

Influence of Different Levels of Bio Stimulant *Bacillus coagulans* with Vitamin E in Diets of Common Carp Fingerlings on Hematological and Biochemical Traits

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Abstract A laboratory experiment was conducted for 60 days by adding three different levels of *Bacillus coagulans* with a constant level of vitamin E to the diets of common carp fingerlings for a goal of study their effects on some blood and biochemical traits. Average weight of Fingerlings were 11.2 ± 0.13 g and treatments included : T1(Control treatment) without addition, T2 vit.E only vit.E (100mg/kg), T3 same ratio of vit.E with the bio stimulant *B.coagulans* (150mg/kg), while T4 vit.E with (200mg/kg) bio- stimulant, and (250mg/kg) vit.E in T5. Experimental results indicated that: Significant differences ($P \leq 0.01$) in all blood indicators studied between different experimental treatments. Control treatment recorded a significant difference at red blood cells (RBC) amounting to $4.20 \pm$ million / ml and hemoglobin (Hb) 6.30 ± 0.05 g/deciliter, packed cell volume (PCV) $22.0 \pm 0.57\%$ and white blood cells (WBC) recorded at 9.20×10^9 /liter, which are values representing the normal basal levels. In contrast, cellular immune indicators improved, represented by the percentages of (lymphocytes, monocytes, eosinophils and neutrophils) in the supplemented treatments. Blood chemistry profile showed superiority by treatment T4 in protein percentage and recorded the lowest blood sugar level. Cholesterol also decreased in all supplemented treatments compared to the control, while T3 recorded highest level of triglycerides and lowest level for enzymes liver.

Keywords: vitamin E; probiotic; *Bacillus coagulans*; common carp.

1. INTRODUCTION

Aquaculture is a rapidly growing field, and plays a crucial role in achieving food security, especially in developing countries. It provides individuals with a suitable source of protein, and contributes to the economic development of coastal and rural areas [1],[2]. However,

overfishing and pollution of natural to production farms fish intensive and sustainable [3]. And developed improvements scientific and technological all aspects aquaculture aquatic life nearly as from expected that will double demand for seafood by by year 2050 ‘And aquaculture will significantly contribute to meeting this demand [4]. Concurrently with the expansion of fish farming, driven by scientific and technological advances to the evolutionary rise of the aquaculture feed industry and the use of feed additives and nutritional supplements and Scenario Growth This, which allows for improvements significant in production of fish farmed and their health and welfare [5],[6]. And shown several studies that feed additives for fish, such as vitamins and minerals and amino acids, are used to ensure the provision of a complete feed for all nutrients nutritional The essential for growth and health of fish farmed [7] . where are considered some additives such as vitamin (E) growth promoters [8] . that fish cannot synthesize all types of vitamin (E) biologically active, and must obtain it from their diet [9]. Also, it is considered in sufficient quantities important for promoting growth, scavenging free radicals, inhibiting lipid peroxidation in cell membranes, and alleviating oxidative stress in fish [10],[11]. Also used currently other additives ‘such as probiotic products in aquaculture, which contain bacterial strains multiple the most important of which are *Lactobacillus sp*, and *Bifidobacterium sp*, and *Bacillus spp*, and *Pseudomonas sp*, and others [12],[13],[14]. Among the species of *Bacillus spp*, *B.coagulans* is the most used [15]. It has a positive effect on the immune activity and growth of many aquatic animals, such as common carp [16] . Specifically, previous reports indicated that adding (*B.coagulans*) to the diet as a probiotic can enhance growth, immune response, and disease resistance in white shrimp [17] .

Also revealed previous studies that adding *B.coagulans* to feed, either alone or with other additives, may improve the growth of tilapia and health by stimulating immune responses and antioxidant capacity, and changing some indicators biochemical and related hematological parameters [18]. As it showed its effect clearly in improving the blood profile (Hematological profile). As these additions lead to an increase significant in red blood cell count and hemoglobin level [19]. And for the purpose of knowing the effect of adding vitamin E with different levels of the probiotic on the hematological and biochemical parameters in common carp fish in this experiment.

