determining hematological and immunological indicators

* Fish were anesthetized with monophenyl ether glycol at a concentration of 150 - 200 ppm before collecting blood samples. Blood samples were collected from tail vein. After a part of the blood was collected and analyzed for its formula and biochemical parameters, the remaining part was centrifuged to collect serum. Fish serum was stored at -80^{o}C to analyze lysozyme content.

- Analysis of white blood cells, red blood cells, hematocrit and hemoglobin were determined using a Sysmex XT-1800i blood analyzer (Sysmex Corporation, Hyogo, Japan). Plasma triglyceride and protein content were measured using a DxC600 general chemistry analyzer.

- Serum lysozyme activity was determined according to the description of Shugar (1952) with adjustments.

* Isolation of leukocytes to determine the phagocytic index and respiratory burst of leukocytes was performed as described by Samai et al (2017) with modifications. Evaluation of these two criteria is applied to experiments 2 and 4.

- Phagocytic activity and phagocytic index of pompano head-kidney tissue white blood cells were determined based on the method of Siwicki et al (1994).

- Respiratory burst activity of pompano's head-kidney cells was determined according to the method of Cheng et al. (2007).

Histological methods and analysis of bone deformities: Evaluation of histological structure was performed in experiment 1 and bone deformities were performed in experiment 3.

- Sectioning according to the histological method described by Coolidge and Howard (1979). Analyze the structure of liver and muscle tissue of fish under a microscope, recording histological changes according to Ferguson (2006).

- Determination of bone morphology was performed according to the method of Schnell (2016). Bone morphology was photographed using a Canon camera (China).

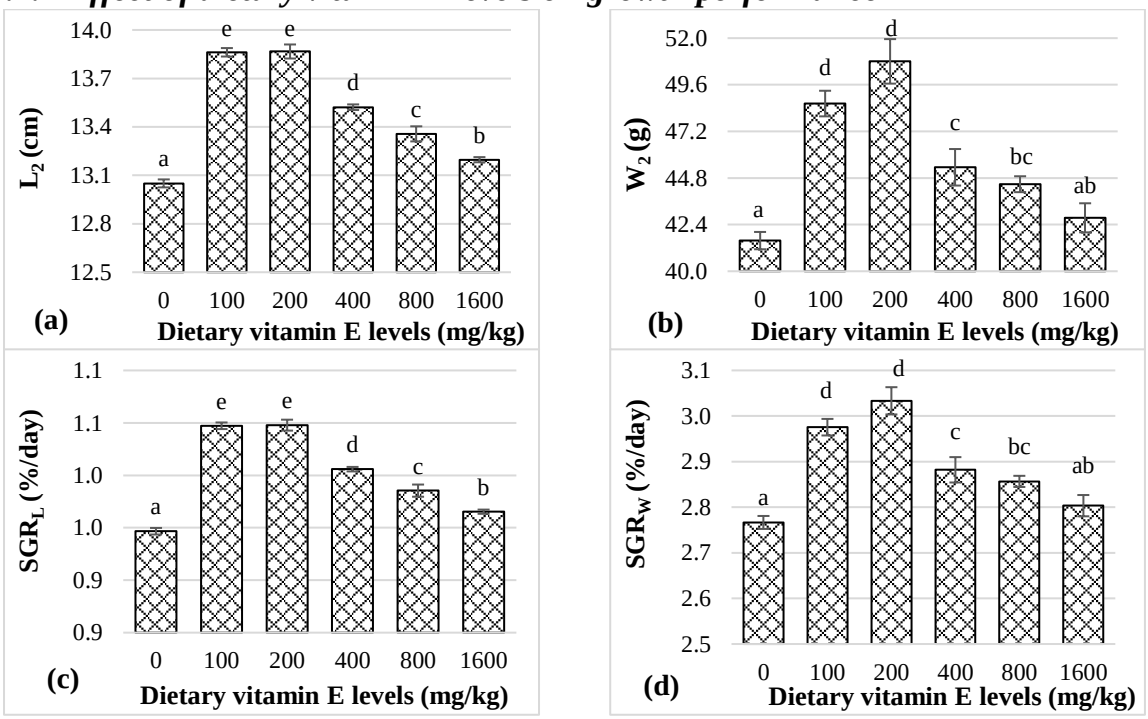
2.4.2 Statistical Analysis

Data were expressed as mean value ± standard error. Data were statistically analyzed using one-way ANOVA (Experiment 1 & 3) and two-way ANOVA (Experiment 2 & 4) with SPSS 22.0 software. The difference between the mean values was determined by the Duncan test with a significance level of $P < 0.05$.

CHAPTER 3. RESULTS AND DISCUSSION

3.1 Effects of dietary vitamin E concentrations on the natural immune response

3.1.1 Effect of dietary vitamin E levels on growth performance



Hình 3.1 Length (a, c) and weight (b, d) of fish fed dietary vitamin E levels

The results presented in Figure 3.1 show that supplementing snubnose pompano diets with vitamin E significantly improves final length (FBL), final weight (FBW), and specific growth rates (SGRL, SGRW). These values increased significantly as the level of vitamin E supplementation rose from 0 to 200 mg/kg diet, reaching their highest at 200 mg/kg diet, with statistically significant differences compared to other levels ($P < 0.05$). However, when supplementation exceeded 200 mg/kg diet, these indicators gradually declined, particularly at 1600 mg/kg diet, indicating that high levels of vitamin E can have adverse effects on growth. Therefore, a vitamin E supplementation level of 200 mg/kg diet is identified as optimal for achieving the best growth performance in terms of length, weight, and specific growth rates.

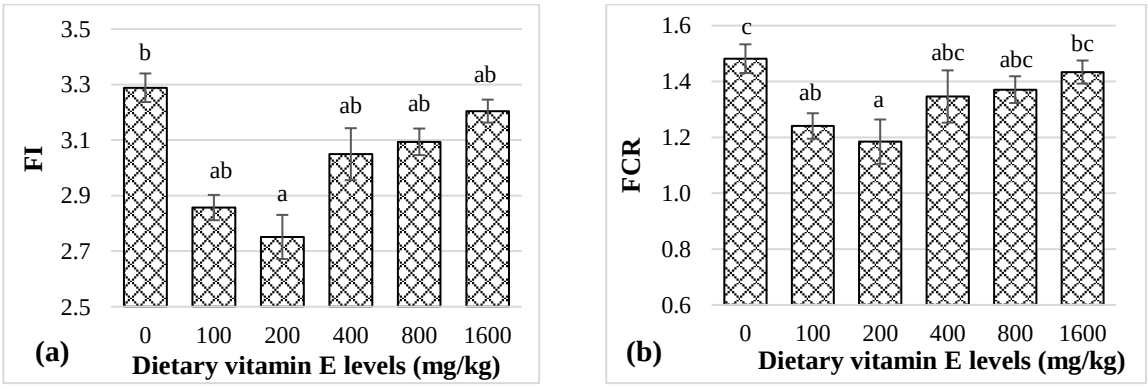


Figure 3.2 Fish feed consumption (a) and feed conversion ratio (b) of fish fed different dietary vitamin E levels

The results in Figure 3.2 show that supplementing vitamin E at a level of 100 mg/kg diet is optimal for reducing feed intake (FI) and improving feed conversion ratio (FCR) in snubnose pompano. Feed intake was lowest at 100 mg/kg diet and increased slightly at supplementation levels ranging from 400 to 1600 mg/kg diet,

although the differences were not statistically significant (P > 0.05). The feed conversion ratio was lowest at 100 mg/kg diet, with statistically significant differences compared to other levels (P < 0.05). This indicates that a supplementation level of 100 mg/kg diet is ideal for improving feed utilization efficiency.

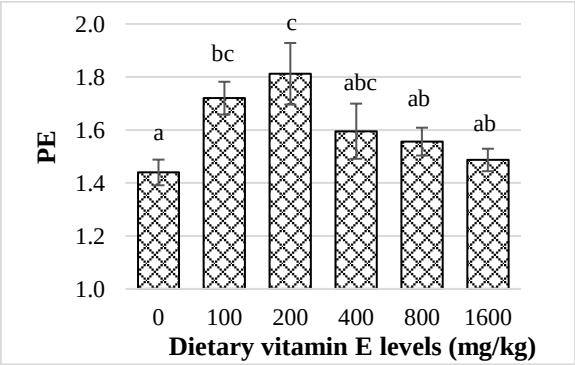


Figure 3.3 Protein utilization efficiency of fish fed different dietary vitamin E levels

Protein efficiency (PE) in fish increased progressively with vitamin E supplementation from 0 to 200 mg/kg diet, reaching the highest value at 200 mg/kg diet, with statistically significant differences (P < 0.05). However, when supplementation exceeded 200 mg/kg diet, protein efficiency gradually declined, particularly at 1600 mg/kg diet. The level of 200 mg/kg diet was identified as optimal for improving protein efficiency, while higher or lower levels did not achieve maximum effectiveness (Figure 3.3).

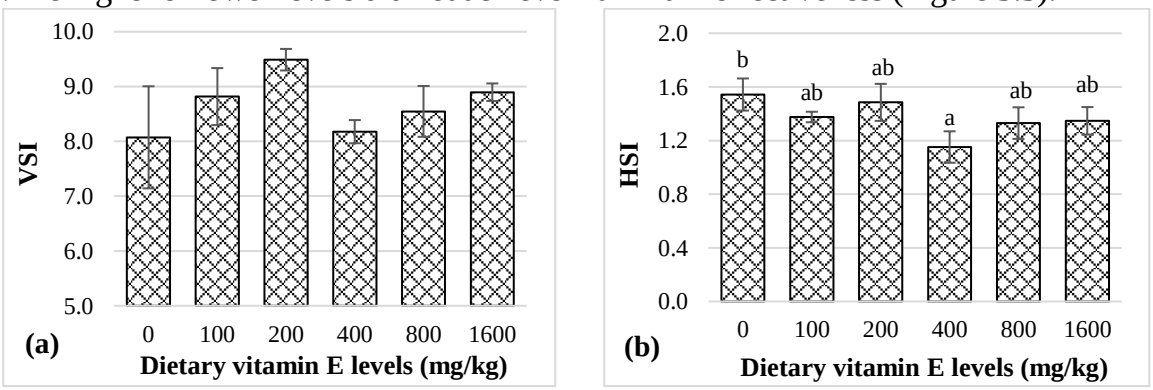


Figure 3.4 Visceral index (a) and liver index (b) of fish fed dietary vitamin E levels

The viscerosomatic index (VSI) of fish increased slightly, peaking at the 200 mg/kg diet vitamin E supplementation level, but there were no statistically significant differences between levels (P > 0.05). The hepatosomatic index (HSI) was lowest at 100 mg/kg diet, with a statistically significant difference compared to 0 mg/kg diet (P < 0.05), while levels of 200 mg/kg diet or higher did not provide additional noticeable benefits. Supplementing with 200 mg/kg diet vitamin E is considered optimal for improving growth and maintaining a balance in the VSI and HSI of fish (Figure 3.4).

3.1.2 Effect of dietary vitamin E levels on the whole body composition

Table 3.1 shows that supplementing with 200 mg/kg diet of vitamin E is optimal for improving the biochemical composition of fish, with the highest levels of protein (19.66 ± 0.80%) and lipid (10.85 ± 0.39%) while maintaining a balance in moisture and ash content. Excessive supplementation (1600 mg/kg diet) reduces protein, lipid, and ash content, providing no additional benefits and potentially causing negative effects.

Table 3.1 Proximate whole-body composition of pompano fed different dietary vitamin E levels

Dietary vitamin E content (mg/kg diet)	Moisture (%)	Ash (%)	Protein (%)	Lipid (%)
0	70.18±0.58^{c}	3.74±0.15^{a}	18.52±0.02^{a}	7.2±0.2^{a}
100	66.54±0.52^{a}	5.40±0.14^{cd}	19.54±0.04^{cd}	10.42±0.17^{c}
200	66.73±0.4^{a}	5.66±0.1^{d}	19.66±0.08^{d}	10.81±0.38^{c}
400	68.1±0.06^{b}	5.07±0.15^{c}	19.31±0.07^{bc}	9.34±0.52^{b}
800	68.31±0.25^{b}	4.43±0.11^{b}	19.26±0.05^{b}	9.27±0.19^{b}
1600	69.62±0.27^{c}	4.17±0.09^{b}	18.51±0.14^{a}	7.47±0.34^{a}

3.1.3 Effect of dietary vitamin E levels on the immune response

Supplementing with 400 mg/kg diet of vitamin E enhances the fish's immune response, with serum lysozyme levels reaching the highest value, significantly higher than other levels ($P < 0.05$). The platelet count (PLT) did not vary significantly among the different levels of vitamin E supplementation ($P > 0.05$) (Figure 3.5).

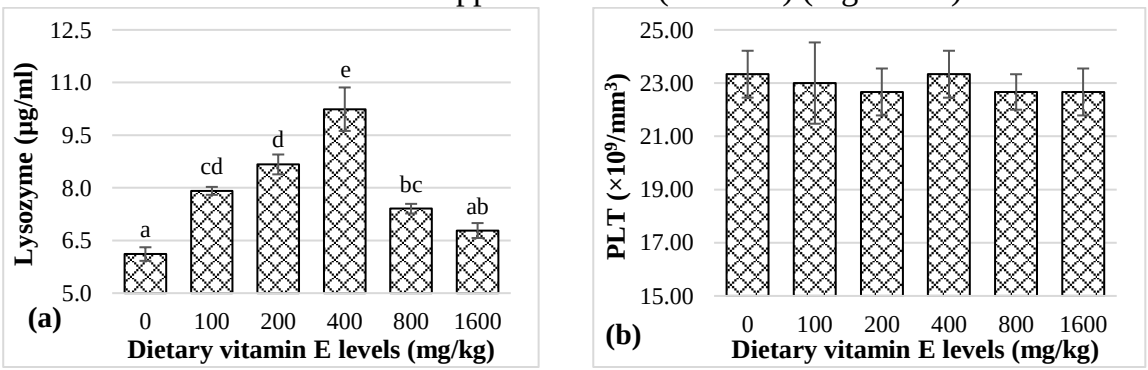


Figure 3.5 Lysozyme content (a) and PLT count (b) of fish fed dietary VE levels

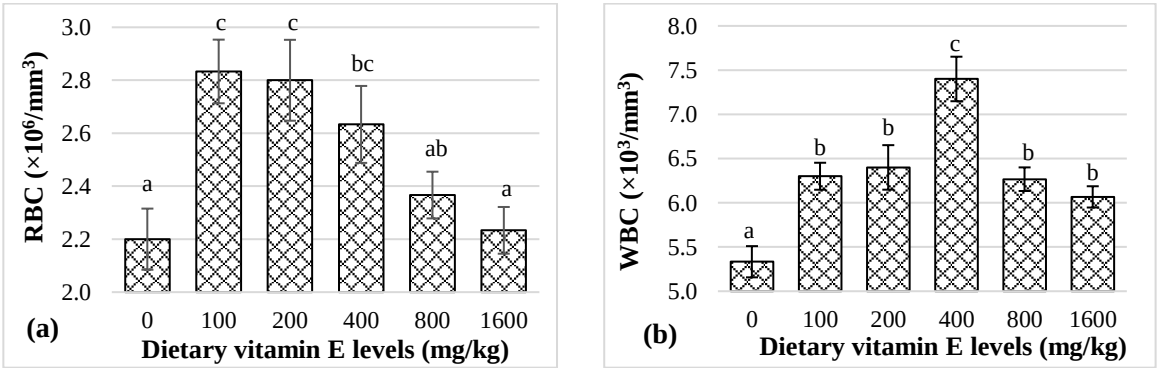


Figure 3.6 RBC (a) and WBC count (b) of fish fed different dietary vitamin E levels

Supplementing with 200 mg/kg diet of vitamin E is optimal for increasing red blood cell (RBC) count, while 400 mg/kg diet is optimal for white blood cell (WBC) count, with statistically significant differences ($P < 0.05$). Supplementing beyond these levels (800–1600 mg/kg diet) provides no additional benefits and reduces these indices (Figure 3.6).

A 200 mg/kg diet vitamin E supplementation is optimal for increasing hemoglobin (Hb) levels and hematocrit (Hct) percentages, with the highest values of 9.0 g/dL and

26.0%, respectively, showing significant differences compared to other levels ($P < 0.05$). Supplementing beyond this level (800–1600 mg/kg diet) reduces these indices (Figure 3.7).