2. MATERIALS AND METHODS

The current study shows the effect of adding three different levels of bacteria *B.coagulans* as a probiotic and vitamin E to the diets of carp common fish on some blood profile parameters and biochemical parameters in the blood of experimental fish. 180 common carp fish common weighing approximately 11.25 grams were used and distributed into 5 treatments, each treatment containing 36 fish divided into three replicates. The experiment duration was 60 days. The first treatment was a control T1 free of additives. The second treatment contained 100 mg/kg vitamin E, the third treatment contained 100 mg/kg vitamin E with 150 mg/kg probiotic, the fourth treatment contained 100 mg/kg vitamin E with 200 mg/kg probiotic agent ‘The fifth treatment contained 100 mg/kg vitamin E with 250 mg/kg probiotic. were fed the diets designated for each treatment at a rate of 3% of the fish weight to standardize the living conditions for all treatments. At the end of the experiment, blood collection tools were prepared from 3 ml medical syringes and tubes for storing blood samples of two types (gel tube and regular tube) and anticoagulant powder EDTA and a

cooler for storing and transporting samples, in addition to gloves and sterilization tools. A small amount of anticoagulant was placed inside each syringe and shaken to ensure adhesion of the powder to its inner walls and prevent and blood clotting. Then, blood samples were drawn directly from the heart. The samples were then divided into two parts. The first part was placed in tubes containing the anticoagulant Ethylene Diamine Tetra Acetic Acid for the purpose of performing hematological profile tests for the fish, which include the count of red blood cells (Red Blood Cells -RBC) and the count of white blood cells (White Blood Cells - WBC), packed cell volume (PCV) which is used to determine the ratio of red blood cell volume to total blood volume, and hemoglobin concentration hemoglobin in the blood (Hb). Meanwhile, the other part of the blood was transferred to gel Tubes) All were placed in the cooler with a temperature ranging between 2-6 degrees. Ciliate for the purpose of transferring it to the laboratory to perform the analysis. After that, the required analyses were performed to count the red blood cells RBC and the white blood cells WBC and packed cells PCV and hemoglobin based on the method that was mentioned by [20]. To calculate the results, a quantity of the experimental fish blood is taken using a Capillary tube containing heparin to prevent coagulation, then one end of the tube is sealed with artificial clay and placed in a centrifuge for five minutes in a device Microcentrifuge at a speed of 3000 rpm. The result is calculated using the special ruler for this test. Also, the ready-made statistical program SASS was used in analyzing the experimental data[21], with a completely randomized design (CRD), and significant differences between the means were compared using Duncan's test [22], at a probability level of ($P \leq 0.01$).

3. RESULTS AND DISCUSSION

Water Quality: Throughout the duration of the experiment, the water quality was within the appropriate range for the Common Carp (*Cyprinus Carpio*), because the water temperature ranged between 24.8-29.5 °C, and dissolved oxygen between 4.8-6.4mg/L, and pH between 6.6-8.7 while the salinity rate ranged between 0.982- 0.711 ppt. All of them were within the ideal limits for the life and growth of Common Carp[23],[24],[25]. These readings agreed with what was mentioned in the study by Hossain & Rahman [26], As shown in Table (1)

Table (1) Physical and chemical properties of the experimental pond water.

Variable	Temperature	Oxygen	pH	Dissolved Salts
First Week	29.2	5.2	7.5	711
Second Week	29.4	5.5	7.5	834
Third Week	28.9	5.5	7.3	874
Fourth Week	28.9	5.3	7.2	902
Fifth Week	27.9	5.1	6.8	937
Sixth Week	26.8	5.1	7.5	930
Seventh Week	24.9	5.1	7.1	970
Eighth Week	25.2	4.9	6.6	982
Ninth Week	24.8	5	7.7	724

Blood parameters: Blood count measurements are among the most important indicators of fish health and well-being. These include red blood cell count, white blood cell count, hemoglobin, and packed cell volume.

White blood cell percentage (%): Fish in T1 recorded the highest white blood cell count $9.20 \pm 0.05 \times 10^9/L$, significantly than the other treatments. In contrast, T4 showed the lowest white blood cell count $4.33 \pm 0.08 \times 10^9/L$, indicating immune stability and a reduction in unnecessary inflammatory responses, or a decrease in antigen passage through the digestive tract wall due to probiotics. Treatments T3 and T5 showed moderate and similar values for this indicator, $7.56 \pm 0.08 \times 10^9/L$ and $7.60 \pm 0.05 \times 10^9/L$, respectively, with no significant difference between them.

Red blood cell count (%): The red blood cell count showed a clear difference between the treatments. T1 recorded the highest count 4.20 ± 1 million/ml, significantly outperforming all other treatments. In contrast, T2 had the lowest red blood cell count 2.13 ± 0.08 million/ml. This suggests that vitamin E may affect the stability of the red blood cell membrane, leading to a decrease in count. T4 showed a significant improvement compared to treatments T2, T3, and T5, recording 3.26 ± 0.06 million/ml. This may indicate that the addition of the bio stimulant at a concentration of 200 mg/kg counteracted the previous effect of the vitamin.

Blood hemoglobin concentration (Hb g/dl): The results of blood hemoglobin measurements in the analytical table showed a significant advantage in T1 over all other treatments, recorded a highest concentration 6.30 ± 0.05 g/dL. This was followed by T2 and T4, with equal values of 6.0 ± 0.05 g/dL for each, T5 and T3, recorded the lowest concentration at 4.30 ± 0.05 g/dL and 5.60 ± 0.05 g/dL, respectively. The decrease in most parameters in T5 indicates the stress that the high dose may cause on the digestive system, thus affecting blood functions.

Packed cell volume (PCV%): The results showed a significant advantage for T1 and T2 in the percentage of packed cells. These two treatments recorded the highest values of $22.0 \pm 0.57\%$ and $20.0 \pm 0.57\%$, respectively, with no significant difference between them. T4, did not show any significant difference compared to T2, which achieving the same value of $20.0 \pm 0.57\%$. T3, recorded the lowest level at $15.0 \pm 0.57\%$, indicating that negatively affected compared to the other treatments.

Table (2) shows the results of the effect of adding vitamin E and the probiotic *B.coagulans* on red and white blood cell counts, hemoglobin, and hematocrit volume in the blood of common carp at the end of the experiment.

Treatments	RBC million\μl	HB g\dl	PCV %	WBC x109 \ L
T1	4.20±0.05 a	6.30±0.05 a	22.0±0.57 a	9.20±0.05 a
T2	2.13±0.08 d	6.0±0.05 b	20.0±0.57 b	5.40±0.05 c
T3	2.80±0.05 c	4.30±0.05 d	15.0±0.57 c	7.56±0.08 b
T4	3.26±0.06 b	6.0±0.05 b	20.0±0.57 b	4.33±0.08 d
T5	2.80±0.05 c	5.60±0.05 c	19.0±0.57 b	7.60±0.05 b

Significance Level	**	**	**	**
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The decrease in blood parameters is explained by the fact that the addition of probiotics with vitamin E affected the physiological balance of the blood due to physiological stress, leading to a modification of the immune response. While probiotics usually enhance immunity, inappropriate doses can lead to relative immunosuppression [27].

Analysis of Cellular Immunity Indicators: Cellular immunity parameters, represented by the white blood cell (WBC) count and the percentages of different cell types (lymphocytes, monocytes, eosinophils, and neutrophils) in common carp at the end of the experiment, indicate the health status of the stock and its response to pathogens.

Total White Blood Cell Count (WBC): The results showed significant variation in total WBC counts among the different treatments. T1 recorded the highest count $9.20 \times 10^9/L$, representing the normal level of immune cells under typical conditions. In contrast, the various supplementation treatments showed a significant decrease in WBC count, while T4 recorded the lowest value $4.33 \times 10^9/L$, followed by T2 at $5.40 \times 10^9/L$. This decrease in the total WBC count in the treated treatments indicates a state of immune homeostasis, agrees with the findings of Ringø et al [28]. (2022), who suggested that it is possible to enhance the immune response without an increase in white blood cell count. T3 and T5 recorded similar values of 7.56 and $7.60 \times 10^9/L$, respectively, indicating that all levels of the activator showed a moderate effect compared to the vit.E only T2 and T4.

Lymphocyte percentage: Lymphocytes are a fundamental component of the adaptive immune system in fish, playing an essential role in both cellular and humoral immunity [28]. The results showed a significant improvement in the percentage of these cells in all supplementation treatments compared to the control treatment, which recorded the highest percentage at $37.0 \pm 0.57\%$. T2 and T5 recorded $34.0 \pm 0.57\%$ and $33.0 \pm 0.57\%$ respectively, and the lowest percentage was observed in treatments T4 and T3, both at $32.0 \pm 0.57\%$. This decrease may reflect an improvement in overall health and a reduction in the intensity of immune stimulation, especially considering the role of probiotics in regulating the immune response and reducing inflammation by affecting gut-associated lymphoid tissue (GALT) and reducing cytokine secretion inflammatory factors [29],[30].

Monocyte percentage: Monocytes are large phagocytic cells that play a role in eliminating pathogens. T1 recorded a percentage of $3.0 \pm 0.0\%$, which was significantly consistent with all treatments except for T3 which showed a significant difference ($P < 0.01$) compared to the other treatments, reaching $4.0 \pm 0.0\%$. This superior monocyte percentage indicates specific stimulation of the innate defense line (phagocytosis) in this particular treatment. This aligns with the findings of Hoseinifar et al [5]. who stated that a higher monocyte percentage indicates clear stimulation of components of innate immunity, particularly phagocytic cell activity. These cells are essential elements of non-specific defense due to their phagocytic ability.