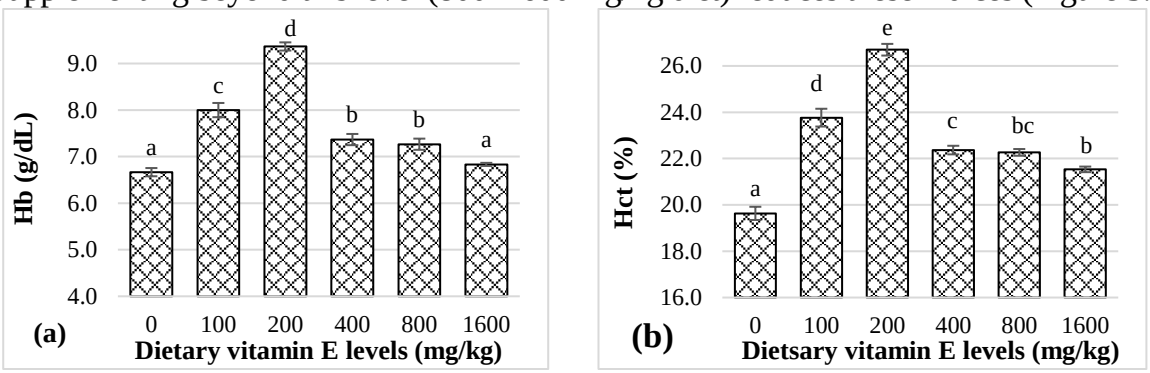


Figure 3.7 Hb content (a) and Hct ratio (b) of fish fed different dietary VE levels

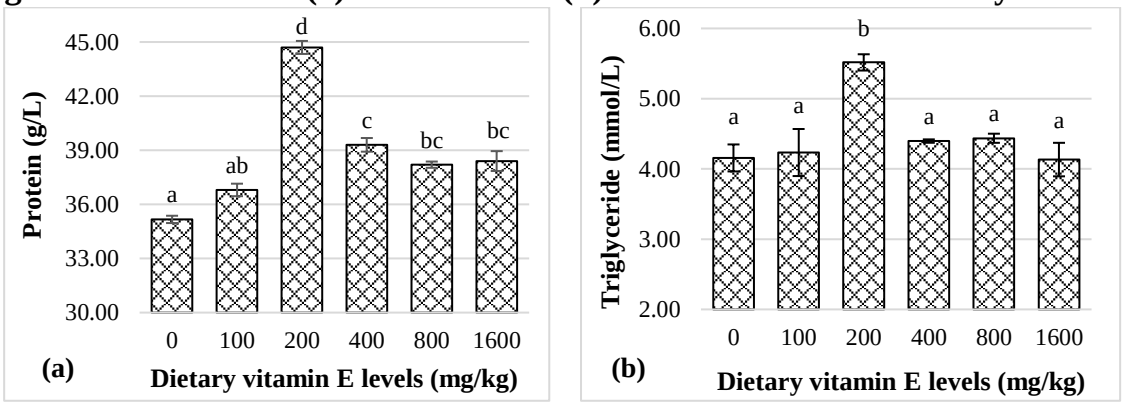


Figure 3.8 Plasma triglyceride (a) and plasma protein (b) content of fish fed different dietary vitamin E levels

Supplementing with 200 mg/kg diet of vitamin E is optimal for increasing plasma protein levels (44.5 g/L), while 400 mg/kg diet is optimal for triglyceride levels (5.5 mmol/L), with statistically significant differences ($P < 0.05$). Supplementing beyond these levels does not provide further improvement and may reduce these indices (Figure 3.8).

3.1.4 Effect of dietary vitamin E levels on liver and muscle organization of pompano

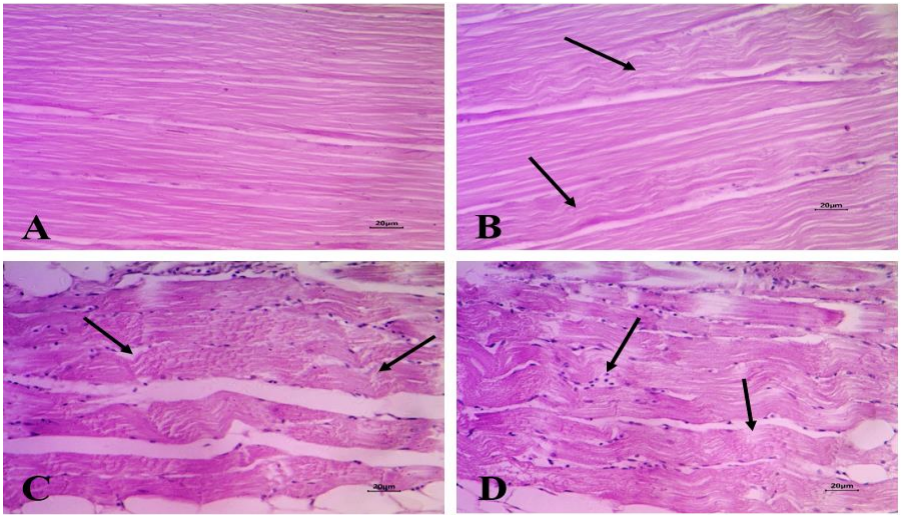


Figure 3.9 Snubnose pompano muscle tissue (H&E).

Moderate supplementation of vitamin E helps maintain healthy muscle tissue structure in snubnose pompano, with clear muscle fibers. However, a level of 800

mg/kg diet begins to cause slight fiber deformation, and 1600 mg/kg diet results in severe damage with necrosis and tissue deformation, highlighting the importance of using an appropriate dosage of vitamin E (Figure 3.9).

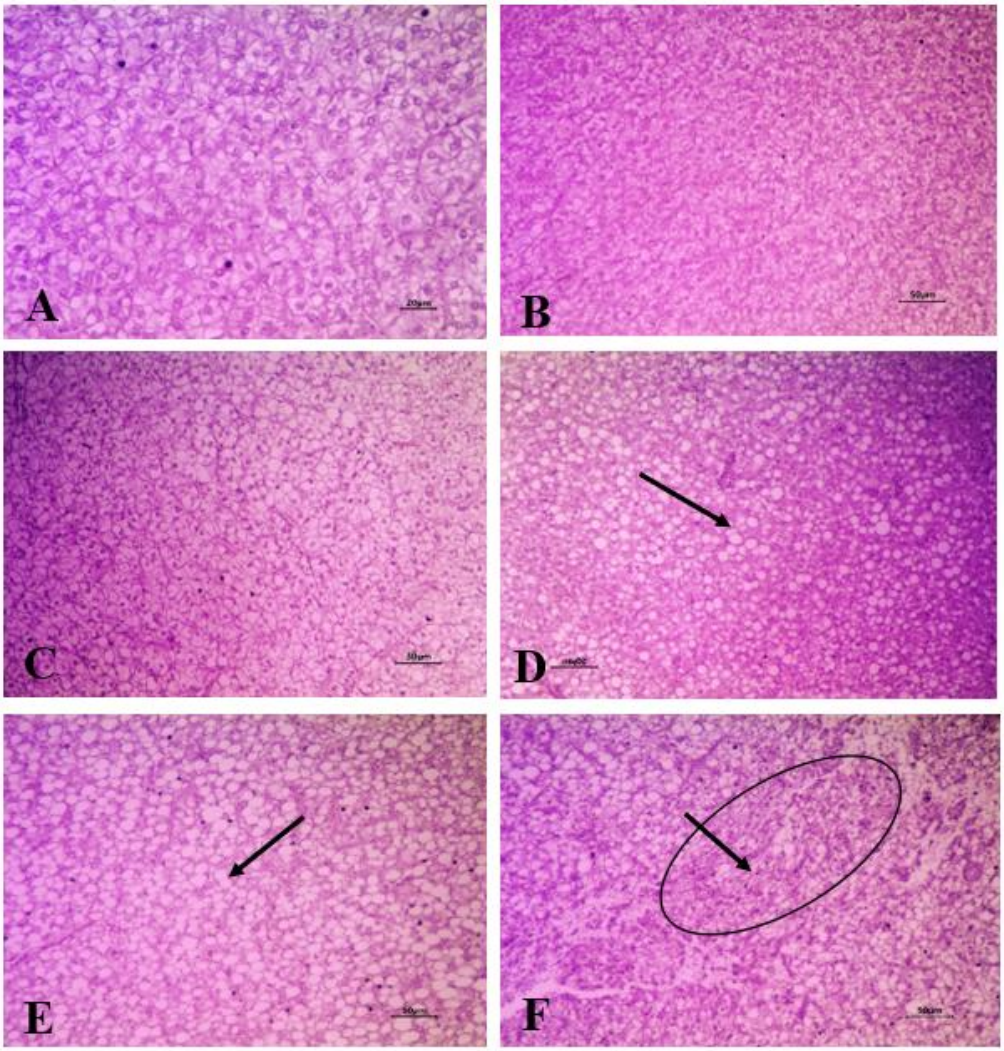


Figure 3.10 Liver tissue of pompano supplemented with VE from 100–400 mg/kg diet (H&E).

Supplementation of vitamin E at 100–200 mg/kg diet maintains normal liver tissue structure with polygonal hepatocytes and clear central nuclei. However, at 400 mg/kg diet, the number of vacuoles increases, and at 1600 mg/kg diet, nuclear degeneration and blood cell infiltration in necrotic liver areas are observed, indicating that excessive supplementation causes liver tissue damage (Figure 3.10).

3.2 Impact of VE levels on the immune response under high temperature conditions

3.2.1 Effects of vitamin E and temperature on growth and food utilization efficiency

A temperature of 34°C allows snubnose pompano to achieve optimal growth and feed utilization efficiency, with final length (14.75 cm), weight (65.6 g), growth rates (SGRL: 1.32%/day, SGRW: 3.89%/day), and protein efficiency (PE: 1.45). Supplementing vitamin E at 400 mg/kg diet significantly improves weight (62.85 g), growth rates (SGRL: 1.3%/day, SGRW: 3.83%/day), and PE (1.4). There is no interaction between temperature and vitamin E, and VSI and HSI indices remain unaffected. High temperature and appropriate vitamin E supplementation support optimal growth and nutritional efficiency in juvenile fish (Table 3.2).

Table 3.2. The effects of vitamin E and temperature on growth and feed utilization efficiency in juvenile snubnose pompano

Treatment	FBL (cm)	FBW (g/fish)	SGR_L (%/day)	SGR_W (%/day)	VSI (%)	HSI (%)	FI (%BW/day)	FCR	PE
28:0	14.32±0.00	57.75±0.53	1.27±0.01	3.7±0.01	6.24±0.16	0.99±0.05	4.28±0.04	1.67±0.02	1.28±0.01
28:400	14.17±0.12	58.83±1.30	1.26±0.01	3.73±0.03	6.31±0.05	1.01±0.03	4.21±0.08	1.64±0.04	1.3±0.03
31:0	14.64±0.10	61.65±0.48	1.3±0.01	3.8±0.01	6.85±0.09	1.07±0.04	4.03±0.03	1.55±0.01	1.37±0.01
31:400	14.69±0.10	63.39±0.89	1.31±0.01	3.84±0.02	7.09±0.09	1.09±0.01	3.93±0.05	1.51±0.02	1.42±0.02
34:0	14.62±0.10	64.89±0.43	1.3±0.01	3.88±0.01	6.99±0.12	1.11±0.03	3.84±0.02	1.47±0.01	1.45±0.01
34:400	14.87±0.12	66.31±0.20	1.33±0.01	3.91±0.00	7.06±0.04	1.13±0.03	3.76±0.01	1.43±0.00	1.49±0.00
Temperature effects									
28	14.25^{A}	58.29^{A}	1.26^{A}	3.71^{A}	6.27^{A}	1.00^{A}	4.24^{C}	1.65^{C}	1.29^{A}
31	14.66^{B}	62.52^{B}	1.31^{B}	3.82^{B}	6.97^{B}	1.08^{B}	3.98^{B}	1.53^{B}	1.39^{B}
34	14.75^{B}	65.6^{C}	1.32^{B}	3.89^{C}	7.03^{B}	1.12^{B}	3.8^{A}	1.45^{A}	1.47^{C}
Vitamin E effects									
0	14.53	61.43^{X}	1.29	3.79^{X}	6.69	1.06	4.05^{X}	1.56^{X}	1.37^{X}
400	14.58	62.85^{Y}	1.3	3.83^{Y}	6.82	1.08	3.97^{Y}	1.53^{Y}	1.4^{Y}
Two-way ANOVA P-values									
Temperature	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Vitamin E (VE)	NS	< 0.05	NS	< 0.05	NS	NS	< 0.05	< 0.05	< 0.05
Temperature xVE	NS	NS	NS	NS	NS	NS	NS	NS	NS

3.2.2 Effects of vitamin E and temperature on the whole body composition

A temperature of 34°C enables snubnose pompano to achieve the highest accumulation of protein (19.81%) and lipid (9.64%), while ash content decreases. Supplementing vitamin E at 400 mg/kg diet also improves protein (19.47%) and lipid (9.47%) levels compared to the non-supplemented group (protein: 18.75%, lipid: 7.24%) while reducing ash content. There is no significant interaction between temperature and vitamin E, but both factors contribute to enhancing the biochemical quality of the fish (Table 3.3).

Table 3.3 Effects of temperature and vitamin E on body composition of fish

Treatments	Ash (%)	Moisture (%)	Protein (%)	Lipid (%)
28:0	5.76±0.39	67.19±0.69	18.45±0.09	5.51^{a}±0.46
28:400	4.2±0.46	67.72±0.68	19.17±0.17	9.37^{c}±0.49
31:0	5.3±0.30	67.48±0.31	18.89±0.29	7.86^{b}±0.22
31:400	4.27±0.08	68.74±0.48	19.3±0.23	9.42^{c}±0.30
34:0	5.43±0.21	67.54±0.30	18.91±0.25	8.33^{bc}±0.39
34:400	4.18±0.17	69.88±0.38	19.92±0.35	10.66^{d}±0.20
Temperature effects				
28	4.98	67.56	18.81	9.5^{B}
31	4.79	68.11	19.1	8.64^{B}
34	4.8	68.71	19.42	7.44^{A}
Vitamin E (VE) effects				
0	5.49	67.4	18.75^{X}	7.24^{X}
400	4.22	68.78	19.47^{Y}	9.82^{Y}
Two-way ANOVA P-values				
Temperature	NS	NS	NS	< 0.05
VE	NS	NS	< 0.05	< 0.05
Temperature x VE	NS	NS	NS	< 0.05

3.2.3 Effects of vitamin E and temperature on the immune response

At 34°C and with vitamin E supplementation (400 mg/kg diet), the natural immunity of snubnose pompano is enhanced, with lysozyme levels (8.38 µg/mL), respiratory burst activity (1.48), phagocytic activity (75.53%), and phagocytic index (2.00) reaching their highest values. This combination optimizes the health and immunity of the fish under aquaculture conditions (Bảng 3.4).

Table 3.4. Effects of vitamin e and temperature on serum lysozyme levels, respiratory burst activity, phagocytic activity, and phagocytic index

Treatment	Lysozyme (µg/mL)	Phagocytic Activity	Phagocytic Index	Respiratory Burst Activity
28:0	6.70±0.33	69.80±1.85	1.78±0.06	1.22±0.04
28:400	7.52±0.59	73.80±2.75	1.90±0.06	1.25±0.03
31:0	7.19±0.42	71.40±2.42	1.86±0.07	1.29±0.02
31:400	7.72±0.24	74.80±1.46	1.85±0.07	1.37±0.03
34:0	7.20±0.27	74.40±1.89	1.96±0.06	1.34±0.03
34:400	9.40±0.63	78.00±1.73	2.02±0.06	1.48±0.03
Temperature effects				
28	7.11^{A}	71.8	1.84	1.23^{A}
31	7.46^{AB}	73.1	1.86	1.33^{B}
34	8.3^{B}	76.2	1.99	1.41^{C}
Vitamin E (VE) effects				
0	7.03^{X}	71.87^{X}	1.87	1.29^{X}
400	8.21^{Y}	75.53^{Y}	1.92	1.36^{Y}
Two-way ANOVA P-values				
Temperature	< 0.05	NS	NS	< 0.05
VE	< 0.05	< 0.05	NS	< 0.05
Temperature x VE	NS	NS	NS	NS

Table 3.5. Effects of temperature and vitamin e on hematological parameters

Treatment	WBC ($\times 10^3/mm^3$)	RBC ($\times 10^6/mm^3$)	Hb (g/dL)	Hct (%)	PLT ($\times 10^3/mm^3$)	Triglyceride (mmol/L)	Protein (g/L)
28:0	5.75^{a}±0.07	1.79^{a}±0.01	6.7±0.08	19.8^{a}±0.45	22.2±0.37	3.99±0.15	36.06^{a}±0.63
28:400	6.51^{b}±0.15	1.87^{a}±0.03	7.12±0.09	22.12^{b}±0.51	23.8±0.58	4.19±0.20	36.2^{a}±0.46
31:0	6.65^{b}±0.13	2.21^{b}±0.03	6.92±0.27	21.92^{b}±0.31	22.4±0.24	4.75±0.34	36.76^{a}±0.39
31:400	7.15^{c}±0.04	2.21^{b}±0.03	7.9±0.34	22.78^{b}±0.40	23.4±0.40	4.92±0.32	39.52^{b}±0.46
34:0	6.35^{b}±0.05	2.21^{b}±0.04	7.42±0.29	22.08^{b}±0.25	22.4±0.51	4.41±0.04	39.24^{b}±0.38
34:400	7.54^{d}±0.13	3.11^{c}±0.05	8.98±0.27	25.68^{c}±0.66	23.2±0.58	5.15±0.38	40.36^{b}±0.56
Temperature effects							
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}
Vitamin E (VE) effects							
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}
Two-way ANOVA P-values							
Temperature	< 0.05	< 0.05	< 0.05	< 0.05	NS	< 0.05	< 0.05
VE	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	NS	< 0.05
Temperature x VE	< 0.05	< 0.05	NS	< 0.05	NS	NS	< 0.05

A temperature of 34°C improved the hematological parameters of snubnose pompano, including WBC (6.95 × $10^3/mm^3$), RBC (2.21 × $10^6/mm^3$), Hb (8.09 g/dL), Hct (25.68%), plasma protein (40.56 g/L), and triglycerides (5.15 mmol/L), reflecting enhanced physiological activity and immunity. Supplementing with 400 mg/kg diet of vitamin E also significantly enhanced these indicators, with RBC (2.44 × $10^6/mm^3$), Hb (8.09 g/dL), Hct (23.53%), and plasma protein (38.69 g/L) higher than the control group without supplementation. No significant interaction between temperature and vitamin E was observed, but both factors independently contributed to improved health and immunity in the fish (Table 3.5).