Eosinophil percentage: Eosinophils play a crucial role in defense against parasites and allergic responses. The results showed that T2 and T5 recorded the highest percentage at $2.0 \pm 0.00\%$, while the remaining treatments recorded only $1.0 \pm 0.00\%$. This relative increase in eosinophil count is attributed to a possible role of vit.E on enhancing the immune response

against parasites. A recent study demonstrated that vit.E enhances phagocytic cell activity and innate immunity in fish [31], or it may reflect a slight response of the fish's body to probiotic supplements at certain levels. Studies have indicated that probiotics can influence eosinophil counts. While one study showed a decrease in their numbers while they remained within normal limits in sturgeon [32], another study indicated changes in their values as an indicator of improved non-specific immunity in Amazonian fish [33]. However, these ratios remain within normal limits, as documented in a study that confirmed blood indicators remained within reference values after probiotic use [32], thus confirming the safety of the additives used and their lack of adverse allergic reactions.

Neutrophil percentage: Neutrophils are among the most important components of innate (non-specific) immunity, constituting the largest proportion of white blood cells in most fish species. They play a key role in phagocytosis and the rapid inflammatory response against pathogens. The results indicate that neutrophil percentages were high in all treatments, ranging between 60.0% and 64.0%. The control treatment recorded the lowest percentage at $60.0 \pm 0.57\%$, while the treated treatments recorded higher percentages. T4 had the highest neutrophil percentage at $64.0 \pm 0.57\%$, followed by T3 at $63.0 \pm 0.57\%$, and finally T2 with the lowest percentage at $61.0 \pm 0.57\%$.

This high neutrophil percentage in all treated treatments compared to the control indicates a significant enhancement of the fish's innate immunity. The high percentages of these cells reflect an improved ability to respond quickly to bacterial and fungal infections. Neutrophils are also among the first immune cells to reach the site of infection [34],[35]. This improvement in neutrophils can also be attributed to the effect of the bio stimulant *B.coagulans* in activating the intestinal mucosal immune system [36],[37], and the role of the Vit.E improves immune cell function by protecting their membranes from oxidative stress [38],[31].

Table (3) shows the results of the effect of adding vitamin E and the bio stimulant *B.coagulans* on the count of white blood cells, lymphocytes, monocytes, and granulocytes in the blood of common carp at the end of the experiment.

Treatments	WBC x109 \ L	Lymphocyte %	Monocyte %	Eosinophil %	Neutrophil %
T1	9.20±0.05 a	37.0±0.57 a	3.0±0.0 b	1.0±0.0 b	60.0±0.57 d
T2	5.40±0.05 c	34.0±0.57 b	3.0±0.0 b	2.0±0.0 a	61.0±0.57 cd
T3	7.56±0.08 b	32.0±0.57 c	4.0±0.0 a	1.0±0.0 b	63.0±0.57 ab
T4	4.33±0.08 d	32.0±0.57 c	3.0±0.0 b	1.0±0.0 b	64.0±0.57 a
T5	7.60±0.05 b	33.0±0.57 bc	3.0±0.0 b	2.0±0.0 a	62.0±0.57 bc
Significance Level	**	**	**	**	**

Biochemical Characteristics: Biochemical indicators of the blood of common carp in the experiment, including total protein, blood glucose, cholesterol, triglycerides, and the liver enzymes GPT and GOT.

Total Protein (WBC) Level: The total protein level in the blood reflects the nutritional and general health status of the organism, as it is closely related to the efficiency of protein metabolism and liver function. The results indicated a significant variation in total protein levels among the different treatments. The control treatment and T3 recorded the lowest values at 3.80 ± 0.05 g/dL, while T4 showed the highest significant value at 4.50 ± 0.05 g/dL, followed by the T5 at 4.30 ± 0.05 g/dL, and then T2 at 4.10 ± 0.05 g/dL.

Blood glucose level: Blood glucose level is an important indicator of the stress status of fish and their carbohydrate metabolism. Highest blood glucose level was recorded in T5 at 110.0 ± 0.57 mg/dL, followed by T2 at 107.0 ± 1.15 mg/dL, with no significant difference between the two. In contrast, T3 and T4 recorded the lowest levels at 100.67 ± 1.45 mg/dL and 99.67 ± 1.20 mg/dL, respectively, while T1 recorded an average level of 104.67 ± 1.20 mg/dL.

Cholesterol level: Blood cholesterol levels reflect the efficiency of lipid metabolism and the function of the liver and endocrine glands [39]. The results showed a significant decrease in cholesterol levels in all treatments compared to the control which recorded the highest value 97.67 ± 0.88 mg/dL. T4 recorded the lowest cholesterol level at 81.0 ± 0.57 mg/dL, followed by T5 at 88.0 ± 0.57 mg/dL, then T2 at 93.0 ± 0.57 mg/dL. T3 recorded a value of 97.0 ± 0.57 mg/dL, almost equal to that of T1.

Triglyceride levels: Triglycerides are a direct indicator of fat metabolism and energy stores in the body. The results shown a significant difference in triglyceride levels among the treatments. Treatment 3 recorded the highest significant level at 259.0 ± 0.57 mg/dL, followed by treatment 2 at 220.0 ± 0.57 mg/dL. In contrast, the control treatment recorded the lowest level at 158.67 ± 0.88 mg/dL, while treatment 4 recorded a level of 168.0 ± 0.57 mg/dL, and treatment 5 a level of 178.0 ± 0.57 mg/dL.

Liver enzymes (GOT and GPT): The liver enzymes GOT and GPT are sensitive indicators of liver cell health and function. Their levels in the blood increase when there is damage or stress in the liver cells.

- **GPT (ALT):** Statistical analysis revealed significant differences ($P < 0.01$) in GPT enzyme levels among the different treatments. T1 and T4 recorded a value of 8.0 ± 0.57 U/L, T2 recorded a value of 9.0 ± 0.57 U/L, T3 recorded a value of 5.0 ± 0.57 U/L, and T5 recorded a value of 4.0 ± 0.57 U/L.

- **GOT (AST):** The results did not show any significant differences (ns) in GOT enzyme levels among all treatments. The recorded values ranged from 3.0 ± 0.57 U/L in T3 to 5.0 ± 0.57 U/L in T2 and T4. While T1 and T5 recorded a value of 4.0 ± 0.57 U/L.

This marked increase in total protein in the blood of the T4 and T5 treatment fish indicates an improvement in the efficiency of protein synthesis processes in the liver, and an increase in the production of albumin and globulins, reflecting an enhancement of the immune nutritional status of the fish [40],[41] [34]. This improvement can be attributed to the synergistic effect of the bio stimulant in enhancing amino acid absorption from the intestines [42],[41]. and vit.E at its role as an antioxidant protecting liver cells from oxidative stress [43],[44],[38] . thus creating an optimal environment for anabolic processes.

The decrease in blood glucose levels in the T3 and T4 parameters indicates a state of metabolic stability and reduced stress levels, as elevated blood glucose is often associated with the stress response resulting from cortisol secretion.

This decrease in cholesterol levels is a positive indicator reflecting improved lipid metabolism and a reduced risk of lipid deposition in blood vessels and tissues [45]. The elevated triglyceride levels in treatments T3 and T2 indicate that vitamin E, alone or with low doses of the probiotic, enhances intestinal fat absorption, leading to increased blood triglyceride levels.

The decrease in GPT enzyme levels in treatments T5 and T3 suggests improved liver function and reduced oxidative stress, reflecting the protective effect of the additives on liver cells. No adverse effects or damage to liver cells were observed, confirming their safety and non-toxicity at the levels used in the study.

Table (4) shows the results of the effect of vitamin E and the probiotic *B.coagulans* on total protein, glucose, cholesterol, and triglycerides in the blood of common carp at the end of the experiment.

Treatment s	Total Protein (g/dl)	Blood Glucose (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	GPT(UL)	GOT(UL)
T1	3.80±0.05 d	104.67±1.20 b	97.67±0.88 a	158.67±0.88 e	8.0±0.57 a	4.0±0.57
T2	4.10±0.05 c	107.0±1.15 ab	93.0±0.57 b	220.0±0.57 b	9.0±0.57 a	5.0±0.57
T3	3.80±0.05 d	100.67±1.45 c	97.0±0.57 a	259.0±0.57 a	5.0±0.57 b	3.0±0.57
T4	4.50±0.05 a	99.67±1.20 c	81.0±0.57 d	168.0±0.57 d	8.0±0.57 a	5.0±0.57
T5	4.30±0.05 b	110.0±0.57 a	88.0±0.57 c	178.0±0.57 c	4.0±0.57 b	4.0±0.57