3.3 Effect of dietary vitamin C levels on the immune response

3.3.1 Effect of vitamin C content on growth and food utilization efficiency

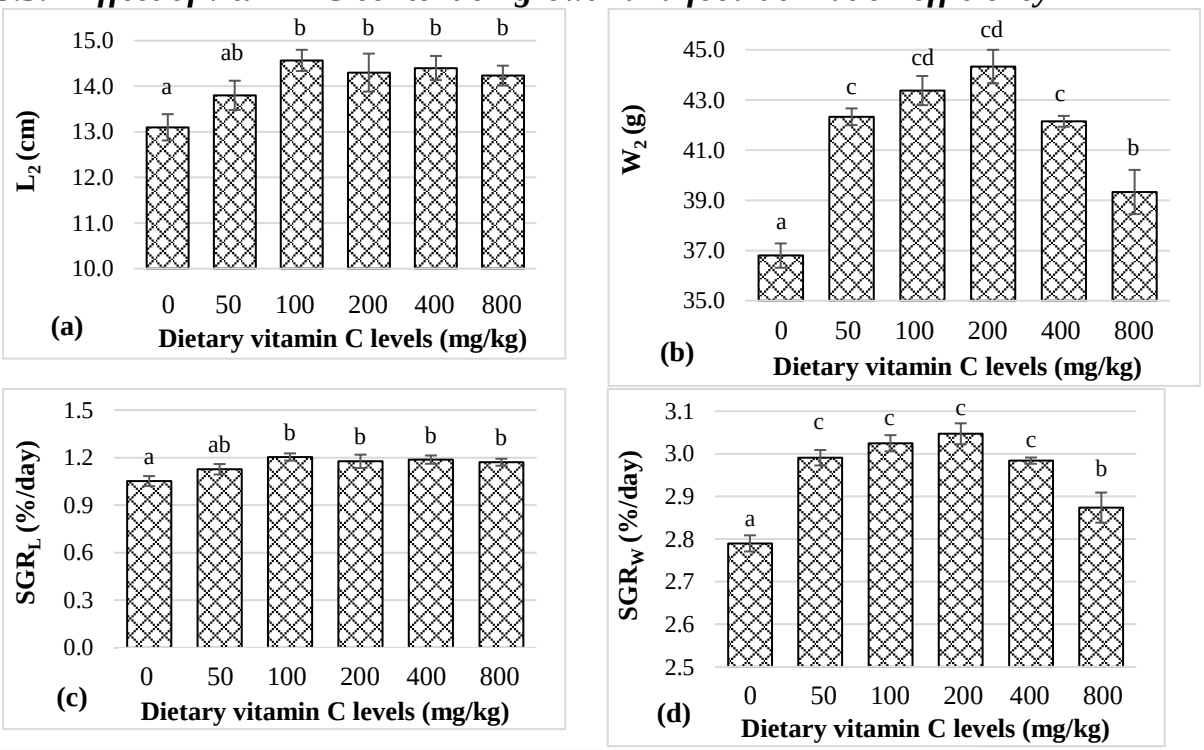


Figure 3.11 Length (a, c) and weight (b, d) of fish fed different dietary vitamin C levels

Supplementing with 100–200 mg/kg diet of vitamin C optimized the length (14.5 cm), weight (43–44 g), specific growth rate (1.4%/day), and relative growth rate (3.0%/day) of snubnose pompano. Exceeding this level did not provide additional benefits and may reduce growth efficiency (Figure 3.11).

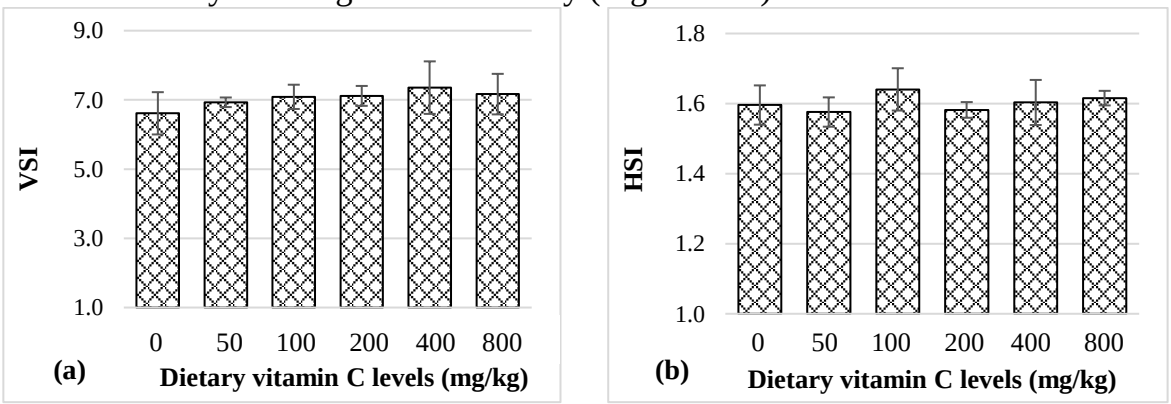


Figure 3.12 Viscerosomatic index (a) & hepatosomatic index (b) of fish fed different dietary vitamin C levels

Supplementing vitamin C from 0–800 mg/kg diet did not significantly affect the viscerosomatic index (VSI) and hepatosomatic index (HSI) of snubnose pompano, with VSI ranging from 7.0–7.5 and HSI from 1.4–1.6, reflecting the stability of organ and liver ratios under experimental conditions (Figure 3.12).

Supplementing vitamin C from 50–200 mg/kg diet improved feed utilization efficiency in snubnose pompano, with the optimal level at 200 mg/kg diet, demonstrated by the lowest feed intake (FI) and feed conversion ratio (FCR). Exceeding this threshold (400–800 mg/kg diet) provided no additional benefits and reduced feed utilization efficiency (Figure 3.13).

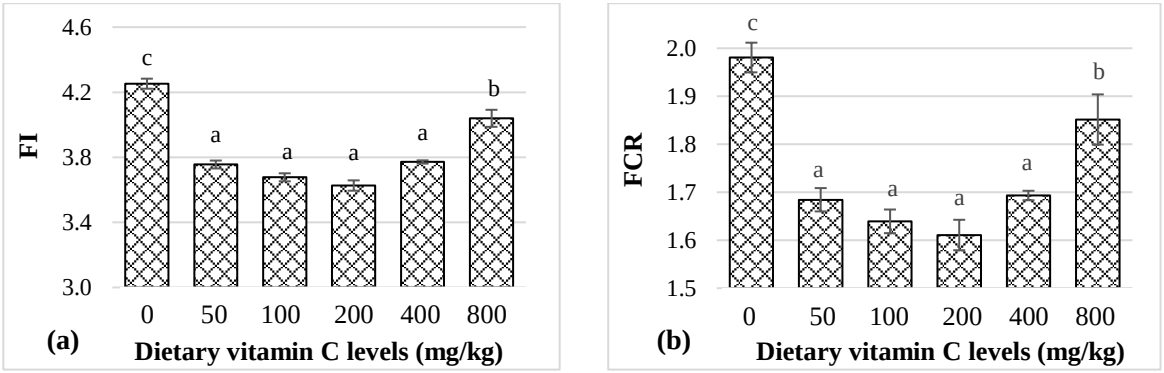


Figure 3.13 Fish feed consumption (a) and feed conversion ratio (b) of fish fed different dietary vitamin C levels

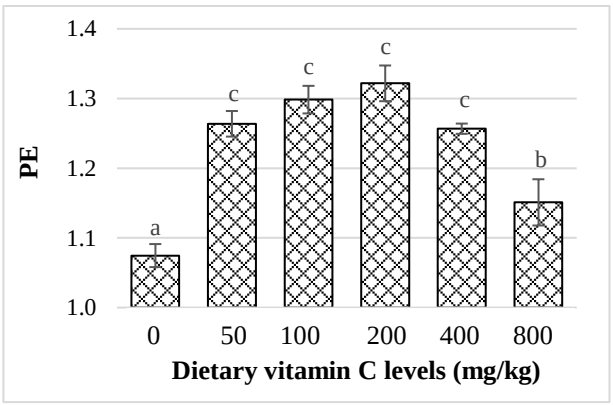


Figure 3.14 Protein utilization efficiency of fish at different dietary VC levels

Supplementing vitamin C at 200 mg/kg diet is optimal for improving the PE of pompano, achieving the highest value of approximately 1.3–1.4. Exceeding this level (400–800 mg/kg diet) provided no additional benefits and reduced PE compared to the optimal level (Figure 3.14).

3.3.2 Effect of dietary vitamin C levels on the whole body composition of pompano

Table 3.6 Body composition of fish fed different dietary vitamin C levels

Dietary vitamin C contents (mg/kg diet)	Moisture (%)	Ash (%)	Protein (%)	Lipid (%)
0	68.85±0.56	5.31±0.32	18.09±0.18^{a}	7.41±0.11^{a}
50	68.98±0.67	4.66±0.62	18.46±0.03^{b}	8.28±0.32^{b}
100	68.66±0.48	5.08±0.55	19.8±0.06^{d}	10.75±0.28^{d}
200	69.46±0.33	4.94±0.17	19.57±0.09^{d}	10.36±0.07^{c}
400	69.5±0.22	4.51±0.12	19.14±0.09^{c}	9.86±0.28^{c}
800	69.27±0.13	4.89±0.37	18.72±0.12^{b}	8.86±0.18^{b}

Supplementing vitamin C at 200 mg/kg diet is optimal for improving the protein content (19.66%) and lipid content (10.75%) in pompano, while reducing ash content (4.51%). Exceeding this level (400–800 mg/kg diet) provides no additional benefits and may reduce the efficiency of protein and lipid accumulation (Table 3.6).

3.3.3 Effect of dietary vitamin C levels on the immune response

Supplementing vitamin C at 200 mg/kg diet significantly increases lysozyme levels to the highest value (9.0 µg/ml), supporting the immune system of pompano. However, PLT is not significantly affected by different levels of vitamin C supplementation (Fig. 3.15).

Supplementing vitamin C at 400–800 mg/kg diet significantly improves RBC count (highest at 3.1×10^{6}/mm³ at 400 mg/kg diet) and WBC count (highest at

7.0×10³/mm³ at 800 mg/kg diet), enhancing oxygen transport and boosting the natural immunity of pompano (Fig. 3.16).

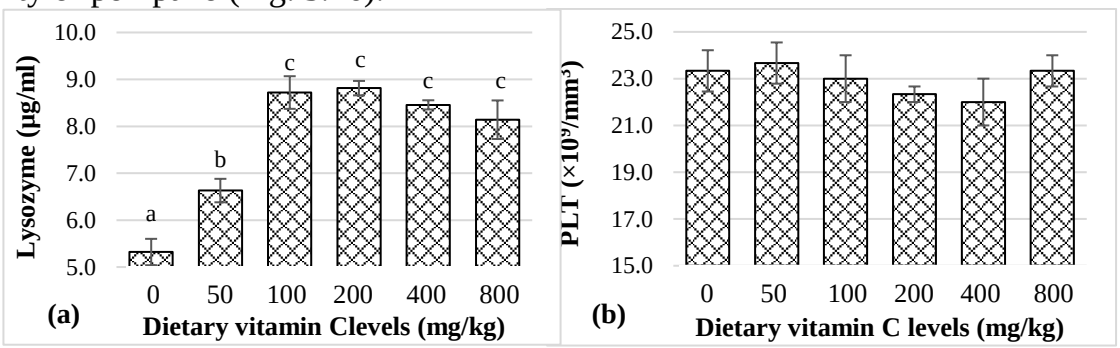


Figure 3.15 Lysozyme content (a) and platelet count (b) at different vitamin C levels

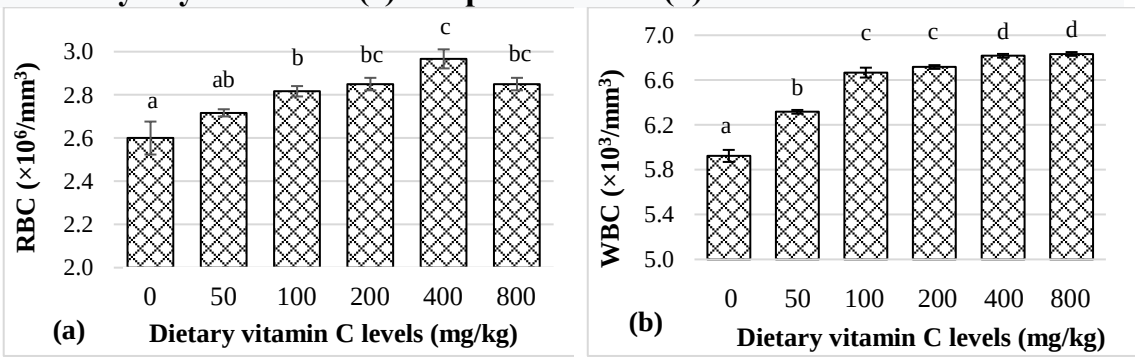


Figure 3.16 Red blood cells (a) and white blood cells (b) at different vitamin C levels

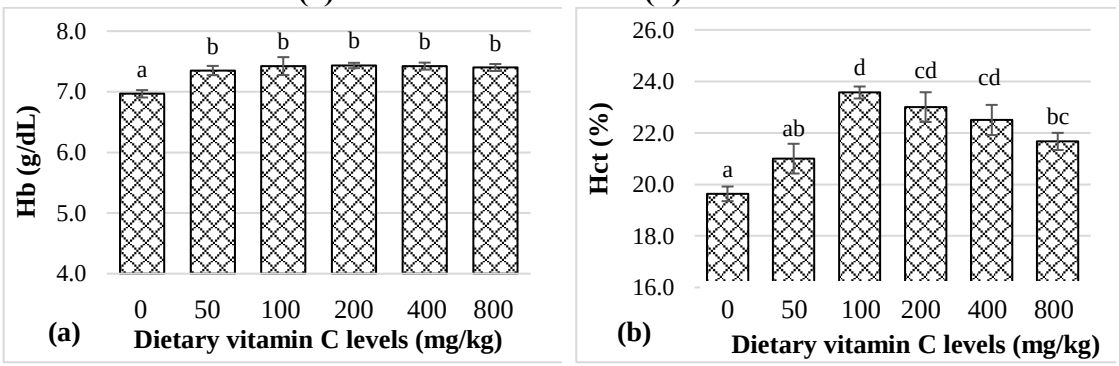


Figure 3.17 Hemoglobin (a) and hematocrit (b) content at different vitamin C levels

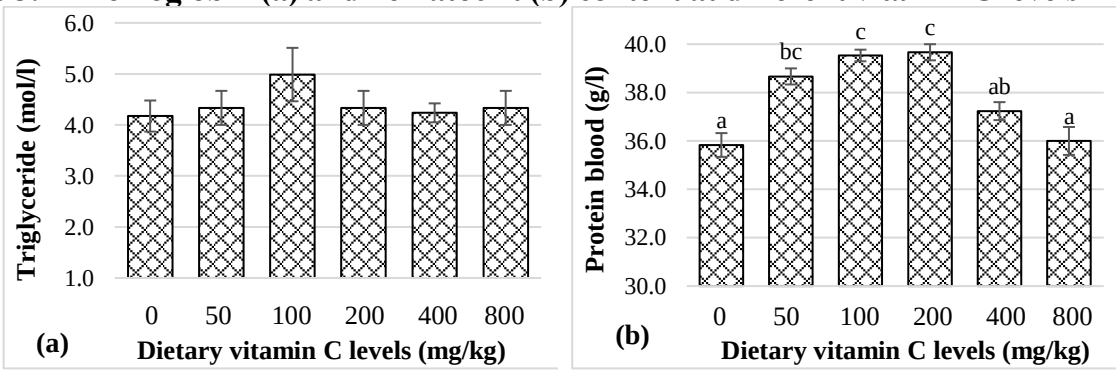


Figure 3.18 Plasma triglyceride (a) and plasma protein (b) content of fish fed different dietary vitamin C levels

Supplementing vitamin C at 50–400 mg/kg diet optimally enhances hemoglobin levels (highest at 7.8 g/dL) and hematocrit ratio (highest at 24%), supporting gas exchange and improving oxygen transport in the blood of pompano (Figure 3.17).

Supplementing vitamin C at 50–200 mg/kg diet significantly improves plasma protein levels in snubnose pompano, reaching the highest value of 39 g/L at 200 mg/kg diet, while triglyceride levels remain unaffected, ranging from 4.5–5.0 mmol/L (Figure 3.18).