4. CONCLUSIONS

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REFERENCES

- [1].FAO. Food And Agriculture Organization. (2018). The State of World Fisheries and Aquaculture (SOFIA); FAO: Rome, Italy, 2018; ISBN 978-92-5-130562-1. [Google Scholar]
- [2].FAO. Food And Agriculture Organization. (2020). The State of World Fisheries and Aquaculture; FAO: Rome, Italy, 2020; ISBN 978-92-5-132692-3. [Google Scholar]
- [3].El-Saadony, M.T., Alagawany, M., Patra, A.K., Kar, I., Tiwari, R., Dawood, M.A.O., Dhama, K., & Abdel-Latif, H.M.R.(2021). The Functionality of Probiotics in Aquaculture: An Overview. Fish Shellfish Immunol. 2021, 117, 36–52. [[Google Scholar](#)] [[CrossRef](#)] [[PubMed](#)]
- [4].Naylor, R.L., Kishore, A., Sumaila, U.R., Issifu, I., Hunter, B.P., Belton, B., Bush, S.R., Cao, L., Gelcich, S., & Gephart, J.A., (2021). Blue Food Demand across Geographic and Temporal Scales. Nat. Commun. , 12, 5413. <https://doi.org/10.1038/s41467-021-25516-4>

- [5].Hoseinifar, S.H., Maradonna, F., Faheem, M., Harikrishnan, R., Devi, G., Ringo, E., Van Doan, H., Ashouri, G., Giocchini, G., & Carnevali, O.(2024). Sustainable ornamental fish aquaculture: The implication of microbial feed additives. *Animals* 2023, 13, 1583. [CrossRef] [PubMed]
- [6].Bu, X., Li, Y., Lai, W., Yao, C., Liu, Y., Wang, Z., Han, S., Zhao, Z., Du, J., Yao, S., et al(2024). Innovation and development of the aquaculture nutrition research and feed industry in China. *Rev. Aquac.* 2024, 16, 759–774. [CrossRef]
- [7].Liang, Q., Yuan, M., Xu, L., Lio, E., Zhang, F., Mou, H., & Secundo, F.(2022). Application of enzymes as a feed additive in aquaculture. *Mar. Life Sci. Technol.* 2022, 4, 208–221. [CrossRef] [PubMed]
- [8].Mori, N.C., Michelotti, B.T., Magnotti, C.C., Bressan, C.A., Bianchin, L.B., Sutili, F.J., Almeida, A.P.G., Kreutz, L.C., Pavanato, M.A., Cerqueira, V.R., et al.(2024). Growth, Metabolic, Hepatic and Redox Parameters, Digestive Enzymes and Innate Immunity in (*Mugil liza*) Fed a Citral-Supplemented Diet. *Fishes* 2024, 9, 240. [CrossRef]
- [9].National Research Council (NRC)(2011): Nutrient requirements of fish and shrimp Springer Nature, Aquaculture International June 2011,20(3) DOI:10.1007/s10499-011-9480-6.
- [10].Liu, B., P.Xu, J. Xie,X. Ge,S. Xia, C. Song & R. Chen. (2014). Effects of emodin and VitaminE on the growth and crowding stress of Wuchang bream (*Megalobramaamblycephala*). *Fish and Shellfish Immunology.* 40(2): 595-602.
- [11].Hamre, K. (2011). Metabolism, interactions, requirements and functions of vitamin E in fish. *Aquaculture Nutrition*, 17, 98–115. <https://doi.org/10.1111/j.1365-2095.2010.00755.x>
- [12].Pérez-Sánchez, T., Ruiz-Zarzuela, I., de Blas, I., & Balcázar, J.L. (2014). Probiotics in aquaculture: a current assessment. *Rev Aquacult.* 6:133–46. doi: 10.1111/raq.12033
- [13].Naiel, M.A., Farag, M.R., Gewida, A.G., Elnakeeb, M.A., Amer, M.S., & Alagawany, M. (2021). Using lactic acid bacteria as an immunostimulants in cultured shrimp with special reference to *Lactobacillus* spp. *Aquac Int.* (2021) 29:219– 31. doi: 10.1007/s10499-020-00620-2
- [14].Abdel-Latif, H.M., Yilmaz, E., Dawood, M.A., Ringø, E., Ahmadifar, E., & Yilmaz, S. (2022) . Shrimp vibriosis and possible control measures using probiotics, postbiotics, prebiotics, and synbiotics: a review. *Aquaculture.* 2022:737951. doi: 10.1016/j.aquaculture.2022.737951
- [15].Jenson I. (2014). BACILLUS | Introduction. In: Batt CA, Tortorello ML, editors. *Encyclopedia of Food Microbiology* (Second Edition). Oxford: Academic Press (2014). p. 111–7.
- [16].Xu, Y., Wang, Y., & Lin, J. (2014). Use of *Bacillus coagulans* as a dietary probiotic for the common Carp, *Cyprinus carpio*. *J World Aquac Soc.* 45:403– 11. doi: 10.1111/jwas.12139