3.3.4 Effect of dietary vitamin C levels on bone morphology of pompano

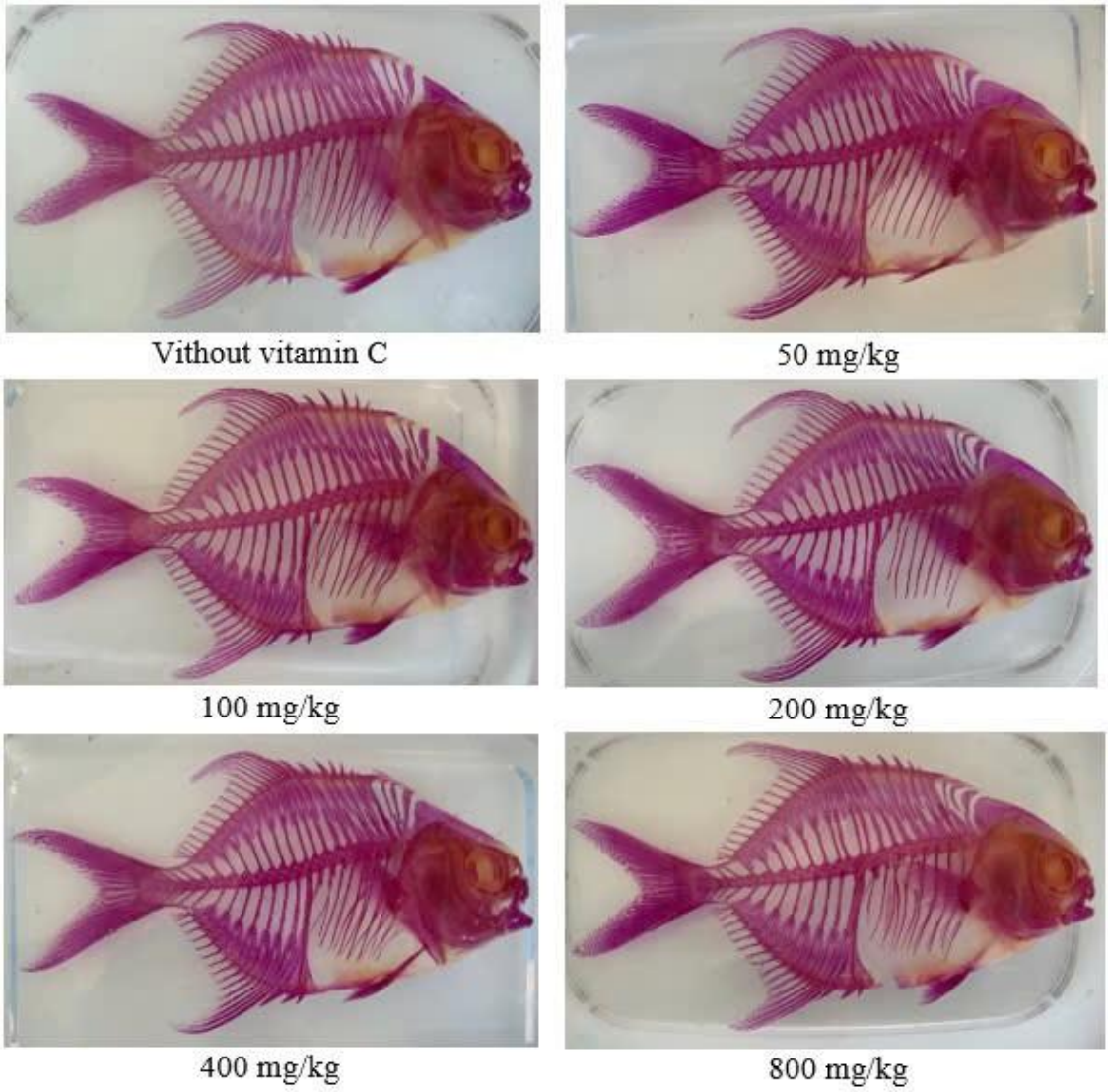


Figure 3.19 Bone morphology of fish fed different dietary vitamin C levels

The bone structure of snubnose pompano shows no significant differences across vitamin C supplementation levels (0–800 mg/kg diet). The skeletal structure is complete, balanced, and free from deformities or deficiencies. This indicates that dietary vitamin C or endogenous reserves adequately meet the fish's needs, ensuring normal skeletal development (Fig. 3.19).

3.4 Impact of different dietary vitamin C levels on the immune response of pompano under high temperature conditions

3.4.1 Effects of vitamin C and temperature on growth and food utilization efficiency

A temperature of 34°C combined with 100 mg/kg diet vitamin C supplementation optimizes growth and feed utilization in subnose pompano. Indicators such as final length, weight, specific growth rates (SGRL, SGRW), and feed conversion ratio (FCR) achieve their highest values under these conditions. Visceral index (VSI) and hepatosomatic index (HSI) are not significantly affected, while feed intake (FI) slightly decreases, indicating more efficient feed utilization (Table 3.7).

Table 3.7 The Effects of vitamin c and temperature on growth and feed utilization efficiency

Treatment	FBL (cm/fish)	FBW (g/fish)	SGR_L (%/day)	SGR_W (%/day)	VSI	HSI	FI	FCR	PE
28:0	13.93±0.10	51.61±0.69	1.48±0.01	4.24±0.03	13.02±0.26	2.42±0.06	4.73±0.06	1.56±0.02	1.36±0.02
28:100	14.16±0.11	52.53±0.65	1.51±0.01	4.28±0.02	12.25±0.15	2.21±0.05	4.65±0.05	1.53±0.02	1.39±0.02
31:0	13.69±0.12	49.11±0.92	1.45±0.02	4.15±0.03	13.21±0.46	2.23±0.04	4.95±0.08	1.66±0.04	1.29±0.03
31:100	13.95±0.12	51.69±0.35	1.49±0.02	4.25±0.01	12.81±0.25	2.11±0.04	4.72±0.03	1.56±0.01	1.36±0.01
34:0	13.77±0.08	52.24±0.47	1.46±0.01	4.27±0.02	12.32±0.55	2.08±0.07	4.68±0.04	1.54±0.02	1.38±0.01
34:100	14.02±0.09	54.51±0.80	1.49±0.01	4.34±0.03	13.21±0.49	2.09±0.03	4.5±0.06	1.47±0.02	1.45±0.02
Temperature effects									
28	14.05	52.07^{AB}	1.49	4.26^{B}	12.63	2.31^{B}	4.7^{A}	1.55^{A}	1.38^{AB}
31	13.82	50.4^{A}	1.46	4.2^{A}	13.01	2.17^{A}	4.8^{B}	1.61^{B}	1.33^{A}
34	13.9	53.37^{B}	1.47	4.3^{B}	12.77	2.08^{A}	4.6^{A}	1.51^{A}	1.41^{B}
Vitamin C (VC) effects									
0	13.8^{X}	50.99^{X}	1.46^{X}	4.22^{X}	12.85	2.24^{X}	4.78^{X}	1.59^{X}	1.34^{X}
100	14.04^{Y}	52.91^{Y}	1.49^{Y}	4.29^{Y}	12.76	2.14^{Y}	4.62^{Y}	1.52^{Y}	1.4^{Y}
Two-way ANOVA P-values									
Temperature	NS	< 0.05	NS	< 0.05	NS	<0.05	< 0.05	< 0.05	< 0.05
VC	<0.05	< 0.05	<0.05	< 0.05	NS	<0.05	< 0.05	< 0.05	< 0.05
Temperature x VC	NS	NS	NS	NS	NS	NS	NS	NS	NS

3.4.2 Effects of vitamin C and temperature on the body composition

A temperature of 34°C combined with 100 mg/kg diet vitamin C supplementation optimizes the biochemical composition of snubnose pompano, with the highest protein (19.44%) and lipid (9.93%) levels while reducing moisture and ash accumulation. This indicates that high temperature and vitamin C supplementation enhance the nutritional quality of the fish (Table 3.8).

Table 3.8 Effects of vitamin C and temperature on the body composition of fish

Treatment	Moisture	Ash	Protein	Lipid
28:0	5.79±0.11	69.14±0.43	18.39±0.05	6.0±0.54
28:100	6.26±0.05	70.25±0.62	18.61±0.04	7.28±0.64
31:0	4.69±0.10	65.55±0.46	19.01±0.03	8.06±0.42
31:100	5.19±0.07	68.17±0.52	19.18±0.02	8.69±0.42
34:0	4.04±0.12	59.82±0.46	19.27±0.02	9.42±0.38
34:100	4.03±0.08	61.29±0.35	19.4±0.04	9.93±0.49
Effect of water temperature				
28	6.03^{C}	69.69^{C}	18.5^{A}	6.64^{A}
31	4.94^{B}	66.86^{B}	19.1^{B}	8.37^{B}
34	4.23^{A}	60.56^{A}	19.33^{C}	9.67^{C}
Effect of VC				
0	4.84	64.84^{X}	18.89	7.83^{X}
100	5.3	66.57^{Y}	19.06	8.63^{Y}
P-values (Two-way ANOVA)				
Temperature	< 0.05	< 0.05	< 0.05	< 0.05
VC	NS	< 0.05	NS	< 0.05
Temperature x VC	NS	NS	NS	NS

3.4.3 Effects of vitamin C and temperature on the immune response of pompano

Bảng 3.9. Ảnh hưởng của nhiệt độ và vitamin C lên lượng lysozyme huyết thanh, hoạt tính thực bào và bùng nổ hô hấp của cá chim vây vàng