- [17].Amoah K, Huang Q-C, Tan B-P, Zhang S, Chi S-Y, Yang Q-H, et al, (2019). Dietary supplementation of probiotic *Bacillus coagulans* ATCC 7050, improves the growth performance, intestinal morphology, microflora, immune response, and disease confrontation of Pacific white shrimp, *Litopenaeus vannamei*. *Fish Shellfish Immunol.* (2019) 87:796–808. doi: 10.1016/j.fsi.2019.02.029
- [18].Fath El-Bab, A.F., Majrashi, K.A., Sheikh, H.M., Shafi, M.E., El-Ratel, I.T., Neamat-Allah, A.N.F., El-Raghi, A.A., Elazem, A.Y.A., Abd-Elghany, M.F., Abdelnour, S.A., Abduh, M.S., Jaremko, M., & Naiel, M.A.E. (2022) Dietary supplementation of Nile tilapia (*Oreochromis niloticus*) with β -glucan and/or *Bacillus coagulans*: Synergistic impacts on performance, immune responses, redox status and expression of some related genes. *Front. Vet. Sci.* 9:1011715. doi: 10.3389/fvets.2022.1011715
- [19].Zhu, F., Zheng, X., Luan, J., Geng, J., & Zhang, H. (2024). Dietary Supplementation of *Bacillus subtilis* as Probiotic Improves Growth, Blood Health, and Immune Responses in Nile Tilapia (*Oreochromis niloticus*). *Animals*, 14(9), 1256.
- [20].Witeska, M., Kondera, E., Ługowska, K., & Bojarski, B. (2022). Hematological methods in fish–Not only for beginners. *Aquaculture*, 547, 737498.
- [21].SAS. Institute (2001). SAS User’s Guide: Statistics version.6.12 ed., SAS. Institute, Inc ., Cary, NC : 7pp.
- [22].Duncan, C.B. (1955). Multiple range and Multiple 'F' test. *Biometric.*,11:1-12.
- [23].Bandyopadhyay, P. (2022). *Advanced analytical techniques in food and feed analysis*. Academic Press.
- [24].Hlordzi, V., Kuebutornye, F. K., Afriyie, G., Abarike, E. D., Lu, Y., Chi, S., & Sakyi, M. A. (2020). The use of Cassava (*Manihot esculenta*) leaf meal as a dietary protein source in the diets of Nile tilapia (*Oreochromis niloticus*): A review. *Aquaculture Reports*, 18, 100483. doi.org/10.1016/j.aqrep.2020.100483
- [25].Siregar, A. F., Sabdono, A., & Pringgenies, D. (2020). Potential of seaweed extracts as an antibacterial agent against fish pathogens. *Journal of Marine Science and Technology*, 28(5), 455–461.
- [26].Hossain, M. A., & Rahman, M. M. (2021). "The Effects of Water Temperature on the Growth and Development of Common Carp (*Cyprinus carpio*)."
Aquaculture Research, 52(2), 678-684
- [27].Dawood, M. A. O., Koshio, S., & Esteban, M. Á. (2021). Beneficial roles of probiotics in fish aquaculture: A review. *Reviews in Aquaculture*, 13(1), 1–20.
- [28].Ringø, E., Hoseinifar, S. H., Ghosh, K., & Van Doan, H. (2022). Probiotics in aquaculture: Effects on gut microbiota and growth. *Aquaculture Nutrition*, 28(2), 365–377.
- [29].Alaryani, F. S., Albaqami, N. M., Elbahnaswy, S., Elshopakey, G. E., Almutairi, L. A., Hendam, B. M., Khattab, M. S., Abd El Megeed, O. H., Mathew, R. T., Abdul Kari, Z., Abd El-Aziz, Y. M., Eissa, M. E. H., Ahmed, A. E., Bahshwan, S. M. A., & Eissa, E. S. H. (2024). Effects of vitamin E and/or selenium nanoparticles on organ histology, hemato-

biochemical parameters, immunity, gene expression, and growth performance in Nile tilapia challenged with *Enterococcus faecalis*. *Aquaculture Reports*, 39, 102514. <https://doi.org/10.1016/j.aqrep.2024.102514>

[30].He, Y., & Li, X. (2024). Vitamin E: A potent antioxidant for aquatic animals. *Reviews in Aquaculture*, 16(2), 754-778. doi.org

[31].Al-Derawi, N. H., Abdel-Latif, H. M. R., & Khalil, R. H. (2024). The potential effects of dietary vitamin E nanoemulsion on growth performance, immune responses, and resistance against *Aeromonas veronii* infection in Nile tilapia (*Oreochromis niloticus*). *BMC Veterinary Research*, 20(1), 123. <https://doi.org/10.1186/s12917-024-03982-6>

[32].Kryuchkova, A. V., Kravtsova, L. Z., & Ivanov, A. A. (2024). Effect of probiotic "Ecoflor" on hematological and biochemical parameters of sturgeon fish. *E3S Web of Conferences*, 539, 01015. <https://doi.org/10.1051/e3sconf/202453901015>

[33].de Oliveira, T. F., de Souza, A. P., & da Silva, M. C. (2020). Probiotic supplementation in *Brycon amazonicus*: Effects on hematological and immunological parameters. *FAO AGRIS – Boletim do Instituto de Pesca*, 46(3), 1-10. (Available via AGRIS database)

[34].Abdel-Latif, H. M. R., Dawood, M. A. O., & El-Sabagh, M. (2023). Neutrophil-mediated immune responses in teleost fish: A review on the role of neutrophils in bacterial and fungal infections. *Fish & Shellfish Immunology*, 132, 108456. <https://doi.org/10.1016/j.fsi.2022.108456>

[35].Wang, P. P., Xu, W. J., & Xiang, J. H. (2022). Hypoxia stress affects the physiological responses, apoptosis and innate immunity of Kuruma shrimp, *Marsupenaeus japonicus*. *Fish & Shellfish Immunology*, 125, 228-237.

[36].Omar, A. A., Gado, M. S., Kandel, H. E., Farrag, F. A., & Shukry, M. (2025). Probiotic efficacy in aquaculture: The role of Technospore® (*Bacillus coagulans*) in improving Nile tilapia (*Oreochromis niloticus*) performance and disease resistance: A study on gut health, immunological response, and gene expression. *Probiotics and Antimicrobial Proteins*, 17(5), 3284-3301. <https://doi.org/10.1007/s12602-024-10279-3>

[37].Wang, Y., Li, J., Lin, Z., Xiao, J., Lu, Y., & Lin, L. (2016). Two highly adhesive lactic acid bacteria strains are protective in zebrafish infected with *Aeromonas hydrophila* by evocation of gut mucosal immunity. *Journal of Applied Microbiology*, 120(2), 441-451. <https://doi.org/10.1111/jam.13002>

[38].El-Sayed, A. M., & Izquierdo, M. (2021). The importance of vitamin E for farmed fish—A review. *Reviews in Aquaculture*, 14(2), 688–703. <https://doi.org/10.1111/raq.12615>

[39].Meng, Y., et al. (2023). Dietary cholesterol supplementation inhibits steroid synthesis but does not affect cholesterol transport in turbot (*Scophthalmus maximus*) and tiger puffer (*Takifugu rubripes*). *Aquaculture Nutrition*, 2023, 2308669. <https://doi.org/10.1155/2023/2308669>

- [40].El-Gohary, M. S., & Diab, A. M. (2014). Some studies on the effect of protexin on immune status of cultured seabass fingerlings. Alexandria Journal of Veterinary Sciences, 41(1), 109-119. <https://doi.org/10.5455/ajvs.153599>
- [41].Xia, Y., Yu, E., Lu, M., & Xie, J. (2020). Effects of probiotic supplementation on gut microbiota as well as metabolite profiles within Nile tilapia, *Oreochromis niloticus*. Aquaculture, 527, 735428. <https://doi.org/10.1016/j.aquaculture.2020.735428>
- [42].Carneiro, G. R. A., Opazo, C., Dos Santos, G. R. C., Pereira, H. M. G., Monnerat, G., & Opazo, R. (2023). Influence of lactic-acid bacteria feed supplementation on free amino acid levels in serum and feces of rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792). Frontiers in Marine Science, 10, 1216488. <https://doi.org/10.3389/fmars.2023.1216488>
- [43].Li, J., Li, D., Xu, J., Zhang, J., Zhang, X., & Du, H. (2025). Effects of vitamin E supplementation on growth performance, antioxidant capacity, and hepatic lipid metabolism in juvenile Chinese sturgeon (*Acipenser sinensis*). Antioxidants, 14(11), 1347. <https://doi.org/10.3390/antiox14111347>
- [44].Liang, D., Zheng, Q., Yang, Q., Tan, B., Dong, X., Chi, S., & Zhang, S. (2021). Alterations on growth performance, antioxidant responses and lipid metabolism in liver for juvenile hybrid grouper (♀ *Epinephelus fuscoguttatus* × ♂ *Epinephelus lanceolatus*) fed dietary vitamin E. Aquaculture Reports, 21, 100862. <https://doi.org/10.1016/j.aqrep.2021.100862>
- [45].Meng, Y., Qian, T., Zhang, Q., Zhou, Y., & Wang, S. (2025). Probiotic supplementation improves lipid metabolism disorders and immune suppression induced by high-fat diets in *Coilia nasus* liver. Biology, 14(4), 381. <https://doi.org/10.3390/biology14040381>