Nghiệm thức	Lysozyme (ug/mL)	Hoạt tính thực bào	Chỉ số thực bào	Bùng nổ hô hấp
28:0	6.25±0.27	66.20±1.46	1.68±0.03	1.18±0.04
28:100	6.72±0.16	68.80±1.24	1.76±0.03	1.23±0.03
31:0	7.00±0.33	70.20±1.83	1.76±0.09	1.33±0.02
31:100	8.01±0.06	69.80±2.15	1.79±0.06	1.31±0.03
34:0	8.23±0.28	72.20±2.56	1.83±0.03	1.36±0.03
34:100	9.04±0.07	75.60±1.60	1.93±0.05	1.43±0.03
Temperature effects				
28	6.48^{A}	67.5^{A}	1.72^{A}	1.2^{A}
31	7.51^{B}	70.0^{AB}	1.78^{AB}	1.32^{B}
34	8.63^{C}	73.9^{B}	1.88^{B}	1.39^{C}
Vitamin C (VC) effects				
0	7.16^{X}	69.53	1.76	1.29
100	7.92^{Y}	71.4	1.83	1.32
Two-way ANOVA P-values				
Temperature	<0.05	<0.05	<0.05	<0.05
VC	<0.05	NS	NS	NS
Temperature x VC	NS	NS	NS	NS

Table 3.10: Effects of vitamin c and temperature on hematological parameters

Treatment	WBC ($\times 10^3/mm^3$)	RBC ($\times 10^6/mm^3$)	Hb (g/dL)	Hct (%)	PLT	Triglyceride (mmol/L)	Protein (g/L)
28:0	5.61±0.22	1.87^{a}±0.06	7.02^{ab}±0.25	19.79±0.40	21.4±0.24	4.15±0.20	35.45^{a}±0.14
28:100	5.97±0.28	1.93^{a}±0.02	6.91^{a}±0.10	21.14±0.63	22.4±0.40	4.04±0.27	36.5^{b}±0.14
31:0	6.34±0.21	2.08^{b}±0.02	7.26^{ab}±0.10	22.0±0.43	22.8±0.20	4.79±0.33	37.08^{c}±0.08
31:100	6.61±0.13	2.24^{c}±0.06	7.45^{b}±0.14	23.25±0.42	23.6±0.24	4.32±0.30	37.92^{d}±0.18
34:0	7.23±0.07	2.76^{d}±0.06	8.72^{c}±0.12	24.74±0.21	23.8±0.20	4.86±0.34	38.68^{e}±0.08
34:100	8.11±0.18	3.15^{e}±0.05	9.38^{d}±0.15	25.64±0.43	24.8±0.20	4.93±0.40	39.11^{f}±0.03
Temperature effects							
28	5.79^{A}	1.9^{A}	6.97^{A}	20.47^{A}	21.9^{A}	4.1^{A}	35.98^{A}
31	6.48^{B}	2.16^{B}	7.35^{B}	22.63^{B}	23.2^{B}	4.56^{AB}	37.5^{B}
34	7.67^{C}	2.95^{C}	9.05^{C}	25.19^{C}	24.3^{C}	4.89^{B}	38.9^{C}
Vitamin C (VC) effects							
0	6.39^{X}	2.24^{X}	7.67^{X}	22.18^{X}	22.67^{X}	4.6	37.07^{X}
100	6.9^{Y}	2.44^{Y}	7.91^{Y}	23.34^{Y}	23.6^{Y}	4.43	37.84^{Y}
P-values (Two-way ANOVA)							
Temperature	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
VC	<0.05	< 0.05	=0.05	< 0.05	< 0.05	NS	< 0.05
Temperature x VC	NS	<0.05	<0.05	NS	NS	NS	<0.05

A water temperature of 34°C and supplementation with 100 mg/kg diet vitamin C significantly improved the natural immunity indicators of snubnose pompano. Serum lysozyme levels (8.63 µg/mL), phagocytic activity (73.9%), phagocytic index (1.93), and respiratory burst activity (1.39) reached their highest values at 34°C. Vitamin C supplementation increased lysozyme levels (7.92 µg/mL) and supported phagocytic activity, phagocytic index, and respiratory burst activity, enhancing the health and disease resistance of the fish (Table 3.9).

A water temperature of 34°C and 100 mg/kg diet vitamin C supplementation also improved hematological parameters in subnose pompano, with WBC ($8.11\times10^3/mm^3$), RBC ($3.15\times10^6/mm^3$), Hb (9.05 g/dL), Hct (25.15%), and plasma protein (38.9 g/L) reaching their highest values. Vitamin C supported immune function and overall health, while PLT and triglyceride levels were minimally affected (Table 3.10).

CHAPTER 4. CONCLUSIONS AND RECOMMENDATIONS

4.1 Conclusions

1) Supplementation of vitamin E at 100–800 mg/kg diet improved growth performance, feed utilization efficiency, and biochemical composition of snubnose pompano, with the optimal range being 100–200 mg/kg diet. Vitamin E at 100–400 mg/kg diet enhanced hematological and immune parameters, with the highest lysozyme activity and white blood cell count observed at 400 mg/kg diet, while red blood cell count, hemoglobin (Hb), hematocrit (Hct), blood protein, and triglyceride levels were optimal at 200 mg/kg diet. Vitamin E had no effect on survival rate, platelet count, VSI, or HSI. However, excessive supplementation (800–1600 mg/kg diet) led to hepatic degeneration, necrosis, and muscle tissue damage.

2) Under experimental conditions, snubnose pompano demonstrated adaptability within a temperature range of 28–34°C. Growth performance, feed utilization efficiency, protein content, moisture levels, hematological indices, serum lysozyme, and respiratory burst activity were highest at 34°C. Temperature did not significantly affect phagocytic activity or the phagocytic index. Vitamin E supplementation at 400 mg/kg diet within this temperature range improved growth, biochemical composition, hematological indices, and immune response. There was no significant interaction between vitamin E and temperature on most parameters, except for total lipid content, white blood cell count, red blood cell count, Hct, and plasma protein levels.

3) Supplementation of vitamin C at 50–800 mg/kg diet improved growth performance, feed utilization efficiency, protein, and lipid content of snubnose pompano, with the optimal level being 100 mg/kg diet. Vitamin C enhanced hematological parameters such as Hb, Hct, red blood cell count, white blood cell count, serum protein, and lysozyme activity, while having no effect on survival rate, platelet count, triglyceride levels, VSI, HSI, moisture content, ash content, or bone morphology after 10 weeks of experimentation.

4) Under experimental conditions, as temperature increased within the range of 28–34°C, growth performance, feed utilization efficiency, total lipid and protein content, along with hematological and innate immune parameters of snubnose pompano, showed an improving trend, with some parameters peaking at 34°C. Vitamin C supplementation at 100 mg/kg diet within this temperature range enhanced growth rate, feed utilization efficiency, total lipid content, and improved hematological and immune indices, particularly red blood cell count, Hb, and serum protein levels. Further research is needed to determine the optimal temperature for snubnose pompano development by assessing the effects of higher temperatures on growth performance, nutrient metabolism, and immune response.

4.2 Recommendations

1) It is necessary to analyze the vitamin E and vitamin C content in the liver and muscle, as well as evaluate the effects of these vitamins and temperature on endogenous antioxidants such as SOD, CAT, and GPx. This will provide a better understanding of the accumulation, metabolism, and antioxidant role of these vitamins in snubnose pompano.

2) The maximum temperature tolerance of snubnose pompano should be determined, and further studies are needed on the effects of temperatures exceeding 34°C on growth, feed utilization efficiency, and immune response. Additionally, it is important to assess the interaction between vitamins and temperature to gain deeper insights into the combined effects of these factors on the development of juvenile snubnose pompano.

LIST OF WORKS RELATED TO THE THESIS

1) Pham TH, Tran VH & Le MH (2024), “Combined effects of vitamin E and temperature on the growth, biochemistry, and natural immune response of juveniles snubnose pompano (*Trachinotus blochii*)”, *Israeli Journal of Aquaculture – Bamidgeh*, 76 (4), pp.204-216.

2) Pham Thi Hanh, Nguyen Tan Khang, Tran Vi Hich & Le Minh Hoang (2023), "The effect of supplementing vitamin C in feed on growth and natural immune response of pompano (*Trachinotus blochii*, Lacepède, 1801)," *Journal of Agriculture & Rural Development*, No. 24/2023, pp. 57-66.

3) Pham Thi Hanh, Khuong V. Dinh, Ngo Van Manh, Le Anh Tuan, Minh-Hoang Le, Tran Vi Hich (2025). Dietary vitamin E enhances the growth, and immunity of snubnose pompano *Trachinotus blochii* juveniles, Fisheries and Aquatic Sciences, Accepted. This paper will be at issue on February 2025.